Biological Synopsis of the Solitary Tunicate
Ciona intestinalis

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BIOLOGICAL SYNOPSIS OF THE SOLITARY TUNICATE

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by

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ABSTRACT

The solitary ascidian *Ciona intestinalis* is native to northern European waters but now occurs worldwide from sub-Arctic to tropical regions. Apart from Scandinavian waters where it may dominate the epibenthic community, it typically occurs as an opportunistic fouling organism on artificial substrates in harbours or in association with aquaculture equipment. Over the last decade population outbreaks have been observed at multiple sites along the south shore of Nova Scotia, and similar outbreaks are now threatening the PEI mussel industry already dealing with an infestation of the clubbed tunicate *Styela clava*. The life history strategy of *C. intestinalis* is characterized by rapid growth (20 $mm.mo^{-1}$), early maturation (8-10 wk) and high reproductive output (>10,000 $eggs.ind^{-1}$). These characteristics, along with the ability to survive under adverse conditions of low flow and/or high turbidity, allow it to exploit and dominate new substrates at the expense of other fouling species. Given that larvae have limited dispersal capability, range extensions are likely facilitated by juveniles or adults hitchhiking on floating debris or the hulls of commercial and recreational vessels. A comprehensive risk management strategy is needed to curb the continued spread of *C. intestinalis*, and control measures are required to mitigate the negative impacts of population outbreaks on the aquaculture industry.

RÉSUMÉ

Carver, C.E., A.L. Mallet and B. Vercaemer. 2006a. Biological Synopsis of the Solitary Tunicate *Ciona intestinalis*. Can. Man. Rep. Fish. Aquat. Sci. 2746: v + 55 p.

L'ascidie solitaire *Ciona intestinalis* est originaire des eaux de l'Europe du Nord mais est désormais répandue depuis les régions subarctiques jusqu'aux régions tropicales. En dehors des eaux scandinaves où cette espèce domine parfois la communauté épibenthique, on la retrouve typiquement, en tant que salissure opportuniste, sur les substrats artificiels dans les ports ou en association avec l'équipement aquacole. Durant la dernière décennie, des explosions de populations ont été observées dans plusieurs sites le long de la côte sud-ouest de la Nouvelle-Ecosse et des explosions similaires menacent désormais l'industrie mytilicole de l'I-P-E qui est déjà aux prises avec l'infestation de l'ascidie plissée *Styela clava*. La stratégie du cycle biologique de *C. intestinalis* est caractérisée par une croissance rapide (20 mm.mois^{-1}), une maturation précoce (8-10 semaines) et une fécondité élevée (>10,000 œufs.ind^{-1}). Ces caractéristiques, autant que sa capacité à survivre dans des conditions défavorables de faible flux et/ou de turbidité élevée, lui permettent d'exploiter et de dominer de nouveaux substrats aux dépens d'autres espèces de salissures. Etant donné que les larves ont une capacité de dispersion limitée, l'expansion de la distribution de l'espèce est probablement facilitée par la capacité des juvéniles ou des adultes à se fixer sur des débris flottants ou la coque des bateaux commerciaux ou récréatifs. Une stratégie de gestion du risque approfondie est nécessaire pour limiter la propagation de *C. intestinalis*, et des mesures de contrôle sont requises pour diminuer les impacts négatifs sur l'industrie aquacole.

1.0   INTRODUCTION

The solitary ascidian *Ciona intestinalis* (Linnaeus 1767) is a temperate species noted for its cosmopolitan distribution and opportunistic behaviour. Population outbreaks of this species have caused substantial biofouling problems for aquaculture operations in South Africa (Hecht and Heasman 1999), New Zealand (Heasman, pers. comm.), Chile (Uribe and Etchepare 2002) and Scotland (Karayucel 1997). Although reported in Canadian waters prior to 1900 (Stimpson 1852), it is only recently that this cryptogenic species has been observed in high densities. The "invasive potential" exhibited by *C. intestinalis* has prompted growing concern with regard to possible ecological implications as well as impacts on marine activities, particularly aquaculture. The purpose of this review is to summarize the information available on the basic biology of this species, focusing primarily on those factors which may control its distribution and survival in Canadian waters. A discussion of possible vectors for dispersion as well as potential impacts on other organisms and industry should provide a basis for developing appropriate risk management strategies.

1.1. NAME AND CLASSIFICATION

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

Phylum: Chordata
Subphylum: Tunicata
Class: Ascidiacea
Order: Enterogona
SubOrder: Phlebobranchia
Family: Cionidae
Genus: *Ciona*
Species: *intestinalis*

Common name: Sea Vase tunicate

Although the ITIS website lists *C. intestinalis* as belonging to the Order Enterogona and the SubOrder Phlebobranchia, certain ascidian taxonomists prefer Lahille's 1886 classification scheme which included this species in the Order Phlebobranchia (Lambert 2005). Other taxonomists, specifically Kott (1990) argue that *C. intestinalis* belongs in the SubOrder Aplousobranchia.

C. intestinalis was first described by Linnaeus in 1767 under the name *Ascidia testinalis*, but other synonyms include *Ascidia tenella* and *Ciona tenella*. The common form is listed as *C. intestinalis* forma *typica* as opposed to forma *longissima* or *gelatinosa* which are found only in sub-Arctic and Arctic waters (Millar 1966). Note that *C. intestinalis* has been frequently confused with its close relative *Ciona savignyi* (Lambert 2003).

1.2. GENERAL DESCRIPTION

Adult specimens of the solitary ascidian or tunicate *C. intestinalis* may grow up to 15 cm in length and 3 cm in diameter (Figure 1). The body is cylindrical with soft, translucent tissues which vary in colour from pale greenish/yellow to orange. In older individuals the outer tunic becomes progressively more leathery and often takes on a brownish hue as a result of algal or bacterial fouling. Two openings or siphons are located at one end of the body; the longer inhalent siphon has eight lobes and the smaller exhalent siphon has six lobes, both with yellow margins and in some cases orange/red pigment spots. When disturbed the organism rapidly retracts these siphons using strong longitudinal muscles located beneath the protective outer tunic. *C. intestinalis* is a sessile filter feeder which is typically observed attached to hard natural or artificial substrates by short projections of the tunic (villi). It may occur in dense aggregations, often as a dominant member of the biofouling community, in enclosed or semi-protected marine embayments.

Typically *C. intestinalis* is distinguished from its congener *C. savignyi*, by the presence of a red rather than a white spot at the end of the sperm duct; however, surveys indicate that *C. intestinalis* from Atlantic coast populations lack this red spot (Lambert, pers. comm.). One distinguishing characteristic of *C. savignyi* is the presence of white pigment specks scattered throughout the body wall (Hoshino and Nishikawa 1985). For more detailed information on the morphological and taxonomic features which distinguish *C. intestinalis* see Van Name (1945), Millar (1966) and Monniot and Monniot (1972).

Figure 1. Photograph of live *Ciona intestinalis*

2.0 DISTRIBUTION

2.1. NATIVE DISTRIBUTION AND ABUNDANCE

C. intestinalis is believed to have originated in the Northeast Atlantic; when first described by Linnaeus in 1767, the Type Locality for the species was listed as the "European oceans". Early records are available for the coasts of Denmark, Norway, France and Britain in the late 1700's and early 1800's (Kott 1990 – see also www.deh.gov.au). Currently *C. intestinalis* occurs all around the coasts of Britain (www.iobis.org) and the Netherlands (www.ascidians.com). Major natural populations are found in shallow protected inlets in Denmark (Petersen and Riisgard 1992), along the west coast of Sweden (Dybern 1965) and extending northwards along the coast of Norway (Dybern 1967, 1969, Gulliksen 1972). Cold water or sub-Arctic records also exist for the Faeroe Islands, the east coast of Greenland and as far north as Spitsbergen and Bear Island (Russian Arctic – 78°N) (Millar 1966).

In terms of the Mediterranean there are records for the east coast of Spain, Italy, Greece, Turkey and Egypt (Millar 1966). In many cases *C. intestinalis* is listed as a fouling organism on artificial substrates such as marinas on the Aegean Sea coast of Turkey (Kocak et al.

1999), gas production platforms in the Black Sea (Zolotarev et al. 1993) and oyster culture operations in southern France (Mazouni et al. 2001).

2.2. NON-NATIVE DISTRIBUTION (EXCLUDING CANADA)

C. intestinalis is believed to have been spread widely throughout all temperate regions by shipping activities, particularly as a hitchhiker on the hulls of vessels (Monniot and Monniot 1994, Lambert and Lambert 1998). The earliest record from the US east coast is for New Bedford, Massachusetts (Agassiz 1850), and it is currently distributed from Maine south to Rhode Island (Plough 1978, see also www.massbay.mit.edu). On the US west coast, it was first recorded in San Diego bay, California in 1915 (Ritter and Forsyth 1917), and is currently a prominent member of the biofouling community in harbours such as San Francisco and along the California coast southwards into the Baja peninsula (Lambert and Lambert 1998, 2003). According to Van Name (1945) its range extends from California north to southern Alaska, but it was not observed in recent comprehensive surveys conducted in Puget Sound, Washington (Cohen et al. 1998) or Alaska (Hines and Ruiz 2000). Two collections, one in Alaska in 1903 (Ritter 1913) and one in British Columbia in 1937 misidentified *C. savignyi* as *C. intestinalis* (Lambert 2003). *C. savignyi*, originally from Japan, was first documented in California in 1985 and is now common from San Diego to Santa Barbara and from Seattle to Tacoma, Washington (Lambert 2003).

In South America, *C. intestinalis* is listed as a member of the invertebrate community in Brazil (Millar 1958), Argentina (www.iobis.org), Peru (www. guiamarina.com), and as a significant biofouling problem for scallop culture operations in Chile (Uribe and Etchepare 1999 2002). In South Africa, it occurs as a fouling organism in Table Bay Harbour (Millard 1952) and has been cited as a major problem for mussel operations in Saldahana Bay (Hecht and Heasman 1999). It may well occur in other coastal regions of South America and Africa but little information is available for these areas. Monniot and Monniot (1994) suggested that while *C. intestinalis* may be periodically and repeatedly introduced into warm water zones such as the Cape Verde Islands (equatorial Atlantic), it is primarily a coldwater species and hence fails to establish persistent populations.

The earliest record for the Australian continent is Hobart, Tasmania (Quoy and Gaimard 1834 in Kott 1990) and there are recent reports of its occurrence on a Tasmanian fish farm

(Tan et al. 2002). According to official surveys it also occurs along the eastern and southern coasts of mainland Australia including Queensland, New South Wales, and Victoria (www.deh.gov.au). Large populations were apparently common in Australian ports from 1950-1970, but numbers have since declined (Kott 1990). Recent publications describe it as a new arrival or an "exotic" in Port Phillip Bay and Westernport, Victoria (Cohen et al. 2000a, 2000b) as well as in western Australia (MacDonald 2004). *C. intestinalis* has been documented in various harbours in New Zealand (Millar 1982), and was considered a major pest for the mussel culture industry in the 1990's (Heasman, pers. comm.). Similar to the Australian experience, however, it seems to have virtually disappeared in recent years.

There are numerous references to the presence of *C. intestinalis* in Asian waters, but it is difficult to establish when this species first appeared or became prominent in this region. NIMPIS (2002) lists *C. intestinalis* as occurring throughout Indonesian waters and northwards along the coasts of China and Japan. This is consistent with various studies which indicate that *C. intestinalis* is a biofouling problem for aquaculture operations in Korea (Na and Lee 1977, Kang et al. 1978), China (Cao et al. 1999, Zhou et al. 2002) and Japan (Yamaguchi 1975, Hoshino and Nishikawa 1985, Arakawa 1990).

2.3. DISTRIBUTION IN CANADA

In Canadian waters, *C. intestinalis* is generally considered "cryptogenic" or of unknown origin; it may be native to this region but was likely introduced from northern Europe. The earliest record of occurrence in Eastern Canada is for Grand Manan Island (Stimpson 1852, Whiteaves 1900), and it is currently listed as a member of the bathyal or deepwater fauna in the Bay of Fundy (www.gmbis.marinebiodiversity.ca). Van Name (1945) reported its occurrence in the Gulf of St. Lawrence and Newfoundland. Brunel et al. (1998) also listed *C. intestinalis* as bathyal in the Gulf of St. Lawrence (specifically the region extending from the Gaspé south to the Baie des Chaleurs), based on records from Van Name (1912) and Jean (1953). Over the last decade, this previously rare species has undergone a population outbreak, particularly along the south coast of Nova Scotia (N.S.) in bays with artificial substrates such as docks, fishing vessels and aquaculture gear (Figure 2). It has also been observed at aquaculture operations in the Ile Madame area of southern Cape Breton, but is

notably absent or very rare along the eastern shore region of N.S. (Clancey and Hinton 2003, Clancey and MacLachlan 2004).
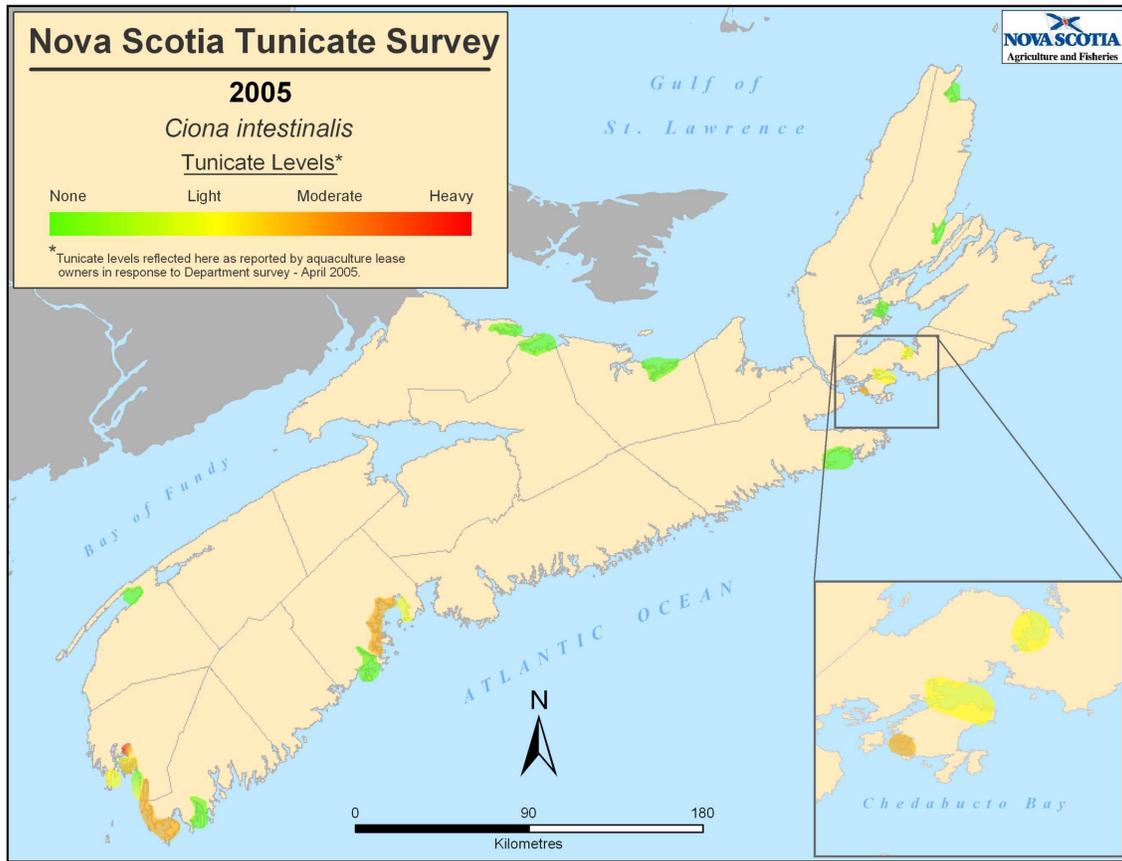
Figure 2. Results of the 2005 tunicate survey in Nova Scotia. Survey was conducted by interviewing aquaculture lease holders (map courtesy of Andrew Bagnall, N.S. Dept. Fisheries).

Observations now suggest that a similar population outbreak is occurring in certain Prince Edward Island (PEI) estuaries; the first record was for the Montague area in October 2004, and it has since appeared in several adjacent bays (Figure 3).

C. intestinalis has been documented in the Canadian Arctic (Atkinson and Wacasey 1976), but this may have been one of the coldwater subspecies, forma *gelatinosa* or forma *longissima*. Whether it occurs along the Labrador coast and up into Hudson Bay is not known. Huntsman (1912) lists *C. intestinalis* as a member of the benthic invertebrate community on the west coast of Canada, but it is possible that specimens of *C. savignyi* may have been misidentified. It was also listed more recently in the deepwater Gwaii Haanas

Invertebrate Survey (www.iobis.org), but again this identification was not confirmed. At present its status on the west coast remains elusive; there are no recent records for shallow coastal waters and no mention of this species as a biofouling problem for aquaculture operations to date (Theriault, pers. comm.).
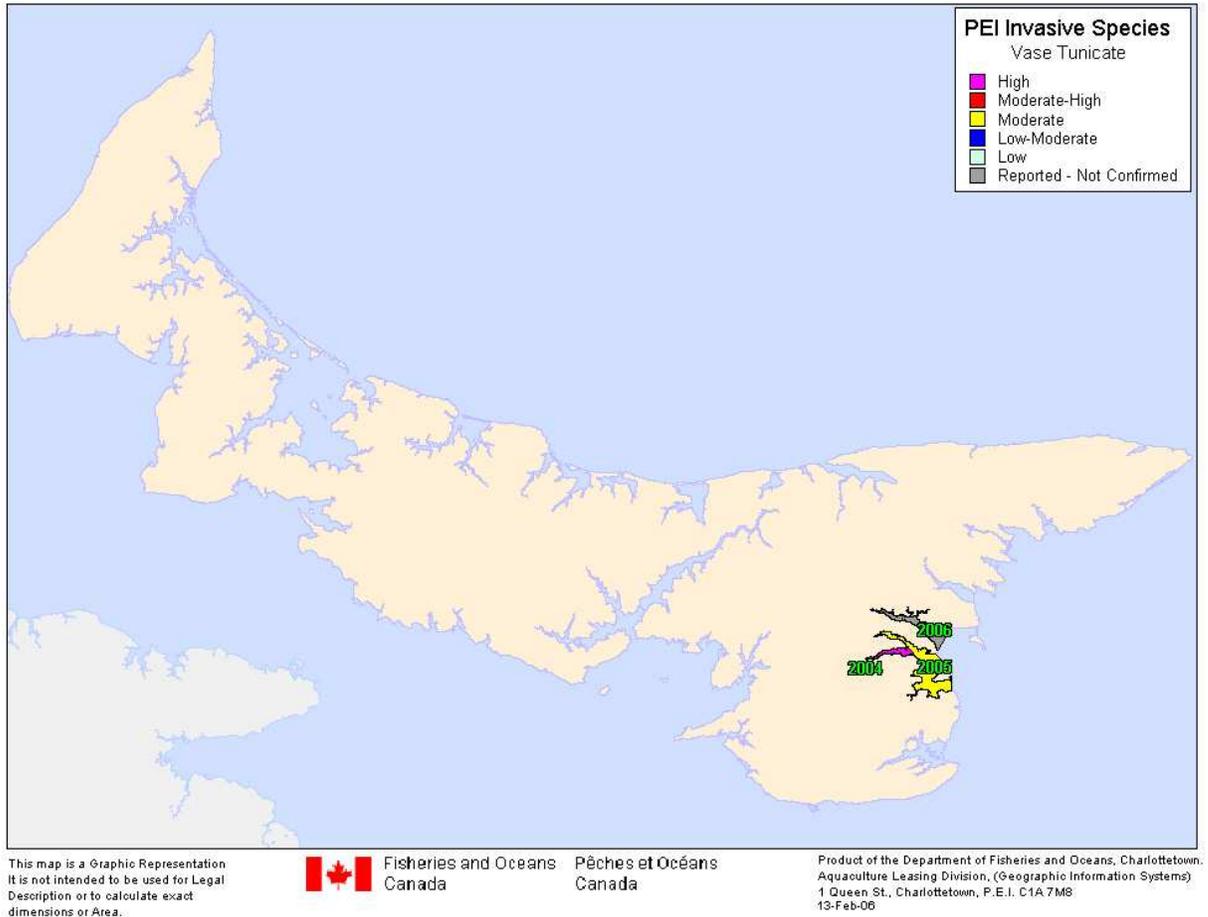
Figure 3. Distribution of *C. intestinalis* in P.E.I. as of February 2006 (map courtesy of Neil MacNair, PEIDAF, Art Smith and Deryck Mills, DFO).

3.0   BIOLOGY AND NATURAL HISTORY

3.1. BODY STRUCTURE

C. intestinalis has a cylindrical body with two siphons and a gelatinous outer tunic composed of tunicin, a polysaccharide material chemically similar to cellulose (Millar 1970) (Figure 4). Inside the tough outer tunic is a thin sac-like membrane composed of an external

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epithelium, connective tissue, muscles and blood vessels which encloses the internal organs (Van Name 1945). Contraction of the longitudinal muscle bands and circular muscle fibres embedded in this membrane allow the organism to retract rapidly when disturbed. The body is divided into a large atrial cavity which contains the branchial sac and a smaller visceral cavity containing the digestive and reproductive organs (Millar 1971). At the upper end the oral (inhalant) or branchial siphon opens into the branchial sac or pharynx which is perforated with openings called stigmata. The structure of the stigmata and the wall of the branchial sac are characteristic of the type of ascidian (for details see Van Name 1945, Millar 1966, Plough 1978).
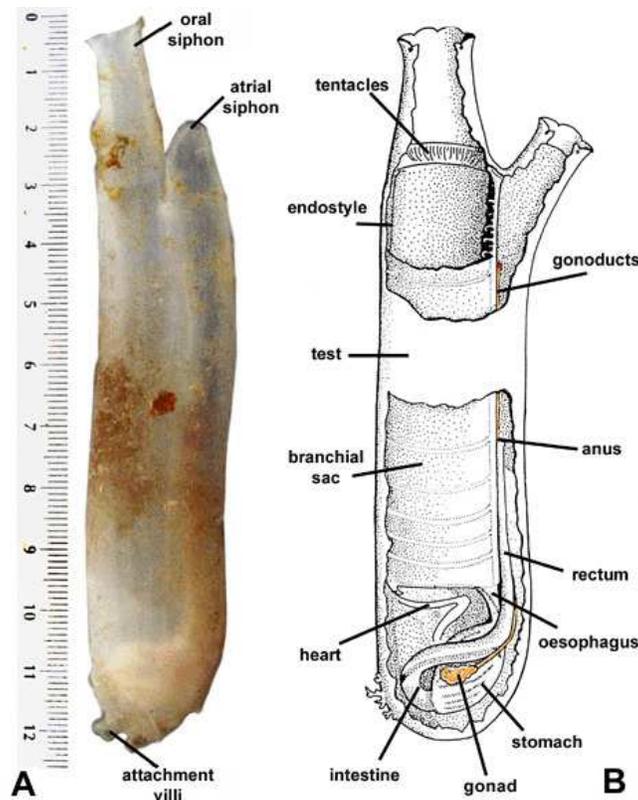
Figure 4. Body structure of *C. intestinalis* from Cirino et al. (2002): A. whole individual, B. internal anatomy.

Cilia located in the stigmatal openings or ostia direct water from the branchial sac into the atrial cavity where it is ejected through the atrial or exhalant siphon (MacGintie 1939). Unlike bivalves which primarily use cilia to sort particles and direct them towards the mouth, *C. intestinalis* is a mucus feeder (Jorgensen et al. 1984). The rod-like endostyle which lies

along the wall of the branchial sac continuously secretes a mucus net or mesh-like structure (Flood and Fiala-Médioni 1981). As cilia transport this net across the wall of the branchial sac, incoming particles become entrapped in the mucus. These mucus-bound particles are gathered into a cord by the dorsal lamina or languets and then transported to the oesophagus and the stomach which lie below the branchial sac (Millar 1970). Material passes into the intestine which curves upward from the base of the stomach to join the rectum lying against the wall of the atrial cavity; faeces discharged into the atrial cavity are expelled through the atrial siphon.

C. intestinalis is classified as a hermaphrodite – individuals possess both male and female reproductive organs (Millar 1970). These structures are located in the lower section of the tunic; the ovary lies between the stomach and the intestine whereas the testis is dispersed in a diffuse mass over the surface of the intestine and part of the stomach. In mature individuals, the ovary has a pinkish appearance and the testis is white. Separate gonoducts (oviduct, sperm duct) run parallel to the rectum but extend further up into the atrial cavity. Gametes discharged into the atrial cavity are expelled or "broadcast" into the environment.

C. intestinalis has an open blood system driven by a tubular heart which lies adjacent to the digestive tract (Plough 1978). Blood from the digestive tissues collects in the ventral sinus below the branchial sac and is forced up through the vessels lying between the stigmata. After several minutes the heartbeat slows and then starts beating in the opposite direction which moves fluid from the dorsal sinus down into the spaces around the viscera. This primitive circulation system distributes dissolved organic material and oxygen throughout the body. The blood may contain high levels of vanadium and other trace metals which are taken up in the branchial sac (Goldberg et al. 1951, Cheney et al. 1997). According to Michibata et al. (2001) ascidians accumulate vanadium in order to produce a specific type of blood cell or vanadocyte.

3.2. FEEDING AND RESPIRATION

As described above *C. intestinalis* is an active suspension feeder or filter feeder with the ability to remove particles from the surrounding environment. It should be noted that most estimates of particle filtration rate are based on laboratory experiments using cultured algae

of uniform size, rather than natural particle distributions. Because physiological rates are frequently expressed in weight-specific terms, i.e. per g total dry weight or per g organ dry weight (body tissues excluding the tunic), a summary of these various relationships has been provided (Appendix 1). For example, a 50-mm individual has a total dry wt of 0.11 g and an organ dry wt of 0.05 g whereas a 100-mm individual has a total dry wt of 0.58 g and an organ dry wt of 0.26 g.

Particle filtration

Fiala-Médoni (1978) reported that *C. intestinalis* does not exhibit any pumping rhythm; water flow through the atrial siphon is basically constant. At a temperature of 15^oC the mean flow velocity for 65-70 mm individuals was 7.6 $cm.s^{-1}$ with a maximum velocity of 27 $cm.s^{-1}$. Filtration rates based on the removal of algal cells were also continuous without any noticeable rhythm. Weight-specific mean filtration rate was estimated at 4.3 $l.h^{-1}.g^{-1}$ or 0.95 $l.h^{-1}$ for individuals of 0.22 g organ dry wt. In a previous study Fiala-Médoni (1974) had estimated a lower weight-specific filtration rate of 3.5 $l.h^{-1}.g^{-1}$ or only 0.46 $l.h^{-1}$ for 70-82 mm individuals (0.13 g organ dry wt) at 15^oC. Subsequent studies focused on clarifying the relationship between filtration rate and body size. Randlov and Riisgard (1979) determined filtration rate ($ml.min^{-1}$) at 10^oC as a function of organ dry weight (g) as $F = 142 (W_{organ})^{0.91}$. For a 70-mm individual (0.13 g organ dry wt), this is equivalent to 1.3 $l.h^{-1}$, or higher than the values reported by Fiala-Médoni (1974, 1978).

Petersen and Riisgard (1992) investigated the same relationship at 15^oC and reported filtration rate ($ml.min^{-1}$) as a function of total dry weight (g) as $F = 118 (W_{total})^{0.68}$ or organ dry weight (g) as $F = 199 (W_{organ})^{0.67}$. In this case, the predicted filtration rate for a 70-mm individual (0.13 g organ dry wt) would be 3.0 $l.h^{-1}$ or again higher than previously reported. In the same study it was determined that within the temperature range of 4 to 21^oC, maximum filtration rate ($ml.min^{-1}.ind^{-1}$) increased linearly with increasing temperature (T_{oC}) according to $F_{max} = 1.46 (T) -1.21$. Above 21^oC filtration rate declined rapidly with increasing temperature. The overall formula for predicting filtration rate ($ml.min^{-1}$) based on organ dry weight (g) and temperature (T_{oC}) was $F = 199 (W_{organ})^{0.67} + 1.46 (T-15)$. For 70-mm individuals (0.13 g organ dry wt), this translates into a maximum filtration rate of 2.6 $l.h^{-1}$ at 10^oC, 3.0 $l.h^{-1}$ at 15^oC and 3.5 $l.h^{-1}$ at 20^oC. Petersen and Riisgard (1992) acknowledged

that these values were up to three times higher than previously reported; they suggested that feeding may have been depressed in earlier trials due to disturbance and/or the use of high algal cell densities (e.g. 20,000 cells.ml^{-1}).

In addition to the impact of body size and temperature on filtration rate, it is important to determine how *C. intestinalis* responds to increases in particle concentration. Robbins (1983) reported that filtration rate declined with increasing concentration of inert inorganic particles. Over most of the range of concentrations and individual sizes examined, the ingestion rate was constant and maximal, with the largest individuals having the highest ingestion rates. Petersen and Riisgard (1992) reported that weight-specific filtration rate decreased logarithmically with increasing algal cell concentrations above 10,000 to 15,000 cells.ml^{-1}, possibly as a result of satiation or filling of the gut. Using microscopical techniques, Petersen et al. (1999) confirmed that the beat frequency of the cilia in the branchial sac decreased with increasing algal cell concentration. At low concentrations flow velocity in the atrial siphon remained stable, whereas at high concentrations, flow velocity was reduced and much less stable. They also confirmed that filtration rate varies in response to gut fullness or gut clearance rate. In a similar experiment, Sigsgaard et al. (2003) confirmed that filtration rates declined and ingestion rates stabilized with increasing algal cell concentration. Unfortunately, little information is available as to how the filtration rate of *C. intestinalis* varies in response to increasing particle concentrations in the field.

Particle selection/Retention efficiency

Compared to bivalves, ascidians are more efficient at retaining small particles (<4 µm) such as bacterioplankton or picoplankton (Jorgensen et al. 1984). Flood and Fiala-Médioni (1981) reported that the mucus net of *C. intestinalis* is constructed of longitudinal and transverse filaments that form a rectangular mesh structure with holes measuring 0.4 µm by 0.7 µm. Feeding trials confirmed that this species is 100% efficient at retaining particles down to 2-3 µm and 70% efficient at retaining 1-µm particles (Randlov and Riisgard 1979, Jorgensen et al. 1984).

Unlike bivalves, *C. intestinalis* does not have the capacity to sort particles and reject unsuitable material as pseudofaeces. Instead, as particle concentrations increase it exhibits an increased frequency of squirting, or muscular contractions which actively expel

accumulated material through the oral siphon thereby preventing clogging of the branchial sac (Jorgensen and Goldberg 1953, Robbins 1984, Petersen et al. 1999). Robbins (1984) concluded that rates of squirting are dependent on food quantity rather than quality; *C. intestinalis* showed no ability to distinguish between inorganic and organic particles.

Other studies, however, suggest that *C. intestinalis* may actively discriminate between particles of different sizes. According to Lesser et al. (1992) this species exhibited preferential selection for large phytoplankton (16 μm) over smaller phytoplankton (3-5 μm) when provided with natural planktonic assemblages. Similarly, Zhang et al. (2001) reported that *C. intestinalis* tended to select larger algal cells (10 μm) over smaller cells (1.5 μm) in feeding selectivity trials. More studies based on natural particle assemblages are required to resolve this issue.

Digestion/Excretion

There is little information on digestion and excretion rates of *C. intestinalis* fed on natural particle assemblages. Fiala-Médioni (1974) estimated a digestion efficiency of 83% on an algal diet of *Monochrysis lutheri* (20,000 cells.ml^{-1}). Sigsgaard et al. (2003) reported evidence of selective digestion of particles by *C. intestinalis* when provided with a high-quality food source, the microalgae *Rhodomonas* sp. and a low-quality food source, particles of eelgrass *Zostera marina*. Weight-specific rates of filtration and ingestion were similar for the two diets, but weight-specific respiration rates were substantially higher when digesting *Rhodomonas* sp. The authors concluded that the energy expended in digestive activity is strongly dependent on the quality of the food ingested.

According to Robbins (1985a) the amount of faeces produced is dependent upon both the quantity and nature of the particulate suspension ingested. Gut residence time is constant and reduced assimilation efficiencies at higher particulate concentrations are thought to be associated with the degree of satiation.

C. intestinalis excretes ammonia but not urea. Markus and Lambert (1983) estimated ammonia (NH$_3$-N) excretion rates as 86 μg NH$_3$-N.g$_{total}^{-1}$.h^{-1} based on total dry weight or 192 μg NH$_3$-N.g$_{organ}^{-1}$.h^{-1} based on organ dry weight. Interestingly, these values were three times higher than ammonia excretion estimates for *S. clava* at the same temperature (20°C).

According to Zhang et al. (2000) *C. intestinalis* exhibits maximum ammonia excretion rates at 18°C.

Respiration/growth

Markus and Lambert (1983) estimated weight-specific oxygen consumption rates for *C. intestinalis* at 20°C as 0.82 ml O_2 .g_{total}^{-1}.h^{-1} based on total dry weight, or 1.71 ml O_2 .g_{organ}^{-1}.h^{-1} based on organ dry weight. This was generally consistent with Shumway (1978) who estimated the oxygen consumption rate of *C. intestinalis* at 10°C as 0.515 ml O_2 .g_{total}^{-1}.h^{-1} (ml O_2 .h^{-1} = 0.515 $(g_{total})^{0.831}$). Similarly, Petersen et al. (1995) observed a maximum weight-specific oxygen consumption rate of 1.08 ml O_2 .g_{organ}^{-1}.h^{-1} at 10°C; they also noted that respiration rate increased sigmoidally with increasing algal cell concentration (0-12,000 cells.ml^{-1}). Zhang and Fang (2000) reported that the relationship between temperature and oxygen consumption in *C. intestinalis* peaks at 18°C.

Comparison of the ratio of tunic weight to organ weight over a range of sizes indicates that these two components exhibit isometric growth; the organs consistently account for 36% of the total wet weight and 45% of the total dry weight (Carver, unpubl. data, Appendix 1). According to Petersen et al. (1995) weight-specific growth rate for the body organs increases sigmoidally up to a maximum of 8% per day. Costs of maintenance are greater in larger individuals, whereas costs of organ growth are similar regardless of size. Growth in terms of increase in body length was estimated at 2.3-2.8% length.d^{-1}, which was similar to Dybern's (1965) estimate of 2-3% length.d^{-1}. Petersen et al. (1995) also suggested that growth rate was predictable based on a "condition index" incorporating the ratio of organ dry weight to total dry weight (CI = W_{organ}/W_{total}).

3.3. REPRODUCTION AND DEVELOPMENT

Gamete production and spawning

C. intestinalis is a simultaneous hermaphrodite with concurrent production of eggs and sperm (Figure 5). In the very early stages of maturity, however, it shows a tendency towards protandric hermaphroditism; sperm production precedes egg production. Reproductive capability is size rather than age dependent (Millar 1952), although size at maturity varies among populations (Dybern 1965). The general consensus for cold temperate populations along the Scandinavian coast and the Atlantic coast of North America is that individuals must

attain 50-80 mm before producing fertile gametes (Dybern 1965, Cirino et al. 2002, Carver et al. 2003). Once individuals reach maturity gametes are produced continually as long as temperatures are suitable. Dybern (1965) suggested that *C. intestinalis* stops spawning in the fall due to degenerative changes in the gonads associated with declining water temperatures. However, Carver et al. (2003) documented the presence of apparently ripe eggs in the ovary from November (7°C) through January (1°C). Signs of egg resorption were evident in February and March (-1°C), but gamete production resumed in April when temperatures increased to 4°C. Dybern (1965) also noted that gonad development may occur before temperatures reach 8°C, or the lower limit for normal embryonic development. The lower temperature limit for spawning activity is reported to be 8°C in Scandinavian populations (Dybern 1965, Gulliksen 1972) which is consistent with observations for Nova Scotian populations (Carver, unpubl. data).

C. intestinalis is classified as oviparous; eggs and sperm are expelled through the atrial siphon and fertilization occurs externally. Spawning can be artificially induced in the laboratory by manipulating light levels, specifically exposure to light following a period of darkness (Whittingham 1967, Lambert and Brandt 1967). Note that exposure to continuous light may inhibit spawning (Georges 1971). Field observations indicate that this species typically spawns at dawn (Berrill 1947), although Yamaguchi (1975) observed spawning at sunset in a Japanese population. A common response to light exposure, or synchronized spawning, may improve the probability of successful egg fertilization.
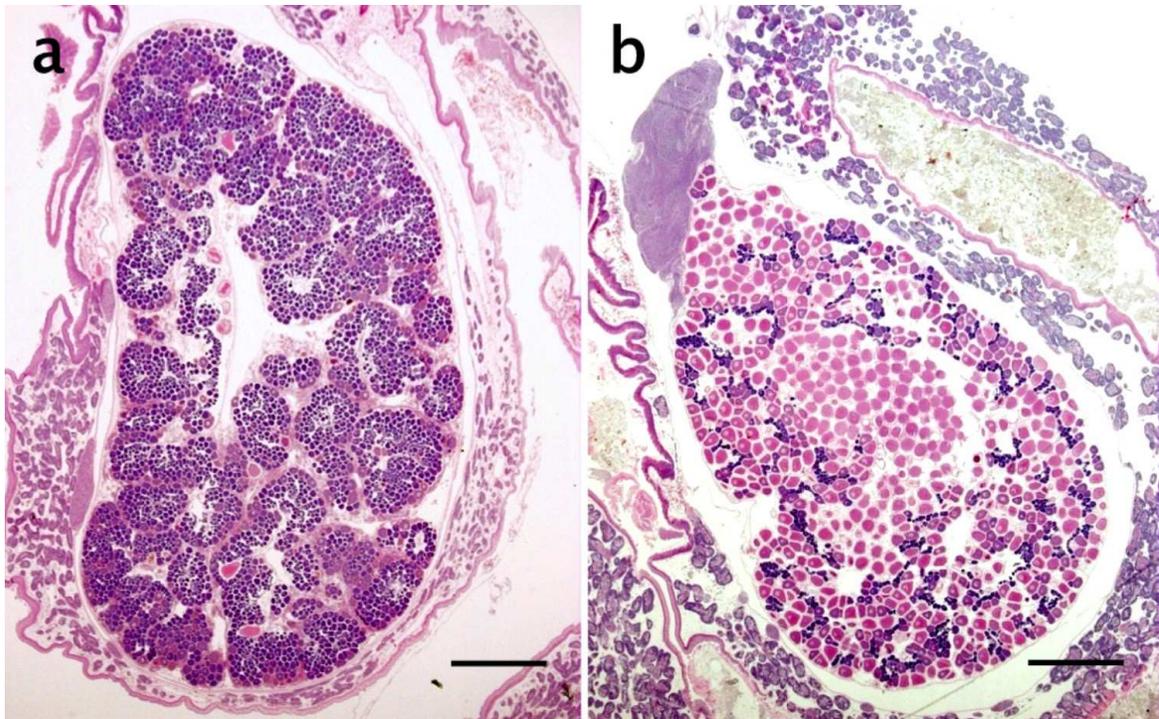
Figure 5. Reproductive status – egg development in the ovary: (a) early development – eggs stained dark purple (April); (b) mature eggs stained pink (May), see also the testis (violet) lying around the outside of the intestine (top-right). The particulate material in the intestine appears greyish-brown. Scale bars are 1 mm.

In terms of fecundity or egg production potential, Yamaguchi (1975) estimated individual rates of 2000-3000 eggs every 2-3 d or approximately 1000 eggs.d^{-1} in a Japanese population. Total egg production over an individual's life span was estimated conservatively at 100,000 eggs. In contrast, Carver et al. (2003) estimated a maximum individual fecundity of 500 eggs d^{-1} for adults of 100-120 mm. Total fecundity was not measured, but observations suggested a conservative lifetime estimate of 12,000 eggs per individual (150 eggs d^{-1} over 60 d); this was consistent with the 10,000 eggs per individual suggested by Petersen and Svane (1995). This variation in reproductive effort among populations may reflect differences in the temperature regime and/or their relative life span (see section 3.4).

C. intestinalis is generally reported to be self-sterile or self-discriminating which means that individuals do not self-fertilize. However, Rosati and Santis (1978) reported that in the Gulf of Naples (Italy) 15% of individuals were self-fertile. Self-sterility and self-fertility are specific properties of the gametes; eggs from self-sterile animals are not fertilized even at very high sperm concentrations. The ability to self-discriminate is established during late

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egg development and is controlled by products in the overlying follicle cells (Pinto et al. 1995).

Ripe eggs of *C. intestinalis* (150-180 μm in diameter) are surrounded by villiform follicles which radiate outwards from the surface (Figure 6). Note that the structure and arrangement of these follicle cells is diagnostic of this species; specifically, *C. intestinalis* eggs have a single large refringent droplet in each follicle as opposed to several small droplets in *C. savignyi* (Byrd and Lambert 2000). Eggs may be released individually or in mucus strings which rapidly become entangled in nearby adults or other substrates such as eelgrass. Petersen and Svane (1995) suggested that the generation of mucus-bound egg strings may improve the probability of fertilization as well as promote the aggregation of populations in suitable areas. Individual eggs not contained in mucus are negatively buoyant in still water and tend to sink and adhere to the substrate where they may remain viable for up to 30 h. During this period, they produce sperm chemoattractants which diffuse rapidly to create an effective egg size (i.e. the distance at which free-swimming sperm detect, and are attracted to, the egg membrane) of 2 mm diameter (Jantzen et al. 2001). It is estimated that if even half of the sperm encountering this chemoattractant halo subsequently reach the egg membrane, fertilization success will increase more than 50-fold. This sperm-attracting activity vanishes when the egg deforms, suggesting that the release of attractant is terminated upon fertilization (Yoshida et al. 1993).
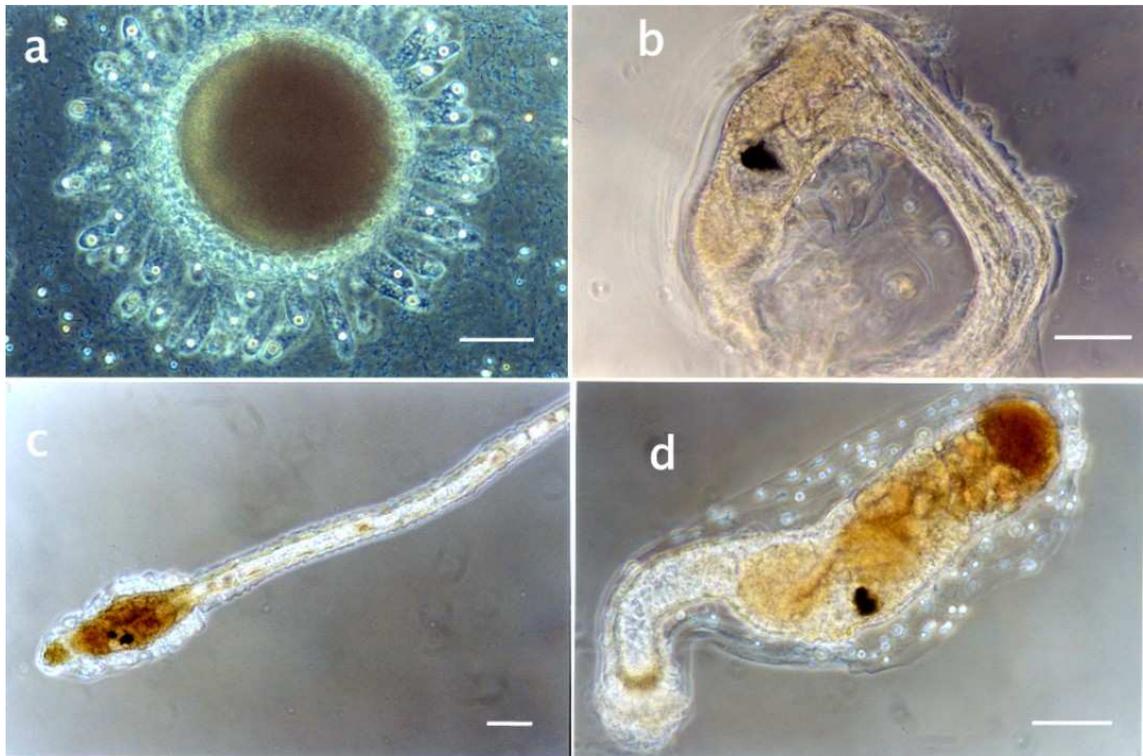
Figure 6. Egg to larva development (a) egg with follicle cells; (b) larva close to hatching; (c) "tadpole" larva with ocellus and statolith; (d) recently-settled larva starting to metamorphose; note that the tail has been resorbed. Scale bars are 50 μm.

Bolton and Havenhand (1996) found that sperm energy reserves are conserved in the absence of homospecific eggs (eggs of the same species). If no eggs are encountered, sperm remain viable for up to 16 h after release, but viability declines to 1.5 h when eggs are present. The sharp decline in sperm longevity when exposed to homospecific egg water corresponded to an increase in sperm activity suggesting a causal link between these characteristics.

Embryonic and larval development

The duration of embryonic development, or the period between egg fertilization and hatching, varies with temperature. Estimates for North Atlantic populations of *C. intestinalis* are generally consistent. In Scandinavia Dybern (1965) estimated 48 h at 12°C versus <24 h at 20°C, while Svane and Havenhand (1993) reported values ranging from 63 h at 9°C to 26 h at 16°C. In New England, Cirino et al. (2002) estimated that embryogenesis required 18 h at 18-20°C while Bullard and Whitlatch (2004) cited 22 h at 20°C. By comparison, Na and Lee

(1977) reported that *C. intestinalis* in Japan exhibited an embryonic development period of only 12 h at 18°C.

The distinctive "tadpole" larva of *C. intestinalis* (0.8–1.2 mm) consists of a trunk (100-200 μm) with a slender muscular tail (800-1000 μm) surrounded by granular test cells (Figure 6). The larva has six developed organ systems (tunic, epidermis, notochord, tail musculature, adhesive organ, and nervous system) and four rudimentary organ systems including a primordial branchial sac (Katz 1983). The notochord, which extends the length of the tail, closely resembles that observed in vertebrate larvae. The trunk has two black spots: a large photosensitive ocellus and a smaller gravity-sensitive statolith (Millar 1970). Adhesive papillae located at the anterior end of the trunk are used to attach to the substrate at settlement. For more details on embryogenesis and larval development see Berrill (1947).

Newly hatched larvae may escape from mucus-bound egg strings to disperse in the plankton (estimated at 40-60%) or may be retained until settlement (Petersen and Svane 1995). According to Millar (1971) they initially swim upward (i.e. positive phototropism and negative geotropism) which tends to promote their distribution. Berrill (1931) estimated that *C. intestinalis* should be capable of moving at 4 mm.s^{-1} based on the size of the larval tail. Nakagawa et al. (1999) reported that newly hatched larvae exhibit an average swimming speed of 1.4 mm.s^{-1} with no response to light stimuli. After 3-4 h swimming speed declines to 0.4 mm.s^{-1} but larvae may be induced to swim more rapidly by sudden decreases in light intensity.

The duration of the tadpole larval phase is temperature dependent and estimates vary widely. Millar (1952) reported time to settlement for British populations to be 6-36 h whereas Jackson (2005) estimated 2-10 d for the same region. Dybern (1965) reported the duration of the larval phase as 4-5 d at 10-12°C or 24-36 h at 18-20°C. Dispersal potential has been estimated at 100-1000 m (Jackson 2005), but this will depend on swimming speed, duration of the tadpole phase and most importantly on the local hydrographic regime. Petersen and Svane (1995) noted that Scandinavian populations tend to be highly localized which may be indicative of limited dispersal. In a study of one shallow Danish inlet, the authors found that recruitment only occurred within the cove suggesting that dispersal to the

outside, as well as influx of larvae through the narrow entrance, was likely limited or non-existent.

Settlement and metamorphosis

Towards the end of the tadpole phase, the larvae tend to sink or swim downwards and become strongly photonegative with a preference for dark or shaded surfaces in zones with reduced water movement and light intensity (Millar 1971, Gulliksen 1972, Schmidt and Warner 1984). Tursi (1980) observed that *C. intestinalis* consistently selected areas located in shadow (96%) versus light (4%) and exhibited a preference for obliquely oriented surfaces (61%) over horizontal (28%) or vertical surfaces (11%). The presence of a biofilm on the substrate may also promote settlement, although observations are mixed. Szewzyk et al. (1991) concluded that larvae of *C. intestinalis* showed no preference for surfaces with a bacterial film over clean hydrophobic or hydrophilic surfaces. In field trials Keough and Raimondi (1996) observed a variable reponse depending on the type of bacterial film. Wieczorek and Todd (1997) reported that numbers of settled larvae generally increased with biofilm age with the highest mean numbers observed on 12 d-old biofilms. It should be noted that larval attachment patterns may reflect the combined effects of both active habitat selection and passive deposition or entrapment of larvae in a "sticky" substratum (Szewzyk et al. 1991).

Havenhand and Svane (1991) concluded that the occurrence of aggregated populations of *C. intestinalis* is related primarily to changes in hydrodynamics rather than gregarious behaviour involving some form of chemoattraction to adults. Laboratory experiments indicated that tadpole larvae were not stimulated to settle in aqueous extracts of adult tunic, nor was there any indication of aggregation on uniform flat substrata. Field experiments with adult *C. intestinalis* and *C. intestinalis* mimics confirmed that larval settlement activity showed a similar positive response to either an increase in the number of adults or the number of mimics.

In terms of timing, the highest levels of settlement occur in the morning (Bullard et al. 2004). The tadpole larva attaches to the substrate with its adhesive papillae, located at the anterior end. After settlement the larval tail is rapidly resorbed into the trunk, reducing the size of the early juvenile from 1.3 mm to 350 μm. After one day a stalk begins to develop

and the first ascidian stage is observed within 10 d (Figure 7). For more details on the juvenile development of *C. intestinalis*, see Berrill (1947), Cirino et al. (2002) and Bullard and Whitlatch (2004).
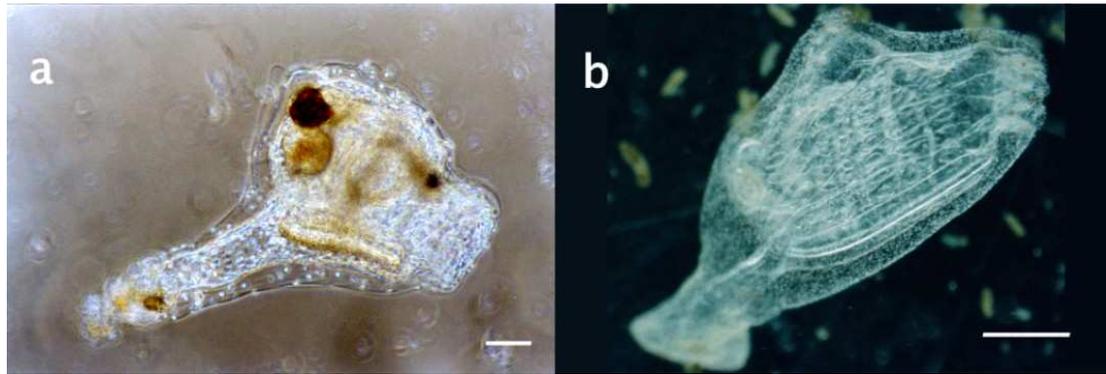

Figure 7. Juvenile development: (a) early juvenile with developing siphons (525 μm), scale bar is 50 μm; (b) juvenile (1.3 mm) with stigmata evident in the branchial sac, scale bar is 500 μm (photo courtesy of Dr. Dan Jackson, DFO).

3.4. LIFE CYCLE: GROWTH, GENERATION TIME AND LONGEVITY

Dybern (1965) reviewed the life history of various populations of *C. intestinalis* and suggested that growth rate, size at maturity and longevity vary depending on cumulative temperature exposure. For example, deep water or boreal populations which rarely experience temperatures exceeding 8°C may reach 150 mm, live 2-3 y and reproduce once a year, or less if water temperatures are unsuitable. This pattern is typical of deep water populations in Scandinavia and sub-Arctic populations.

Cold temperate populations, such as those which occur in Scotland (Millar 1952) or in the shallow regions of the Scandinavian coast (Dybern 1965), experience winter temperatures of -1°C with summer temperatures of 15-20°C, or approximately 7-8 mo at >8°C (April – November). These coastal populations typically survive for 12-18 mo and produce two generations per year. The first is recruited early in the season as soon as water temperatures are suitable; when these individuals reach maturity later in the summer they give rise to the second generation. The two generations co-exist through the winter until the first spawning period of the following year at which time the older individuals spawn and die. The second

generation grows during the fall but growth ceases with declining water temperatures; these individuals do not reach sexual maturity until the following spring.

Temperate populations, such as those in southern Britain or the cooler regions of the Mediterranean which experience water temperatures of 5-20°C, may spawn almost continuously throughout the year. Berrill (1947) estimated a life span for *C. intestinalis* in southern Britain of 1-2 y with as many as three co-existing generations and a very short non-spawning period. By comparison, *C. intestinalis* populations which never experience water temperatures below 10°C, such as those in Japan or the warmer regions of the Mediterranean, exhibit a shorter life span and a reduction in maximum body length. Yamaguchi (1975) estimated the life span of Japanese populations as 6 mo in the winter (14-19°C) and 3 mo in the summer (20-25°C). Maximum body length is 60 mm for both winter and summer populations, but sexual maturity (20 mm) is achieved in 2 mo (10 mm.mo^{-1}) in the winter and 1 mo in the summer (20 mm.mo^{-1}). Under these conditions, 2-3 generations may overlap at any one time and there may be more than 4 generations a year. Mediterranean populations have similar life history characteristics; life span is 3-6 mo at temperatures of 15-25°C with maximal growth occurring at 15-20°C (Dybern 1965, Tursi 1980). In South Africa where water temperatures are cooler but stable (12-14°C year-round), there may be 2-3 generations per year with growth rates as high as 50 mm.mo^{-1} (Millard 1952).

Water temperature profiles for the cooler coastal regions of Atlantic Canada resemble the shallow waters of Denmark, Sweden and Norway (-1°C to 20°C). Observations on the life history of the *C. intestinalis* population in Lunenburg Bay, NS (Carver et al. 2003) were consistent with a pattern of two generations or recruitment peaks per year (Dybern 1965). One recruitment event was observed in May-June and a second in August-September 2000. Monitoring programs conducted in subsequent years (2002-2005) in nearby Mahone Bay (Howes 2005) generally indicated two peaks, but there was a shift in the timing of the first settlement from May to July and in the second from August to October. In the Lunenburg population, maximum body length was 150 mm and size at maturity was estimated at 50-60 mm; new recruits attained this size after approximately 2.5 mo or an average growth rate of 20 mm.mo^{-1} at 10-20°C (Carver et al. 2003). This growth estimate was consistent with values obtained at similar temperatures: 10-20 mm.mo^{-1} in Sweden (Petersen et al. 1995) and 12-21 mm.mo^{-1} in Chile (Uribe and Etchepare 2002).

Observations on the longevity of the Lunenburg *C. intestinalis* population (Carver et al. 2003) were consistent with a predicted life span of 12-18 mo, depending on the timing of settlement (Dybern 1965). It appears that the first generation which settles in May/June/July reaches maturity and spawns in August/September/October; a certain proportion of these individuals, possibly the oldest, do not survive the winter while the remainder contribute to the first recruitment peak the following year and then die (10-12 mo). The second generation which settles in the late summer/fall may reach maturity before the winter or gamete production may be delayed until the following spring/summer depending on water temperatures and food levels. This generation contributes to the first recruitment peak and possibly the second peak before dying through the subsequent fall and winter (12-18 mo).

3.5. HABITAT AND ENVIRONMENTAL TOLERANCES

The distribution and survival of *C. intestinalis* in various habitats reflects its ability to cope with a wide range of environmental conditions. Although often listed as a member of the subtidal bathyal or deep water fauna down to 500 m (e.g. Brunel et al. 1998), substantial populations of this species are frequently observed in shallow coastal waters. In particular, this species appears as a prominent member of the biofouling community on artificial substrates such as floating docks (Cohen et al. 2000a, Lambert and Lambert 1998, 2003), and aquaculture gear (Karayucel 1997, Hecht and Heasman 1999, Mazouni et al. 2001). Natural epifaunal populations are found on hard substrates including bedrock, boulders, macroalgae or shelled organisms but not on soft sandy or muddy bottoms. In shallow protected inlets in Scandinavian waters, *C. intestinalis* may occur in densely aggregated populations which dominate the benthic eelgrass community (Petersen and Riisgard 1992), or it may be confined to steeply sloping rock walls and overhanging ledges (Gulliksen 1973). Various factors which may be responsible for controlling the distribution and survival of *C. intestinalis* in Atlantic Canada are discussed below.

Temperature

C. intestinalis is generally considered a coldwater or temperate species with occasional populations appearing in tropical harbours, although these are likely transitory (Monniot and Monniot 1994). As with many aspects of the life history of this species, temperature tolerance varies among geographical populations or ecotypes. In Japan or the warmer

regions of the Mediterranean, populations regularly experience 25-28°C, but the upper temperature tolerance of coldwater-adapted populations, such as those in Scandinavia or Atlantic Canada, is not well documented. Dybern (1965) lists 30°C as the upper temperature limit, but Petersen and Riisgard (1992) noted that filtration rates declined above 21°C suggesting that higher temperatures may be stressful. Other studies have also indicated that *C. intestinalis* exhibits a decline in ammonia excretion rate and oxygen consumption rate above 18°C (Zhang and Fang 2000, Zhang et al. (2000).

Tolerance for low temperatures also varies among geographical populations. In the Mediterranean most of the adults die when temperatures fall below 10°C; the population is maintained by the survival of younger individuals which are more cold tolerant (Marin et al. 1987). Monitoring of Scandinavian populations indicated a high mortality of *C. intestinalis* during the coldest period of the year (Dybern 1965). It should be noted these Scandinavian populations rarely experience temperatures below 1°C, whereas those in Atlantic Canada may encounter -1°C conditions for 1-2 mo (Carver et al. 2003). Reports of significant overwintering mortality in Mahone Bay, N.S. (Darnell, pers. comm.) suggest that survival may be negatively impacted by exposure to low water temperatures and/or low food levels. It is conceivable that the harsher the winter conditions, the fewer large adults from the previous year's first generation survive to reproduce the following summer.

Within a population or ecotype temperature tolerance may vary depending on the life history stage. In Scandinavian populations, normal egg development requires temperatures of 8-22°C whereas normal larval development can occur in the 6-24°C range (Dybern 1965). In situations where water temperatures fluctuate due to unseasonable weather conditions, these developmental tolerances may play a role in controlling the success of recruitment. Nomaguchi et al. (1997) found that embryos of *Ciona savignyi* could acclimate to temperature.

It should be noted that the developmental stages of *C. intestinalis* in deep water or Arctic regions may be more cold-tolerant than those of warmer water populations.

Salinity

C. intestinalis is classified as euryhaline with a high salinity tolerance range (12-40‰), although it typically occurs in full salinity conditions (>30‰). Dybern (1967) found that the

lower salinity limit for adults and developmental stages in Scandinavian populations was 11‰. Optimal salinities likely vary among ecotypes; for example, the optimal salinity for Mediterranean populations is 35‰ (Marin et al. 1987) which is higher than would normally be experienced by northern Atlantic coastal populations. Lambert and Lambert (1998) reported that *C. intestinalis* populations on floating docks in southern California harbours were vulnerable to pulses of low salinity; winter rains resulted in massive die-offs followed by recolonization in the spring, probably via recruits from deeper water populations. *C. intestinalis* may also have the ability to withstand short term salinity fluctuations. Shumway (1978) reported that oxygen consumption rate declined with decreasing salinity and ceased entirely when the external seawater concentration reached 19‰. During periods of decreased salinity, the siphons were tightly closed and oxygen consumption was zero. More research is required to establish the lower salinity limit for Atlantic Canadian populations, as well as determine their ability to withstand freshwater pulses.

Oxygen/Turbidity

The ability of *C. intestinalis* to withstand decreasing oxygen levels has not been well documented. Mazouni et al. (2001) noted that whereas oysters (*Crassostrea gigas*) can survive short term exposure to periods of anoxia (Thau Lagoon, France), the associated biofouling community dominated by *C. intestinalis* suffered heavy mortality. Observations on the frequent occurrence of *C. intestinalis* in relatively "polluted" harbours suggest, however, that this species has a high tolerance for turbid conditions (i.e. high suspended sediment loads) and possibly reduced oxygen levels. Naranjo et al. (1996) explained this distribution on the basis of body structure; specifically *C. intestinalis* has siphons with wide apertures which reduce the risk of blocking, and a shape which allows it to reach above the sediment layer and thereby survive smothering. Based on distribution studies in certain industrialized harbours in Spain, the authors suggested that *C. intestinalis* could be considered an indicator species for highly polluted areas which have been subject to intense stress (i.e. substrate transformation, water stagnation and excess sedimentation) over long periods of time. Kocak and Kucuksezgin (2000) also noted that *C. intestinalis* was one of the rapid breeding opportunistic species which tended to be dominant in Turkish harbours enriched by organic pollutants.

Despite these observations, the turbidity tolerance level for this species is not well established. Robbins (1985b) found that continual exposure to elevated levels of inorganic particulates (>25 mg.l^{-1}) arrested the growth rate of *C. intestinalis*, whereas exposure to 600 mg.l^{-1} resulted in 50% mortality after 12-15 d and 100% mortality after 3 wk. It was suggested that because this species can only "squirt" to clear the branchial sac, it may be vulnerable to clogging under heavy sediment loads. Petersen et al. (1997) concluded that because *C. intestinali*s lacks the ability to sort particles, it was less suited to the turbid environment of an eelgrass meadow than the mussel *Mytilus edulis*.

Water flow rates/wave exposure

C. intestinalis is typically found in areas of low flow or minimal wave exposure, although it can reportedly withstand flow rates up to 3 kt (Jackson 2005). If dislodged, juveniles and adults have a limited capability to re-attach, given calm conditions and prolonged contact with the new substrate (Millar 1971, Carver, pers. obs.). Although *C. intestinalis* requires some water movement to ensure sufficient access to food, its ability to tolerate very low flow rates gives it a competitive advantage in areas of minimal water exchange such as harbours, marinas and docks. Mazola and Riggio (1977) concluded that the presence of a biofouling community dominated by *C. intestinalis* was indicative of low water exchange rather than polluted conditions. In a fouling community study in Palermo Harbour (Italy), they initially noted that the "clean" docks with high water exchange had a rich diverse community, whereas the "polluted" docks were dominated by *C. intestinalis*. However, the interruption of warm water flow from the local thermal power plant in the "clean" area altered the hydrodynamic conditions such that many of the original species failed to recruit successfully and *C. intestinalis* became the dominant species.

3.6. ECOLOGY AND POPULATION DYNAMICS

Extreme environmental conditions (e.g. very low winter temperatures, high spring runoff) may account for some of the spatial and temporal variability in the abundance of *C. intestinalis.* However, this species has a reputation for sporadic population outbreaks, or irregular intense peaks of recruitment which are not apparently linked to changes in environmental conditions (Keough 1983, Cayer et al. 1999). It is also noted for its patchy distribution, both in its native Scandinavian region where it tends to be confined to shallow

inlets (Petersen and Svane 1995), as well as outside its native range where it occurs predominantly on artificial substrates in harbours (Monniot and Monniot 1994, Lambert and Lambert 2003). The purpose of the following section is to discuss several factors which may play a role in controlling population dynamics, or the variability in recruitment success within a particular environment.

Substrate availability

One of the major factors in the establishment and composition of marine epifaunal communities is the availability of suitable substrate for recruitment. A series of studies in Australian harbors have highlighted how the introduction of manmade or artificial structures has changed the composition of biofouling communities (Glasby 1999, Connell and Glasby 1999, Connell 2000, Holloway and Connell 2002). Typically natural habitats offer stable substrates which develop a complex highly diverse community which may be relatively resistant to invasion (Stachowicz et al. 1999, Stachowicz et al. 2002). By comparison, artificial substrates which are frequently disrupted due to seasonal maintenance or fluctuating environmental conditions provide ideal habitat for highly opportunistic species. For example, Koechlin (1977) reported that *C. intestinalis* rapidly colonized floating metal tanks in a newly constructed harbor at Lezardrieux (France) at densities up to 2000 ind.m^{-2}.

Heavy fouling of aquaculture operations is also likely related to increases in the availability of appropriate substrate. By changing the equipment or minimizing the development of fouling communities through maintenance activities, growers effectively renew the supply of fresh substrate for *C. intestinalis.* Another factor which likely promotes the settlement of this species on aquaculture structures is the tendency for larvae to settle in low flow environments. Schmidt and Warner (1984) noted that larvae showed a strong preference for caged structures with reduced light intensity and water movement. They concluded that the impact of caging *per se* on larval behaviour accounted for the subsequent dominance of *C. intestinalis.*

While aquaculture operations effectively encourage recruitment by providing an abundance of available substrate, this increase in substrate alone cannot account for the observed fluctuations in population dynamics. For example, *C. intestinalis* was recorded as a major pest species for mussel culture in New Zealand over several years, and then virtually

disappeared (Heasman, pers. comm.). Likewise, in South Africa *C. intestinalis* was listed as the major biofouling problem for mussel culture by Hecht and Heasman (1999), but has since become relatively rare (Wood, pers. comm.). In Australia, Kott (1990) noted that *C. intestinalis* populations which dominated port areas in 1950's and 1960's have since declined. It appears that even though substrate availability may remain relatively constant from year-to-year, recruitment success may be extremely variable. Environmental factors likely contribute to this variability, but it may be argued that interspecific interactions such as competition and predation also play a critical role in determining the population dynamics of *C. intestinalis*.

Interspecific interactions

The potential impact of predation on the planktonic eggs and larvae of *C. intestinalis* has not been extensively studied but likely varies both temporally and spatially. Petersen and Svane (1995) reported that insignificant numbers of eggs and larvae were recorded in regular plankton samples from their Danish site, possibly due to jellyfish (*Aurelia aurita*) grazing pressure. Competing filter-feeders such as oysters (*Crassostrea gigas*), which are capable of clearing ciliates and large zooplankton (Dupuy et al. 2000), may also exert pressure on planktonic stages. Given that mussels (*Mytilus edulis*) have the capacity to filter out particles up to 6 mm in size (Davenport et al. 2000), it is highly probable that dense accumulations of bivalves can reduce the concentration of eggs and larvae in their vicinity. Anecdotal observations from a mussel culture operation in Mahone Bay, N.S. suggest that increasing the seed density in the sleeve may reduce subsequent settlement rates of *C. intestinalis* (Darnell, pers. comm.).

Another factor which may affect survival of *C. intestinalis* eggs and larvae are chemical interactions between species. For example, Granmo et al. (1988) reported that blooms of the planktonic green algae *Chrysochromulina polylepsis* are acutely toxic to eggs and larvae of *C. intestinalis*; fertilisation and development is completely inhibited. Interestingly, Holmstroem et al. (1992) isolated a surface-colonizing bacteria from the tunic of adult *C. intestinalis* which releases a toxic component that inhibits larval settlement; aged biofilms containing this bacteria were more toxic to the larvae than unaged films. Although *C.*

intestinalis juveniles do settle on adults (Carver, pers. obs.), this strategy may not be advantageous as they risk becoming detached when these older individuals die off.

Conversely, interspecific interactions may also serve to promote larval settlement. Schmidt (1983) observed that growths of the hydroid *Tubularia larynx* on fouling panels greatly enhanced the settlement of *C. intestinalis* to the extent that it subsequently monopolized the entire surface. Schmidt and Warner (1984) concluded that this enhanced settlement was not a chemical interaction but rather related to a reduction in water flow. They cautioned that these types of interactions may be a causative factor in ascidian blooms, and should be taken into account before population outbreaks are attributed to eutrophication or other environmental factors.

At the juvenile stage or post-settlement, factors such as competition for space and food resources become important. Marshall and Keough (2003a) found that size at settlement affected survival; specifically larger individuals survived better than smaller settlers, within and among groups of siblings. Increases in the density of juveniles decreased survival, but these density-dependent effects were much stronger for smaller individuals. Although this study focused on intra-specific competition, the results can be extrapolated to biofouling communities where various species of filter-feeding organisms including ascidians may be competing for resources. Species such as *C. intestinalis* which settle early in the season may outcompete species which settle later simply because their larger size allows for greater energetic reserves and/or greater feeding capacity.

Osman and Whitlatch (2004) suggested that predation on ascidians at the early post-settlement stage may be more important than recruitment in determining the composition of epibenthic communities. They reported that the small predatory gastropods *Anachis* spp and *Mitrella lunata* play an important role in determining the survival of various ascidian species including *C. intestinalis* in New England waters. Specifically, Osman and Whitlatch (1995) estimated a predation rate of >200 newly-settled ascidians per snail per day. *M. lunata* (or *Astyris lunata*) is listed as a member of the epibenthic fauna in the Bay of Fundy (www.gmbis.marinebiodiversity.ca) and the southern Gulf of St. Lawrence (Brunel et al. 1998), but *Anachis* spp are not mentioned. Other potential predator species in New England include nudibranchs, flatworms, nemerteans, small crustaceans and certain polychaetes

(Osman and Whitlatch 2004). There is little information, however, as to the abundance of these various predators in Atlantic Canada, or their potential impact on the survival of juvenile populations of *C. intestinalis*.

As *C. intestinalis* grows it reaches a size refuge from small gastropods but becomes increasingly vulnerable to larger invertebrate predators. Osman and Whitlatch (2004) reported that solitary ascidians, including *C. intestinalis*, survived on panels suspended on racks but not on pilings where they were likely accessible to sea stars and crabs. Heavy grazing of the sea star *Asterias rubens* on adult *C. intestinalis* has been reported from eelgrass beds in Norway (Gulliksen and Skaeveland 1973) and Sweden (Svane 1983). In Atlantic Canada, Carver et al. (2003) observed that both the green crab (*Carcinus maenas*) and the rock crab (*Cancer irroratus*) fed on juvenile (20-50 mm) and adult (50-100 mm) *C. intestinalis* in laboratory trials. The rock crab showed a greater preference for consuming this species than did the green crab; rock crab feeding rates were estimated at 11 adult *C. intestinalis*.d^{-1} at 18°C. It should be noted that neither sea stars nor crabs consume the outer tunic which is highly acidic; sea stars digest the body tissues through the siphon (Gulliksen and Skaeveland, 1973), whereas crabs tear open the tunic with their pincers and excise the organs (Carver et al. 2003). Rock crabs were observed to consume juvenile tunicates (2-10 mm) whole, but then immediately reject the remains of the tunic.

The third group of predators which may play a role in controlling the population dynamics of *C. intestinalis* are surface or bottom-feeding fish species. In Norway, various species are reported to prey on epibenthic populations of *C. intestinalis* including dab (*Limanda limanda*), plaice (*Pleuronectes platessa*) and cod (*Gadus morhua*) (Gulliksen 1972, Lande 1976). Petersen and Svane (1995) observed that the stickleback (*Gasterosteus aculeatus*) preys on *C. intestinalis* in Danish coastal inlets. Yamaguchi (1975) noted that *C. intestinalis* in Japan was confined to sheltered substrates due to predation by browsing fish. Osman and Whitlatch (2004) listed the cunner (*Tautogolabrous adspersus*) as one of the predators potentially responsible for the elimination of *C. intestinalis* from open surfaces in New England waters. Another possible candidate is the common mummichog (*Fundulus heteroclitus*) which was observed to feed on young recruits of the sea grape tunicate (*Molgula manhattensis*) when confined in trays (Flimlin and Mathis 1993).

Although browsing fish species such as cunner and mummichogs are commonly observed in shallow waters along the Atlantic coast of N.S., they do not appear to exert significant grazing pressure on *C. intestinalis* populations associated with suspended mussel culture operations. This may be related to food preferences, i.e. other species may be more palatable than *C. intestinalis*. Pisut and Pawlik (2002) found that certain solitary ascidians (though not specifically *C. intestinalis*) produce compounds in their gonads which deter feeding by bluehead wrasse (*Thalassoma bifasciatum*). Lambert (2005) noted that the presence of sulfuric or hydrochloric acid in the tunic of ascidians may also function as an anti-predator defense mechanism.

Whereas browsing fish may not be important, observations suggest that crabs and/or other benthic predators may actively control the local abundance and distribution of *C. intestinalis*. Benthic surveys in Lunenburg N.S. (MacDonald, pers. comm.) as well as anecdotal evidence indicate that this species is extremely rare or absent from benthic communities despite the availability of suitable hard substrate. *C. intestinalis* was only observed on artificial predator-free substrates such as floating docks, boat hulls, mussel sleeves or oyster bags which were raised above the bottom on tables (Carver et al. 2003). Interestingly, it was noticeably absent from shallow eelgrass communities, very similar to those in Scandinavia where it is often the dominant epibenthic species (Gulliksen 1972, Petersen and Riisgard 1992). Similar to Lunenburg, surveys in California (Fay and Johnson 1971) suggested that *C. intestinalis* only occurred on floats and pilings and not on natural substrates. These observations suggest that outside of its native range, the distribution of *C. intestinalis* is constrained to artificial substrates possibly by competition and/or predation pressure.

3.7. DISEASES AND PARASITES

No diseases have yet been documented in *C. intestinalis*, but the species is known to possess parasitic or commensal copepods belonging to the order Doropygidae (Millar 1971). Becheikh et al. (1996) documented the presence of the copepod *Pachypygus gibber* inside the branchial sac of *C. intestinalis*. Even though this species apparently lives at the expense of its host's feeding activity, it has no impact on tunicate survival or growth and thus qualifies as a non-costly partner or commensal. Pastore (2001) isolated eight species of copepods from

the branchial sac of *C. intestinalis* in the Mediterranean Sea; the three most common were *P. gibber*, *Hermannella rostrata* and *Lichomolgus canui*. Ooishi and O'Reilly (2004) isolated another copepod species, *Haplostoma eruca*, from the intestine of *C. intestinalis* in Scotland.

4.0   HUMAN USES

The majority of the scientific research on *C. intestinalis* has focused not on its ecology, but on the molecular development of the notochord and the dorsal neural tube in the tadpole larva (e.g. Meinertzhagen 2005). Because this larval form has many features resembling vertebrates, it provides a valuable model for studying organ development uncomplicated by the variations and elaboration in other organisms whose organs are constructed of many more cells (Katz 1981). Researchers believe that the tadpole larva represents the closest living form to the ancestral chordate, and thus provides insights into chordate origins and evolution. According to Satoh (2003) this species is also an excellent model for studying the evolution of gene function and regulation because it has an unduplicated compact genome, the sequence of which has recently been published (Dehal et al. 2002), and short gene-regulatory regions.

Another area of research where *C. intestinalis* has shown some promise is in the development of novel pharmacological or commercially useful products. Solitary ascidians possess a wide range of antimicrobial agents in their blood and other tissues, some of which have been purified and characterized (Johnson and Chapman 1970, Jackson and Smith 1993, Jackson et al. 1993). For example, Findlay and Smith (1995) reported that the circulating blood cells, specifically the morula cells of *C. intestinalis* exhibited potent anti-bacterial activity when challenged with a range of bacteria. Given that these morula cells are known to participate in a variety of cellular host defence responses, it is probable that they play a role in the neutralisation of bacteria, and possibly other micro-organisms, which invade tissues.

C. intestinalis may also prove valuable in the development of anti-cancer therapeutics (Rhinehart 2000). Parrinello et al. (1993) reported that hemocytes of *C. intestinalis* showed a natural cytotoxic capacity when assayed *in vitro* against human erythrocytes. Peddie and Smith (1994) demonstrated that the cytotoxic activity of these hemocytes against leukemic cells is mediated by mechanisms similar to those of vertebrate cytotoxic cells.

Environmental researchers have suggested that *C. intestinalis* may be useful as an indicator of marine pollution (Papadopoulou and Kanias 1977), because it tends to accumulate and therefore sequester trace elements such as the heavy metals vanadium, manganese, zinc and iron (Goldberg et al. 1951, Swinehart et al. 1974, Michibata 1984, Cheney et al. 1997). Bellas et al. (2001) suggested that the developmental performance of *C. intestinalis* (i.e. rates of embryogenesis or larval attachment success) could be used as an indicator of seawater quality, specifically heavy metal concentrations (mercury, copper and chromium).

Some ascidians are edible without their tunic and may be cultivated or harvested for sale in Asian food markets (e.g. *Halocynthia roretzi, Halocynthia aurantium* in Japan and *Styela clava* in Korea). However, *C. intestinalis* and other species of Phlebobranchia are not considered edible (Lambert, pers. comm.).

5.0 POTENTIAL VECTORS FOR INTRODUCTION

Unlike the larvae of bivalves which may be dispersed over long distances during their 20-30 d planktonic phase, *C. intestinalis* larvae are non-feeding with a relatively short planktonic phase and limited potential for natural dispersion. Laboratory studies generally report that the duration of the period from egg release to larval settlement does not exceed 1 wk at temperatures of 10-20°C (Dybern 1965, Cirino et al. 2002). This may be extended in the field if egg fertilization is delayed and/or larvae cannot find suitable substrate for settlement; Jackson (2005) suggested a maximum of 10 d for British waters, but few field data are available. Marshall and Keough (2003c) suggested that larger non feeding invertebrate larvae can delay settlement and this is likely to influence dispersion and survival. Another characteristic of this species which may limit its dispersion potential is the tendency for a large proportion (50%) of the eggs and larvae to remain trapped in mucus throughout the developmental phase (Petersen and Svane 1995).

Given that *C. intestinalis* has limited dispersion potential during its larval phase and remains attached for most of its life cycle, range extensions likely involve hitchhiking on natural or artificial substrates. Other tunicate species have been observed to raft on natural floating substrates such as eelgrass or macroalgae (Worcester 1994). In a recent tunicate survey on the South shore of Nova Scotia, *C. intestinalis* was repeatedly found rafting on

clumps of the fleece alga *Codium fragile* (Vercaemer, pers. obs.). Hull fouling of slow-moving vessels such as floating barges, small fishing or recreational water craft is likely responsible for regional dispersal within many coastal areas. Lambert and Lambert (2003) noted that although *C. intestinalis* was probably introduced into San Francisco Bay (California) via commercial shipping, regional traffic was likely responsible for its subsequent dispersal along the California coast and south into the Baha region (Mexico). Researchers in Australia argued that recreational boating was one of the most likely vectors for the dispersion of *C. intestinalis* along their coast (Cohen et al. 2000b, MacDonald 2004). Floerl and Inglis (2003) noted that the reduced circulation or greater residence time of water within protected marina areas may enhance the localized retention of larvae, therby exacerbating the risk of hull fouling and subsequent species dispersal.

Countries such as Australia are actively developing risk minimization strategies to deal with the regional spread of "exotic" species by local shipping (Rainer 1995, Hewitt and Martin 1996, Hayes and Hewitt 2000). One strategy, the widespread use of antifouling paints, may contribute to a reduction in the risk of dispersion, but this requires that regulations be established to ensure that fishing and recreational vessels engage in regular hull maintenance. In a hull fouling survey of recreational vessels entering New Zealand waters, age of antifouling paint was a key factor in predicting the degree of fouling as well as the risk of introducing non-indigenous species (Hayden, pers. comm.). Floerl et al. (2005) cautioned that antifouling paints may provide effective protection against barnacles and bivalves, but other taxa were repelled for only a few months. Manual hull cleaning was found to be relatively ineffective and apparently enhanced the risk of subsequent fouling by colonial and solitary ascidians. *In situ* cleaning of recreational boat hulls by scuba divers is a rapidly expanding small business and is becoming a problem around the Pacific North-West (Lambert, pers. comm.). Recruitment studies with *S. clava* in PEI (Darbyson 2006) suggested that larvae did not settle on surfaces covered with commercial antifouling paint, but preferred untreated surfaces such as the aluminum hulls of aquaculture working craft. The behaviour of *C. intestinalis* larvae with regard to various substrates has not been documented, but would be valuable in assessing the risk of local dispersion by small vessels.

Attachment to the hull of faster-moving ships such as commercial container vessels and bulk carriers is likely restricted to sheltered locations such as sea chests (water intake areas

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which are continually flooded) or poorly-drained anchor lockers. Given that *C. intestinalis* can only withstand currents up to 3 kt (Jackson 2005), it would be unlikely to survive transport on the exposed hull of a commercial vessel moving at 10 kt. Transport in the ballast water of large vessels is another potential vector for this species, although it only has a short larval period and would have to survive uptake and discharge through the ship's ballast water pump. If it should settle on the walls of the ballast tank, it would be unlikely to survive dessication following discharge of the ballast water. Incomplete emptying of ballast water tanks could provide a refuge zone for settlement, but conditions would likely be unsuitable for longterm survival.

One significant potential vector is the movement of aquaculture product or fouled equipment among sites. Cohen et al. (2000b) argued that translocation of mussel ropes was a likely vector in the introduction of *C. intestinalis* into Westernport Australia from nearby Port Phillip Bay. The ability of juveniles and adults of this species to withstand dessication has not been well documented, but under damp conditions it may well survive for hours, if not days. Lutzen (1999) found that *S. clava*, which has a much heavier tunic than *C. intestinalis,* can survive for up to 3 d out of water if kept moist. Preparation or cleaning protocols should be devised for mussel seed as well as other commercial species to reduce the risk of transferring *C. intestinalis* and other fouling organisms among regions of Atlantic Canada. Management strategies are also required to mitigate the risks associated with plant effluent when processing shellfish infested with *C. intestinalis.* Research currently underway in PEI suggests that large numbers of eggs released during the mussel sleeve stripping process may be discharged into the local environment (Bourque, pers. comm.).

6.0   IMPACTS ASSOCIATED WITH INTRODUCTIONS

6.1. IMPACTS ON THE ENVIRONMENT

The introduction of a species with the potential for explosive population growth may have negative ramifications for the local environment. Modelling of dense populations of *C. intestinalis* in a shallow cove with restricted circulation (Kertinge Nor, Denmark) indicated the potential for significant depletion of food resources (Petersen and Riisgaard 1992, Riisgard et al. 1996, 1998). In cases where *C. intestinalis* is a dominant member of a biofouling community, it may also contribute substantially to the deposition of faecal matter

to the bottom. Eventually this ongoing organic enrichment may lead to the development of anoxic sediments, the accumulation of hydrogen sulfide, and the degradation of the benthic community. For example, Stenton-Dozy et al. (2001) noted that mussel raft culture in South Africa had significant long-term negative impacts on the benthic environment. One likely contributor to this impact was the fouling community dominated by *C. intestinalis* which had an overall biomass (wet weight) of 78 tons.raft[-1] compared to only 27 tons.raft[-1] for the cultured mussels. If the biomass of tunicates and other fouling organisms is routinely discarded to the bottom during cleaning of the equipment and/or harvesting activities, the risk of localized enrichment and benthic habitat degradation increases.

One scenario where the appearance of *C. intestinalis* may have a positive impact on the quality of the environment is in the case of eutrophic systems with excess nutrient loading. Conley et al. (2000) argued that benthic filter-feeders such as *C. intestinalis* may play an important role in Danish estuaries by restricting the development of phytoplankton blooms which are associated with hypoxia and anoxic events. Similarly, the grazing activities of *C. intestinalis* growing on cultured oysters in the eutrophic Thau Lagoon (French Mediterranean coast) may contribute to reducing the risk of anoxic events, increasing nutrient regeneration rates and promoting water clarity thereby extending the range of macrophytes into deeper waters (Deslou-Paoli et al. 1998).

6.2. IMPACTS ON OTHER SPECIES

The filter-feeding activity of *C. intestinalis* may negatively impact the abundance of microzooplankton, such as ciliates which typically feed on bacteria, as well as the planktonic larvae of bivalves (Bingham and Walters 1989). Osman et al. (1989) reported that *C. intestinalis* preyed directly on oyster larvae, and inhibited oyster settlement by covering and thereby reducing the amount of free substrate available for attachment. Post-settlement survivorship and growth were also strongly affected by the presence of this and other sessile species. Similarly, in laboratory growth trials, Zajac et al. (1989) indicated that 44 d after settlement, juvenile oysters had reduced survivorship and growth when ascidian competitors were present regardless of food level. Gulliksen (1980) reported that a heavy settlement of *C. intestinalis* on the rocky bottom of a Norwegian fjord effectively altered the composition of the available substrate to the extent that it was no longer suitable for the settlement of other

species. Only when *C. intestinalis* disappeared did the composition of the benthic fauna return to its original state

In terms of trophic competition, comparisons of particle retention efficiency have suggested that whereas ascidians can efficiently retain all >2-µm particles and 70% of 1-µm particles, bivalves exhibit a decline in retention efficiency below 4 µm (Jorgensen et al. 1984). Stuart and Klump (1984) suggested that this difference may be indicative of food-resource partitioning between bivalves and ascidians. Mazouni et al. (2001) argued that *C. intestinalis* growing on cultured oysters in the Thau Lagoon (France) may consume picophytoplankton (<1 µm) which account for 20% of the phytoplankton biomass but are unavailable to the oysters. Although resource partitioning may occur when the majority of particles are <4 µm, in many cases ascidians likely compete directly with bivalves for the same food resources (Riisgard et al. 1996, Petersen et al. 1997). On an individual basis, Lesser et al. (1992) found that *C. intestinalis* had significantly lower clearance rates than mussels for all particle types and size classes; specifically, three *C. intestinalis* cleared approximately the same volume as one *M. edulis*. They concluded that interspecific competition for food by the associated fouling community should not significantly limit the yield of mussels as long as food was not a limiting factor. In one recent experiment (Daigle, pers. comm.) mussels from lines lightly fouled with *C. intestinalis* (70 g wet wt.m^{-1}) exhibited a small (1.2%) but significantly higher meat yield than those from heavily fouled lines (450 g wet wt.m^{-1}).

Although bivalves and ascidians may be trophic competitors, there are numerous instances where they apparently coexist without interference. Dalby and Young (1993) found that adult oysters in Florida were quite tolerant of ascidian overgrowth, whereas MacNair (pers. comm.) found that mussels overgrown by the colonial violet tunicate (*Botrylloides violaceus*) in P.E.I. showed no reduction in growth performance. According to Mazouni et al. (2001) oyster growers in the Thau Lagoon (France) consider the development of an ascidian biofouling community as indicative of a good harvest. Clearly, more field trials are required to elucidate the impact of various levels of *C. intestinalis* biofouling on cultured bivalve populations.

The dominant position of *C. intestinalis* in many fouling communities suggests that it has the potential to outcompete or displace other sessile filter-feeders, effectively depriving them of food and/or space when these resources are limited. In the case of floating substrates where predators are absent or unable to access the community, this advantage may be accentuated. Lambert and Lambert (1998) noted that since *C. intestinalis* appeared in southern California in 1917, there has been a steady decline in the abundance of native ascidian species which previously dominated the harbour areas. Jackson (2005) lists *C. intestinalis* as a potential competitor for the clubbed tunicate *S. clava*, although it is unclear whether one species is likely to become dominant. Ascidian species which may benefit from the presence of *C. intestinalis* are the rapid-growing colonial forms such as the golden star tunicate *Botryllus schlosseri* and the violet tunicate *Botrylloides violaceus* which can settle and overgrow solitary species (Lutzen 1999).

6.3. IMPACTS ON INDUSTRY

C. intestinalis has been listed as a nuisance fouling organism for aquaculture operations in many areas of the world including suspended mussel culture in North America (Lesser et al. 1992, Cayer et al. 1999) and South Africa (Hecht and Heasman 1999), scallop culture in Chile (Uribe and Etchepare 1999, 2002) and oyster culture in Korea (Kang et al. 1978) and Japan (Arakawa 1990). In South Africa, problems with *C. intestinalis* biofouling forced one company to completely re-sleeve their mussel inventory immediately after the main recruitment event. In the case of Chile, a sudden outbreak of this species had a major deleterious effect on the culture of the scallop *Argopecten purpuratus*. Of 300,000 suspended culture units maintained by one company, 105,000 units were fouled by *C. intestinalis* and more than 50,000 units collapsed. This fouling significantly raised production costs, and resulted in high scallop mortality when dissolved oxygen and food resources became limited due to the decrease in water circulation.

In the context of fish farming, the fouling of cages and netting by *C. intestinalis* is costly to remove and may lead to equipment failure. Heavy infestations reduce flow rates, thereby interfering with oxygen exchange and potentially jeopardizing fish health and yield. Tan et al. (2002) reported that biofouling of nets by *C. intestinalis* increased the disease risk to farmed salmon in Tasmania. Monitoring for the disease agent responsible for Amoebic Gill

Disease (AGD) indicated that *C. intestinalis* was a repository for the amoeba and therefore a risk factor. According to Braithwaite and McEvoy (2004) the rapid expansion of the aquaculture industry, coupled with the tightening of legislation on the use of antifouling biocides means a steady increase in the problems of fish farm biofouling.

In Atlantic Canada, the earliest record of *C. intestinalis* becoming a significant fouling problem for aquaculture was in the summer of 1997 at a mussel farm in Lunenburg, NS (Cayer et al. 1999). The overwhelming settlement of this tunicate negatively affected the growth performance of the mussels and eventually resulted in the loss of the crop (Cook, pers. comm.). In 1998-1999 there were continuing problems with *C. intestinalis* fouling on oyster and scallop culture bags in Lunenburg (Carver et al. 2003), as well as anecdotal reports of similar fouling problems at various sites along the southwest coast of NS. In 2000 the problem was observed at a mussel farm in Mahone Bay, N.S. as well as at mussel and scallop operations in the nearby Chester area. Anecdotal reports suggest that fish growers along the south coast of N.S. (e.g. Shelburne) are experiencing similar biofouling problems. The recent arrival of *C. intestinalis* in the Montague area of P.E.I. in 2004 has further complicated the ongoing biofouling problems associated with the earlier introduction of the non-indigenous clubbed tunicate *S. clava*. Not only must growers now deal with the increased operational costs of removing this tunicate infestation from their lines, but the common practice of moving seed mussels within the province has been severely curtailed in an attempt to restrict the dispersion of these species (MacNair, pers. comm.). Mussel processors must separate increasing volumes of unwanted tunicate biomass from the marketable product and bear the cost of disposal to the landfill.

It should be noted that *C. intestinalis* biofouling has also become an issue for other users of the marine environment. For example, the hulls of fishing and recreational vessels rapidly become infested if they remain in inshore areas through the summer months. In one case of hull fouling, Elroi and Komarovsky (1961) documented 2500-10,000 *C. intestinalis* individuals.m^{-2} at an estimated weight of 140 kg.m^{-2}. Not only does this level of biofouling lead to a reduction in hull speed and maneuverability due to increased drag, but it increases maintenance costs. Similarly, navigational buoys, floating docks and work barges require more frequent cleaning and may be much heavier to remove from the water at the end of the season. Intake pipelines for processing plants and research facilities (e.g. Bedford Institute of

Oceanography in Dartmouth, N.S.) also clog more rapidly and require more frequent maintenance.

6.4. CONTROL METHODS

The preferred strategy for dealing with tunicate biofouling is preventive biological control or identifying and exploiting the activity of natural predators. Carver et al. (2003) found that the rock crab (*C. irroratus*) was very efficient at cutting open and extracting the body tissues of *C. intestinalis* in laboratory trials. Switching from suspension culture to oyster rack and bag culture allowed crabs to access the tunicates on the outside of the bags, thereby contributing to a reduction in the intensity of tunicate biofouling. Researchers in PEI have also documented rock crabs feeding on clubbed tunicates (*S. clava*) attached to mussel sleeves. Allowing mussel sleeves to touch bottom to encourage crab predation may, however, increase the risk of sea star predation on the mussels (MacNair, pers. comm.).

Another group of natural predators which may prove to be worth investigating are the small gastropods such as *Mitrella lunata* or *Anachis* spp. which feed on recently settled juvenile tunicates (Osman and Whitlatch 1995, 2004). The natural distribution of these species in Canadian waters is not well documented but the lunar dovesnail *M. lunata* is listed as a common member of the benthic invertebrate community (www.gmbis.marinebiodiversity.ca). Other common gastropods such as the periwinkle *Littorina littorea* may dislodge juvenile tunicates during normal grazing activities (Frey 1986, Petersen and Svane 1995); unlike crabs, this herbivorous species can be inserted inside bags without risk to the shellfish (Enright, 1983, 1993, Carver and Mallet, pers. obs.). Another species, the common mummichog *F. heteroclitus,* was recommended as a biological agent for controlling fouling by sea grape tunicates (*Molgula manhattensis*) inside trays containing juvenile clams (Flimlin and Mathis 1993).

Various chemical and/or mechanical methods have been suggested for eliminating *C. intestinalis* from aquaculture gear such as cages or nets, but there are very few options for treating sleeved mussels due to the risk of fall-off. Arakawa (1990) recommended that fouled nets be treated with fresh water or concentrated brine, or subjected to abrasive methods such as hand-scraping or pressure-washing. Shearer and MacKenzie (1997) suggested exposing tunicates to air, fresh water, lime, or saturated brine dips (90-100‰)

followed by air. MacNair and Smith (1998) recommended saturated brine coupled with a dipping in quicklime solution for eliminating the tunicate *Molgula* sp. from oyster spat collector units. Carver et al. (2003) compared various treatments for eliminating *C. intestinalis* biofouling including hypochlorite, lime, saturated brine, and hot water (40°C); results of laboratory and field trials suggested that dipping or spraying tunicates with a solution of ordinary vinegar (4% acetic acid) was the most effective strategy. Preliminary experimental trials showed no effect of bentonite clay solutions on *C. intestinalis* survival (12-80 mg.l^{-1}) (Daigle, pers. comm.).

The NZMIC (New Zealand Mussel Industry Council) has developed a Tunicate Treatment Technology for eliminating *C. intestinalis* from continuous mussel sleeving at an estimated cost of $1 per metre of line. Apparently this unit is very effective at killing *C. intestinalis* as well as colonial sea squirts such as *Aplidium* sp. (Heasman, pers. comm. in Coutts and Sinner 2004). Preliminary trials conducted at Indian Point Marine Farms (Mahone Bay, N.S) in the fall 2005 generally confirmed the effectiveness of the technology (Carver and Mallet, pers. obs.) Further trials are required to improve the operational efficiency of the system and to identify the costs associated with treatment. Other practical issues which need to be addressed include the risk of gamete dispersion during treatment as well as determining the most appropriate time to undertake the treatment program. If the system proves to be cost-efficient it could play a key role in developing a tunicate management strategy for mussel culture.

Another approach to tunicate control is to develop biological management strategies which exploit aspects of the species ecology or life history characteristics. Arakawa (1990) suggested monitoring the plankton to forecast larval settlement, raising or lowering the gear to minimize recruitment, and deploying decoy plates to distract larvae. Obtaining information on the timing of larval settlement is critical to developing a site-specific management plan. For example, growers in South Africa opted to re-sleeve their mussels immediately following the major recruitment of *C. intestinalis* (Hecht and Heasman 1999). One company in N.S. adjusted their maintenance schedule to reduce the impact of *C. intestinalis* biofouling; specifically, they postponed the annual gear changing/cleaning operation from May to September when the heaviest settlement had passed (Carver et al. 2003). One strategy currently being explored by mussel growers in Atlantic Canada is

increasing the density of mussels in order to reduce the level of tunicate fouling (Darnell, pers. comm., MacNair, pers. comm.). Preliminary observations of reduced settlement intensity may be attributable to hydrodynamic interference or direct ingestion of the eggs and/or tadpole larvae by the mussels. Davenport et al. (2000) reported that *M. edulis* has the potential to ingest significant quantities of mesoplankton up to 6 mm in length.

6.5. IMPACT SUMMARY

The absence of large communities of *C. intestinalis* on natural substrates in Atlantic Canada is consistent with observations from other regions where this species is either cryptogenic or introduced. Specifically, it tends to be confined to artificial substrates with limited predator access and/or zones with adverse environmental conditions which are unsuitable for many other species (Naranjo et al. 1996, Lambert 2005). Within harbours or bays with extensive aquaculture operations, it can rapidly occupy all the available space, monopolize the food resources and potentially displace or inhibit the growth of competing species. Outside of these areas, *C. intestinalis* does not appear to pose a significant threat to other organisms, possibly because of predation pressure and/or greater competition for space in diverse natural communities.

7.0 CONSERVATION STATUS

C. intestinalis is generally considered to be "globally secure" or without conservation status.

8.0 SUMMARY

C. intestinalis is an opportunistic species with a short life cycle and high reproductive capacity rendering it capable of rapidly exploiting new substrate. In Atlantic Canada it may initiate spawning relatively early in the year and continues to produce gametes over several weeks to months. This first generation reaches maturity within 8-10 wk and then gives rise to a second generation within the same year. With increases in water temperature due to global warming, the duration of the reproductive period may be extended. *C. intestinalis* tolerates a wide range of environmental conditions including the high levels of suspended sediment and pollutants which often occur in highly impacted harbour waters. The larvae show a settlement preference for shaded zones with low flow rates, conditions which are

often associated with aquaculture equipment. The recent outbreak of *C. intestinalis* in Atlantic Canada has seriously increased the operational costs associated with aquaculture activities and is negatively impacting the economic outlook for this industry. A serious effort is required to target the potential vectors which may facilitate the regional dispersal of this species, and devise appropriate risk management strategies and control measures to minimize its future impact.

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Appendix 1. Relationships between live length (extended), total weight, tunic weight and
organ weight (Carver, unpubl. data)

Table 1. Wet and dry weight estimates for *C.intestinalis* ranging in length from 5-150 mm.

Extended Length (mm)	Total wet wt (g)	Tunic wet wt (g)	Organ wet wt (g)	Organ wet wt (%)	Total dry wt (g)	Tunic dry wt (g)	Organ dry wt (g)	Organ dry wt (%)
5	0.008	0.004	0.003	38.2	0.0004	0.0002	0.00019	46.4
10	0.042	0.024	0.016	37.7	0.0021	0.0011	0.00099	46.1
20	0.227	0.130	0.084	37.1	0.0116	0.0061	0.00532	45.8
50	2.081	1.225	0.759	36.5	0.1077	0.0568	0.04886	45.4
80	6.491	3.876	2.345	36.1	0.3374	0.1787	0.15239	45.2
100	11.138	6.696	4.007	36.0	0.5803	0.3080	0.26151	45.1
120	17.316	10.467	6.206	35.8	0.9037	0.4805	0.40655	45.0
150	29.714	18.082	10.602	35.7	1.5543	0.8283	0.69764	44.9

Wet Weight Conversions:

Total wet wt (g) as a function of length (mm): $W(total\text{-}wet) = 0.000161\ (L)^{2.42}$ $R^2 = 0.92$
Tunic wet wt (g) as a function of length (mm): $W(tunic\text{-}wet) = 0.0000843\ (L)^{2.45}$ $R^2 = 0.91$
Organ wet wt (g) as a function of length (mm): $W(organ\text{-}wet) = 0.0000635\ (L)^{2.40}$ $R^2 = 0.86$

Organ wet wt (g) as a function of total wet wt (g): $W(organ\text{-}wet) = 0.366\ W(total)\ R^2 = 0.95$

Dry Weight Conversions:

Total dry wt (g) as a function of length (mm): $W(total\text{-}dry) = 0.000008\ (L)^{2.43}$ $R^2 = 0.93$
Tunic dry wt (g) as a function of length (mm): $W(tunic\text{-}dry) = 0.0000041\ (L)^{2.44}$ $R^2 = 0.92$
Organ dry wt (g) as a function of length (mm): $W(organ\text{-}dry) = 0.0000038\ (L)^{2.42}$ $R^2 = 0.89$

Organ dry wt (g) as a function of total dry wt (g): $W(organ\text{-}dry) = 0.454\ W(total\text{-}dry)$
$R^2 = 0.98$

Extended (live) length (mm) as a function of diameter (mm) when contracted:
$L = -11.4 + 4.55(D)$