A GUIDE TO THE LARVAL AND JUVENILE STAGES OF COMMON LONG ISLAND SOUND ASCIDIANS AND BRYOZOANS

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CONTENTS

INTRODUCTION

This guide provides descriptions and keys to the larval and juvenile stages of common Long Island Sound (LIS) ascidians and bryozoans. It has been designed to allow investigators with limited taxonomic experience to identify larvae and newly settled recruits to the species level. Ascidians and bryozoans are among the most abundant members of subtidal benthic communities and are very common in LIS (Osman & Whitlatch 1995a & b, Witman & Dayton 2001, Steneck & Carlton 2001). Although taxonomic guides are readily available for the identification of adult ascidians and bryozoans (e.g., Weiss 1995), few guides have been developed for larval and juvenile stages (but see Smith & Johnson 1996, Shanks 2001).

Larvae and juveniles are critical links in establishing and maintaining benthic populations (Morgan 1995, Hunt & Scheibling 1997, Underwood & Keough 2001). Thus, ecological factors that affect the early life stages of organisms can have dramatic influences on adult populations (Gaines & Roughgarden 1987, Roughgarden *et al.* 1988, Osman & Whitlatch 1995c, Hunt & Scheibling 1998, Moksnes 2002). Because LIS researchers have previously been unable to identify the early life stages of ascidians and bryozoans, few studies have been conducted to determine how the ecology of larvae and juveniles affect Long Island Sound (LIS) populations (but see Osman & Whitlatch 1995a & b).

In addition to allowing researchers to identify the early life stages of species that currently reside in LIS, this guide may also assist in the monitoring of LIS for newly-arrived invasive species. Species invasions are common in New England and LIS (Steneck & Carlton 2001), with many invasions being caused by the inadvertent transport of competent larvae and juveniles in ballast water and on ship hulls (Ruiz *et al.* 2000, Wonham et al. 2001). Although invasive species often arrive in LIS as larvae and pass through subsequent juvenile stages, identification of potentially invasive organisms has previously been delayed until recruits become large enough to be identified with existing adult keys. Thus, this guide may also assist researchers in recognizing unfamiliar and hence potentially invasive species.

Information for this guide was collected in conjunction with a Long Term Research in Environmental Biology (LTREB) project conducted at the University of Connecticut's Avery Point campus, Groton, Connecticut. The scope of this monitoring project is to develop baseline data for invertebrate recruitment in LIS. All recruits are counted on PVC settlement panels (100 cm^2) deployed weekly at several sites near Groton, Connecticut. Thus, the species included in the key are those commonly found in northeast LIS. Researchers should be aware that other species of ascidians and bryozoans reside in LIS (Weiss 1995) and may be abundant near their sampling locations. Additionally, the included descriptions come from living specimens (juveniles) or samples preserved for less than one day (larvae). Specimens that have been preserved for longer periods may look somewhat different from those described in this guide.

DEFINITIONS

Diagrammatic representations of common structures in larval and juvenile ascidians and bryozoans are shown in Figure 1 (page 21).

Adhesive papillae (ascidian larvae): Structures located at the anterior end of an ascidian larva's body used to attach the larva to the substratum.

Ancestrula (bryozoan juveniles): The original zooid of a bryozoan colony. Frequently morphologically different from subsequently produced zooids.

Body (ascidian larvae): The rounded, or bulbous portion of an ascidian larva.

Ocellus (ascidian larvae): Light sensing organ. Visible as a round or oblong pigment spot near the center of the body of an ascidian larva. Commonly called an eyespot.

Statolith (ascidian larvae): Gravity sensing organ. Visible as a round or oblong pigment spot near the center of the body of an ascidian larva. Ventral to ocellus if both structures are present. Commonly called an eyespot.

Zooid (bryozoan juveniles): Individual in a bryozoan colony.

Zooecium (bryozoan juveniles): Skeleton surrounding zooid.

KEY TO ASCIDIAN LARVAE

1. Colonial or Solitary
   - Colonial: relatively large (usually 1.10 mm or longer, but occasionally smaller) transparent or translucent rounded body..... 2
   - Solitary: relatively small (usually less than 1.10 mm)..... 6
2. Presence of a Frill
   - A frill of small finger-like projections around the equator of the body..... *Botrylloides violaceus*
   - No frill..... 3
3. Morphology of Adhesive Papillae
   - Adhesive papillae project well beyond the body..... *Botryllus schlosseri*
   - Adhesive papillae do not project well beyond the body or none visible..... 4
4. Presence of spots
   - Body covered with iridescent spots..... *Aplidium constellatum*
   - Few spots on body..... 5
5. Presence of Lateral Ampullae
   - Six additional structures resembling adhesive papillae (lateral ampullae) present in a second row behind three anterior adhesive papillae..... *Didemnum* sp.
   - Lateral ampullae absent..... *Diplosoma listerianum*
6. Number of Pigmented "Eyespots" Visible
   - One eyespot visible..... 7
   - Two eyespots visible..... 9
7. Body Shape
   - Body rounded and bulbous with the pigmented portion being fully enclosed by a transparent capsule..... *Molgula manhattensis*
   - Body narrow and/or snake-like..... 8
8. Cells Interspersed about the Body and Tail
   - Numerous cells interspersed about the body and tail such that larva appears to be decaying or falling apart..... *Ciona intestinalis*; see also "Distinguishing between morphologically-similar ascidian larvae"
   - No cells interspersed about the body and tail..... *Styela clava*; see also "Distinguishing between morphologically-similar ascidian larvae"
9. Body Shape
   - Body oval or seed-shaped..... *Ciona intestinalis*; see also "Distinguishing between morphologically similar ascidian larvae"
   - Body squarish..... *Ascidiella aspersa*; see also "Distinguishing between morphologically similar ascidian larvae"

KEY TO 1-DAY OLD ASCIDIAN JUVENILES

1. Size and Shape
   - Very small (about 0.30 mm), spherical..... 2
   - Small (between 0.50 – 1.50 mm), amorphous..... 5
   - Very large (> 2.00 mm), orange or dark red..... *Botrylloides violaceus*
2. Sand Grains & Debris
   - Sand grains or debris firmly attached to surface..... 3
   - No sand grains or debris on surface.. 4
3. "Pseudopods" and Coloration
   - Pseudopods present and/or body is opaque pink..... *Styela clava*
   - No pseudopods; body transparent or translucent grey..... *Molgula manhattensis*

4. Presence of a Stalk
   - Body attached to substratum via a stalk; body moves back and forth if squirted with a water-filled pipette..... *Ciona intestinalis*
   - Stalk absent; body firmly attached to substratum..... *Ascidiella aspersa*
5. Appearance
   - Single well-developed, sideways-lying zooid embedded in a gelatinous mass..... *Aplidium constellatum*
   - Multiple zooids embedded in a transparent gelatinous mass covered with large blue-grey spots.....*Diplosoma listerianum*
   - Semi-spherical transparent mass with a yellow colored central portion; two opaque white structures adjacent to the yellow center; appears slimy..... *Didemnum* sp.
   - Translucent grey, covered with blue-grey spots; eyespot visible..... *Botryllus schlosseri*

KEY TO 7-DAY OLD ASCIDIAN JUVENILES

1. Size and Shape
   - Covered with dense mat-like arrangement of sand grains..... *Styela clava*
   - Very large (> 3.00 mm), orange or dark red..... *Botrylloides violaceus*
   - Spherical..... 2
   - Amorphous..... 4
2. Sand Grains & Debris
   - Sand grains or debris firmly attached to surface..... *Molgula manhattensis*
   - No sand grains or debris on surface..... 3
3. Presence of a Stalk
   - Body attached to substratum via a stalk; body moves back and forth if squirted with a water-filled pipette..... *Ciona intestinalis*
   - Stalk absent; body firmly attached to substratum..... *Ascidiella aspersa*
4. Appearance
   - Single well-developed, sideways lying zooid embedded in a gelatinous mass..... *Aplidium constellatum*
   - Multiple zooids embedded in a transparent gelatinous mass covered with large blue-grey spots..... *Diplosoma listerianum*
   - Entirely covered by small, opaque white spicules..... *Didemnum* sp.
   - Large single zooid; overall color blue-green; many portions transparent..... *Botryllus schlosseri*

KEY TO BRYOZOAN LARVAE

1. Cyphonautes or Coronate
   - Cyphonautes: With shell, bilaterally compressed..... 2
   - Coronate: No shell, spherical or barrel shaped..... 4
2. Shape
   - Oval; encrusted with granules..... *Electra crustulenta*
   - Triangular..... 3
3. Aspects of Triangle
   - Basal points of triangle rounded..... *Electra pilosa*
   - Basal points of triangle pointed; brown spots along basal margin..... *Membranipora membranacea*
4. Location of Cilia
   - Cilia confined to equatorial band..... *Cryptosula pallasiana*
   - Cilia covering all or most of the body..... 5
5. Color
   - Purple..... *Bugula neritina*
   - Tan or straw colored..... 6

6. "Eyespots" Visible
   - Eyespots visible..... *Bugula turrita*
   - No eyespots..... 7
7. Presence of Flagella and Shape
   - Flagella present, larva round or square..... *Bugula simplex*
   - Flagella absent, larva barrel-shaped..... *Bowerbankia gracilis*

KEY TO 1 DAY OLD BRYOZOAN JUVENILES

1. Number of Zooids
   - Single zooid..... 2
   - Two small twined (attached together) zooids..... *Membranipora membranacea*
2. Zooecium Shape
   - Shield-shaped..... 3
   - Circular..... 4
   - Bubble-like..... 5
   - Tubular or tapering..... *Bowerbankia gracilis*
3. Presence of Spines
   - 8 spines surrounding the operculum..... *Schizoporella errata*
   - No spines; operculum shaped like a slice of bread..... *Cryptosula pallasiana*
4. Presence of Spines and Appearance
   - No spines; zooecium appears flat..... *Electra crustulenta*
   - Spines surround operculum and point inward toward the zooid; zooecium appears raised..... *Electra pilosa*
5. Color
   - Purple..... *Bugula neritina*
   - Straw or tan colored..... *Bugula simplex* or *Bugula turrita* (they cannot be distinguished)

KEY TO 7-DAY OLD BRYOZOAN JUVENILES

1. Appearance
   - Erect, projecting vertically from the substratum..... 2
   - Cluster of round or shield-shaped zooecia 3
   - Tubular zooecia radiating from a central attachment point..... *Bowerbankia gracilis*
2. Color
   - Purple..... *Bugula neritina*
   - Straw or tan colored..... *Bugula simplex* or *Bugula turrita* (they cannot be distinguished)
3. Zooecium Shape
   - Shield-shaped..... 4
   - Circular or oval..... 5
4. Spines on the Ancestrula
   - Ancestrula with 8 spines surrounding the operculum..... *Schizoporella errata*
   - Ancestrula without spines..... *Cryptosula pallasiana*
5. Presence of Spines
   - Vertical spines at margin of operculums do not point inward toward the zooids..... *Electra crustulenta*
   - Spines surround operculums and point inward toward the zooids..... *Electra pilosa*

SPECIES DESCRIPTIONS

ASCIDIANS (Figures 2, 3, 5, 6 & Color Plate 1)

Note: When a single size is listed in a description rather than a mean ± standard error, this represents the size of the individual that was drawn (Figures 2-3). Mean sizes were not calculated. However, the drawn specimen was selected because it was considered "typical" of the species.

Diplosoma listerianum (Milne-Edwards, 1841) (Figure 2)

Larvae: Large tadpole with bulbous, transparent body. Mean total length = 1.29 ± 0.01 mm (n = 3, range = 1.28-1.32 mm). Body transparent with several lobe-like organs. Ocellus and statolith present, however, close together and often appear as single spot at low magnification. Three large adhesive papillae; papillae do not project beyond margin of body. Stalks run from adhesive papillae to lobe-like organs in center of larva. Scattered granular spots visible in body. Tail opaque light orange-brown. Coloration of central lobes yellow-orange with brown accents.

Aids for Identifying Larvae: *Diplosoma* larvae superficially resemble *Aplidium* larvae because of the presence of yellow-orange lobes. However, *Aplidium* larvae are covered in numerous spots and stalked adhesive glands are rarely visible.

One-Day Old Juvenile: Two zooids embedded in gelatinous mass. Overall width = 1.24 mm. Brachial baskets rounded and iridescent purple. Intestinal regions at base of brachial baskets orange-brown. Overall coloration transparent. Fairly large blue-grey spots evenly spaced throughout gelatinous mass.

Additional note: Due to a cold winter, *Diplosoma* was very rare in Southeastern New England during the summer of 2003 (e.g., Stachowicz et al 2002). The described juveniles were collected from floating docks bathed by the warm water plume of a power plant in Salem, MA. Only 20-30 juveniles were collected and these were at various stages of development. The smallest juveniles (which based upon past personal experience were one-day old) had two zooids. No juveniles with a single zooid were observed. Given the small sample size, however, it is not clear whether a one zooid stage is included in *Diplosoma*'s life history or if all newly settled juveniles have two zooids.

Week-Old Juvenile: Multiple zooids embedded in gelatinous mass. Overall width = 2.76 mm. Incurrent siphons visible; excurrent siphons not visible. Brachial baskets iridescent purple. Developing zooids and opaque orange-brown structures with white speckles (possibly developing reproductive organs) located at base of brachial baskets. Translucent orange-brown digestive regions located underneath brachial baskets. Transparent gelatinous mass interspersed with fairly large blue-grey spots.

Spawning: *Diplosoma* is difficult to spawn. It does not appear to respond to light shocking and instead may release a steady trickle of larvae over the course of its adult life. Larvae can be obtained by dissecting well developed embryos from adult colonies. Methods have not been developed to allow investigators to collect larvae on demand, although it may be possible to collect larvae from spawning chambers as per *Botryllus* (see below). Additionally, larvae can be readily collected by dragging plankton nets near locations with high adult densities.

Didemnum sp. (lahillei?) (Figure 2)

Larvae: Medium to large tadpole. Total length = 1.560 mm. Ocellus and statolith present. Body transparent with very few spots. Three adhesive papillae on anterior side of body; papillae do not project beyond body margin. Lateral ampullae (which resemble adhesive papillae) present in second row behind adhesive papillae and project from colored mass in center of body. Dark yellow lobes in body (digestive structures of developing zooid). Overall coloration yellowish. Lateral ampullae and posterior region of central mass light yellow.

Aids for Identifying Larvae: *Diplosoma* larvae superficially resemble *Didemnum* larvae because of the presence of yellow-orange lobes. However, *Didemnum* possesses lateral ampullae which *Diplosoma* lack.

One Day Old Juvenile: Semi-spherical transparent mass with yellow colored central portion. Overall width = 0.93 mm. Width of yellow central portion = 0.63 mm. Beginnings of a zooid often present at posterior end of central portion. Two "eyespots" often visible on anterior end of central portion. Two opaque white structures present on either side of central portion. White structures appear composed of (and possibly produce)

spicules. Spicules absent form other areas of body. Overall appearance "slimy."

Week-Old Juvenile: Amorphous body entirely covered by small, opaque white spicules. Overall width = 1.73 mm. Very difficult to distinguish internal features, although some internal structures appear as faint white or yellow blotches. Siphons present, but difficult to distinguish. Morphology very variable. Juveniles may have many different shapes, however, most semi-circular with rounded lobes along outer margins.

Spawning: *Didemnum* is difficult to spawn. Only a single larva was obtained by following the "light shocking without cycles" protocol (Page 17). Adult colonies were collected from the field Aug 5, 2003 and placed in the dark for approximately 15 hr before being exposed to light. After about 15 min in the light the single larva was found in the spawning dish. It is possible that the larva was fortuitously released at this time rather than being released as a result of the spawning protocols. The beginning of August seems to be relatively early in *Didemnum*'s spawning season (Whitlatch, *unpublished data*). Thus, few developed larvae may have been present in the adult colonies at this time. "Light shocking without cycles" may produce better results if conducted between mid-August and late September.

Aplidium constellatum (Verrill, 1871) (Figure 2)

Larvae: Medium sized tadpole covered with blue-gray iridescent spots. Mean total length = 1.27 ± 0.05 mm (n = 9; range = 1.12–1.56mm). Body fairly long. Ocellus and statolith present. In fresh samples parts of statolith may be obscured so that three "eyespots" appear present (Fig. 2). Occasionally, tips of three adhesive papillae visible as small protrusions at front of body; often, papillae not visible. Stalks of papillae rarely visible. Lobes visible in body (digestive structures of developing zooid). Center-most lobe dark orange. Two flanking lobes lighter yellow-orange. Tail very light orange.

Aids for Identifying Larvae: *Aplidium* larvae superficially resemble *Diplosoma* larvae because of the presence of yellow-orange lobes. However, *Diplosoma* larvae have very few spots and three distinct adhesive glands with stalks.

Larval Behavior: Larvae swim erratically. After spawning in the laboratory, most larvae swam near water surface. It is unclear whether larvae in the field behave similarly.

One-Day Old Juvenile: Single small, sideways lying zooid embedded in gelatinous mass. Overall width = 1.10 mm. Length of zooid = 0.77 mm. Brachial basket iridescent purple. Posterior end of zooid light orange with dark orange stripe (the endostyle) along margin. Gelatinous mass surrounding zooid filled with iridescent blue-green spots.

Week-Old Juvenile: Single well-developed, sideways lying zooid embedded in gelatinous mass. Overall length = 1.32 mm. Zooid transparent with iridescent openings on brachial basket. Overall coloration light grey. Few iridescent spots visible in gelatinous mass.

Spawning: Spawning was conducted by following the "light shocking without cycles" protocol (Page 17). Colonies were placed in the dark around 12:00 July 15, 2003. Around 13:00 July 16, colonies were placed in dishes and exposed to halogen light. Larvae started to be released after 15 min and were released intermittently at a rate of about 1-2/min thereafter.

Botrylloides violaceus Oka, 1927 (Figure 2)

Larvae: Very large tadpole with bulbous body. Total length = 3.02 mm. No eyespots or statoliths visible. Frill composed of numerous finger-like projections runs around equator of body. Portion of body anterior to frill transparent. Pigmented cone-like structure visible in the center of body. Several adhesive papillae located on anterior portion of body. Portion of the body posterior to the frill contains numerous pigmented structures. Coloration bright orange to deep red.

Larval Behavior: The changing morphology of *Botrylloides* larvae makes it easy to determine how close a larva is to settlement. The frill of the larva acts much like the legs of a lunar lander. In newly released larvae the frill encircles the equator of the body. As the larva gets closer to settlement, the finger-like projections of the frill begin to extend and the anterior portion of the body retracts. When settlement is imminent, the fingers project beyond the end of the body and look much like the fingers of a grasping hand. Upon attachment to the substratum, the fingers form the outer margin of the juvenile *Botrylloides* colony.

One-Day Old Juvenile: Very large single zooid surrounded by frill of finger-like projections. Overall width = 2.35 mm. Brachial basket visible. Endostyle visible as dark orange line inside central zooid extending between two siphons. Developing zooids appear as small buds around the large central zooid. Coloration bright orange to

dark red. More heavily pigmented area located posterior to incurrent siphon.

Week-Old Juvenile: Cluster of large zooids (often four zooids) surrounded by frill of finger-like projections. Overall width = 4.25 mm. Each zooid has its own incurrent siphon, but zooids may share a common excurrent siphon. All aspects of juvenile bright orange to dark red.

Spawning: Spawning was conducted by following the "light shocking without cycles" protocol (Page 17). Colonies were collected and placed in containers at approximately 18:00 July 7, 2000. By 20:00 several larvae were noticed and 8 juvenile colonies had settled on container walls. The containers were covered until 10:00 July 8. Upon opening, 20 new juveniles were found on the inside of the containers and several larvae were swimming in the water column. Over the course of the next 3 hours, larvae were found in the water column at the rate of about 30-40 larvae/hour.

Botryllus schlosseri (Pallas, 1774) (Figure 2)

Larvae: Medium sized tadpole. Well developed adhesive glands on anterior portion of body that project well beyond body. Total length = 1.14 ± 0.02 mm (n = 10, range = 1.06-1.22 mm). Anterior portion of body transparent; posterior portion pigmented. Body can range in shape from round to oval. Ocellus present, but visible from only one side and difficult to detect; ocellus visible in 3 of 11 larvae at 16x magnification. The tail opaque or translucent white. Coloration variable (as are adult colonies); larvae examined light orange.

Aids for Identifying Larvae: Although similar in size to *Ciona*, *Ciona* has a more narrow and seed-shaped body.

One Day Old Juvenile: Small translucent grey body. Overall width = 0.52 ± 0.02 mm (n = 15, range = 0.38-0.63 mm). Most visible feature three lines running most of the length of juvenile (endostyle and sides of brachial basket). Brachial basket iridescent pink-purple; endostyle more opaque and covered with spots. Eyespot usually visible between endostyle and brachial basket. Siphons usually visible, but small. Overall color semi-translucent grey with blue-grey spots.

Additional note: Although it is very difficult to see on PVC panels, juveniles are surrounded by a transparent gelatinous halo. Numerous transparent finger-like projections run from the zooid to the margin of halo similar to the finger-like projections found in *Botrylloides*. Halo roughly doubles overall width of juveniles. Halo not shown in drawing (Fig. 2) and difficult to see in photograph (Color Plate 1).

Week Old Juvenile: Large blue-green single zooid. Overall width = 2.93 ± 0.23 mm (n = 12, range = 1.27-4.18 mm). Brachial basket light pink. Newly developing zooids appear as small round "blobs" at base of main zooid. Closely packed blue-green dots form line between incurrent and excurrent siphons. Groups of closely packed blue-green dots present along outer edge of juvenile. Overall color blue-green, although many portions transparent.

Spawning: Spawning *Botryllus* is sometimes difficult. During a "significant" reproductive period for *Botryllus* (J. Terwin, *personal communication*) (approximately August 1, 2000), several attempts were made to spawn *Botryllus* using the "light shocking without cycles" protocols (Page 17). None of these spawnings were successful. Larvae were obtained from *Botryllus* by placing colonies in enclosed spawning chambers and placing them *in situ* off the Avery Point dock for 24 h (August 6, 2000). At the end of this time, many larvae had settled to the inside of the spawning chamber and active larvae were obtained by filtering water from the chambers through Nitex mesh.

Additional efforts at "light shocking without cycles" proved successful Oct 7, 2003. Colonies were collected Oct 6 and kept in the dark for approximately 22 hours before being exposed to sunlight. Spawning started very slowly, but numerous larvae (10's) were seen after approximately 20 min. Significant numbers of larvae (100's) were seen after approximately 1.5 hours. Given this success, it is unclear why initial efforts at "light shocking without cycles" failed.

Styela clava Herdman, 1881 (Figure 3)

Larvae: Medium sized, snake-like tadpole. Mean total length = 0.85 ± 0.02 mm (n = 9, range =0.80-0.92 mm). Body merges directly with tail; overall aspect of larva snake-like. Single large, round ocellus present. Numerous structures visible near ocellus and appear as bands of pigments at different depths. Body darkly pigmented, including tail. Tail very muscular.

Aids for Identifying Larvae: Single large ocellus. No cells interspersed about body or tail. See also «distinguishing between morphologically similar ascidian larvae» section (Page 15).

One-Day Old Juvenile: Small opaque, pink body. Overall width = 0.29 mm. Eyespot present. Often small lateral, transparent, pseudopod-like structures run outward from body. Small amounts of sand grains and debris attached to body and do not blow away if squirted with a water-filled pipette. Overall coloration opaque granular

pink.

Week-Old Juvenile: Body surrounded by dense, mat-like covering of sand grains. Overall width = 0.54 mm. Siphons visible, but very difficult to see. Juveniles look very much like benthic amphipod tubes and can be easily overlooked. Dense sand grains appear glued together and form mat over body (this contrasts with *Molgula* where sand grains appear to be stuck haphazardly to the surface of the juvenile). Overall color highly transparent; sand grains provide coloration.

Spawning: *Styela* is difficult to spawn. It does not appear to respond to light shocking, but larvae can be obtained using the "spawning via gonad removal" protocol (Page 17). To obtain larvae, *Styela* were collected around 17:00 July 20, 2000. In the lab, a longitudinal incision was made along the tunic. Gametangia were found just beneath the tunic. Pieces of gametangia were removed from 5-6 individuals and placed in a glass dish. Once transferred, eggs and sperm were well mixed by fluxing the water with a pipette. The gametes were allowed to stand overnight. Larvae were found at 8:30 July 21, 20 hours later.

Note of caution: During spawning, pieces of the brachial basket and gut were accidentally transferred to the dish with the gametes. As a precaution, some eggs were transferred to a separate container of clean water while most eggs remained in the original dish. When examined 20 hours later, live larvae were found in the clean container while only dead larvae were found in the container with tunicate fragments. Thus, it seems vital to keep newly released gametes in clean water.

An additional spawning was conducted on Oct 7, 2003. Gonads were dissected from *Styela* at 17:30. The spawning box was checked hourly for larvae starting after 14 hours. Larvae started to hatch at 11:30 Oct 8, 16 hours after spawning. Water temperature was 18.5 ^{o}C.

Molgula manhattensis (Dekay, 1843) (Figure 3)

Larvae: Small larva with bulbous body. Mean total length = 0.48 ± 0.01 mm (n = 3, range = 0.46-0.50 mm). Well developed capsule fully encloses body. Because capsule fairly thick, smooth, and uniform, the larva has the appearance of wearing a space helmet. Ocellus present near center of body. Downward pointing adhesive papillae present on anterior portion of body; papillae enclosed within capsule and do not protrude beyond margin of body. Tail usually sticks out directly behind larva with little bending.

One Day Old Juvenile: Small transparent to translucent light grey ball. Sand grains and debris attached to body do not blow away if squirted with a water-filled pipette. Overall width = 0.22 mm. Eyespot visible. On close inspection, body of juvenile appears to be composed of translucent light grey ball inside larger transparent ball.

Week Old Juvenile: Translucent grey body. Sand grains and debris attached to body do not blow away if squirted with a water-filled pipette. Overall width = 0.88 mm. Center portion of body nearly spherical. Siphons at opposite ends of body. Angle of siphons usually between 120-180 degrees; when viewed from above angle of siphons makes juvenile look like a person with outstretched arms. Brachial basket nearly spherical and iridescent. Eyespot usually visible, but small. Overall color light grey.

Spawning: Spawning was conducted by following the "light shocking with cycles" protocol (Page 16). Individuals were placed in a water table under direct lighting around 19:00 July 12, 2000. At 13:00 July 13, the lighting was removed and individuals were placed in the dark. At 19:00 July 13, the covers were removed and the *Molgula* were placed in a glass dish under direct lighting. Within about 10 min of light exposure, colonies began to shed gametes. Eggs were seen, but sperm was not observed. About 30 min after spawning began, numerous eggs were present in the spawning dish. Adult *Molgula* were removed from the dish, the dish was covered, and the gametes were allowed to stand overnight. When the dish was examined at 10:00 July 14, no eggs were present, but only 4 larvae were collected. A great deal of detrital material was present in the spawning dish making it difficult to determine whether small juveniles were present. It is likely, however, that larvae hatched and settled during the 14 hour period between spawning (ending around 20:00 the previous evening) and inspection.

Ascidiella aspersa (O F Müller, 1776) (Figure 3)

Larvae: Small tadpole with squarish body. Mean total length = 0.85 ± 0.01 mm (n = 9, range = 0.82-0.90 mm). From side, body squarish and uniformly pigmented. Ocellus and statolith present; both spherical and approximately equal in size. Often ocellus and statolith appear located in distinct "pouch" (the cerebral vesicle). Three large adhesive papillae project well beyond body. Posterior portion of body appears flat. Cells may be interspersed about body and tail. However, if present, cells are fairly large and relatively scarce. Tail somewhat short relative to body. Outer cuticle of tail appears wavy.

Aids for Identifying Larvae: See "distinguishing between morphologically-similar ascidian larvae" section

(Page 17).

One-Day Old Juvenile: Translucent grey sphere, without sand grains, does not wobble if squirted with a water-filled pipette. Overall width = 0.27 mm. Firmly attached to substratum without stalk. Eyespot present. Brachial basket poorly developed. Developing brachial basket appears as several lateral grey stripes near eyespot. Often opaque grey ball-like structure near posterior end of juvenile.

Week Old Juvenile: Highly transparent juvenile, without sand grains, does not wobble if squirted with a water-filled pipette. Overall width = 0.80 mm. Eyespot sometimes visible. Brachial basket iridescent purple and green. Although body highly transparent, iridescent blue-green spots scattered over body surface.

Spawning: Spawning was carried out according to the "light shocking with cycles" protocol (Page 16). Individuals were placed in a water table under direct lighting at approximately 19:00 July 12, 2000. At 13:00 July 13 the lighting was removed and individuals were placed in the dark. At 19:00 July 13, the individuals were placed in a glass dish under direct lighting. Within about 10 min of light exposure, colonies began to shed gametes. Eggs were seen, but sperm was not observed. About 30 min after spawning began, numerous eggs were present in the bowl. Many of these eggs were buoyant, with approximately 50% floating on the water surface. Adult *Ascidiella* were removed from the spawning dish, the dish was covered, and the gametes were allowed to stand overnight at 21 °C. Eggs were checked frequently starting about 14 hours after spawning. Free swimming larvae were found 16 hours after spawning.

Ciona intestinalis (Linnaeus, 1767) (Figure 3)

Larvae: Narrow tadpole with dark, granular appearance and cells interspersed about body and tail. Mean total length = 1.12 ± 0.04 mm (n = 10, range = 0.88-1.28 mm). Body oval or seed shaped. Cells interspersed about body and tail giving appearance that larva is sloughing cells or decaying. Adhesive papillae located on anterior side of body. Papillae downwardly directed, project beyond the margin of body, and appear glove-like. Large ocellus and smaller statolith present; both often visible (both visible in 7/12 larvae at 50x magnification), although occasionally only ocellus can be seen. Fins on tail often visible at low magnification, but difficult to see at higher magnification. Coloration dark tan.

Aids for Identifying Larvae: See "distinguishing between morphologically-similar ascidian larvae" section (Page 15).

Larval Behavior: Larvae move awkwardly. When held in glass dish, many larvae remain motionless on bottom. Swimming appears to be inefficient; a great deal of side-side thrashing motion occurs with little forward progress. Larvae in the process of selecting settlement sites often thrash wildly with their tails while their adhesive papillae are attached to the bottom of dish. Thus, they often look like they are driving themselves against the substratum.

One-Day Old Juvenile: Transparent sphere attached to substratum via stalk; juvenile will wobble if squirted with a water-filled pipette. Overall width = 0.27 ± .02 mm (n = 5, range = 0.24-0.34 mm). At least one eyespot visible, sometimes two. Overall color light grey. Posterior portion of the juvenile contains round, opaque white region.

Week-Old Juvenile: Transparent sphere attached to substratum via stalk; juvenile will wobble if squirted with a water-filled pipette. Overall width = 0.76 mm. Eyespot present. Endostyle appears as light grey line along mid-line. Incurrent and excurrent siphons at right angle to each other; incurrent siphon at apex of body, excurrent siphon on side of body. Brachial basket iridescent pink. Body highly transparent. Most visible feature of juvenile the black eyespot.

Spawning: Spawning was conducted by following the "light shocking with cycles" protocols (Page 16). Individuals were placed in lighted water tables for 12 hours. After this time, the light was turned off and they were left in the dark for 6 h. Individuals were then placed under halogen light. Spawning began almost immediately. Individuals released both eggs and sperm, with single individuals occasionally expelling gametes several times. Eggs and sperm of multiple individuals were pipetted out of the large dish and into several small dishes. First cell division was observed after 45 min at approximately 21 °C; subsequent divisions occurred every 15 min thereafter. Fully formed larvae present after 22 h.

From Millar (1953): Larvae are initially positively phototactic and subsequently become negatively phototactic and positively geotactic. Settlement occurs after 6-36 h. In England, the breeding season is April - September.

BRYOZOANS (Figures 4, 5, 6 & Color Plate 2)

Note: When a single size is listed in a description rather than a mean ± standard error, this represents the size of the individual that was drawn (Figure 4). Mean sizes were not calculated. However, the drawn specimen was selected because it was considered "typical" of the species.

Bugula simplex Hincks, 1886 (Figure 4)

Larvae: Tan colored coronate larva with cilia covering most of body. Mean size 0.20 ± 0.01 mm (n = 12). Larvae roundish to squarish and slightly blunted at both ends. Corona present on dorsal surface; at low magnification corona appears to completely wrap around larva forming a "girdle." When viewed from above, corona makes larva appear somewhat mushroom-shaped. At high magnification, set of longer cilia with several branches (flagella) appear to originate at base of corona (flagellae visible on 5/9 larvae at high magnification). Larvae uniformly tan in color; color matches color of adult colonies.

Aids for Identifying Larvae: No eyespots. Long flagella often visible at high magnification. Usually smaller than *B. turrita*.

Larval Behavior: Larvae move in slow spiral.

One-Day Old Juvenile: Small yellow-brown, bubble-like test with few distinct features. Size = 0.17 mm. Nearly opaque; appears shiny. Color matches that of adult colonies.

Week-Old Juvenile: Tan or straw colored branching colony standing upright on substrata. Size = 2.00 mm. Spines present at edge of zooecia; it is unclear, however, whether spines are unique to *B. simplex*. Zooids brownish; darker at base of colony. Tentacles white with iridescent highlights. At this age, uppermost zooids beginning to spread outward to form fan-like whorl characteristic of *B. simplex*.

Spawning: *Bugula simplex* colonies were spawned using "light shocking without cycles" protocol (Page 17). Colonies were collected at approximately 18:00 July 10, 2000 from floating docks at Avery Point and placed in plastic buckets in the laboratory with aeration. Black plastic sheeting was placed around the buckets and colonies were left in the dark until 9:00 July 11, 2000. The sheeting was then removed and the colonies placed in direct sunlight. After about 5 min, some larvae were observed. After 10 min, larvae were very numerous. In contrast, colonies of *B. turrita* that had been treated identically (in separate buckets) did not begin to release larvae until 12 min after light shocking.

Bugula turrita (Desor, 1848) (Figure 4)

Larvae: Tan colored coronate larva with cilia covering most of body. Mean size 0.24 ± 0.01 mm long (n= 10). Larvae roundish to squarish and slightly blunted at both ends. One red eyespot on each side of larva and two eyespots on anterior ventral portion of larva. At high magnification, a set of longer cilia appear to originate at the base of two anterior ventral eyespots. Corona present on dorsal surface midway between two sets of eyespots. From the side, larvae appear cup-shaped. Larvae uniformly tan in color; color matches color of adult colonies. Larvae lose color rapidly when preserved in formalin. After 3 hours in 3% formalin, larvae appear white with orange eyespots. Eyespots may be lost after several weeks exposure to formalin.

Aids for Identifying Larvae: Eyespots present. Larger on average than *B. simplex*.

Larval Behavior: Larvae move in slow spiral. Often observed near bottom of container swimming with two anterior eyespots pressed toward substrata; apparently searching for settlement sites. After 2.5 h most larvae were still swimming although some had settled.

One-Day Old Juvenile: Small yellow-brown, bubble-like test with few distinct features. Size = 0.17 mm. Nearly opaque; appears shiny. Color matches that of adult colonies.

Week-Old Juvenile: Tan or straw colored branching colony standing upright on the substrata. Size = 2.53 mm. No spines present. At low magnification zooids appear as two circular structures connected together.

Spawning: Spawning was carried out according to the "light shocking without cycles" protocol (Page 17). Colonies were collected at approximately 18:00 on July 7, 2000 from floating docks at Avery Point. Colonies were placed in plastic buckets in the laboratory with aeration. Black plastic sheeting was placed around the buckets and colonies were left in the dark until approximately 10:00 July 8, 2000. At the end of this time, the sheeting was removed and the colonies were placed in direct sunlight. After about 15 min in the light some larvae were observed. After 30 min larvae were very numerous.

Bugula neritina Linnaeus, 1758 (Fig. 4)

Larvae: Purple coronate larva with reddish highlights. Size = 0.29 mm. Barrel shaped (when preserved in formalin). Covered with longitudinal bands of cilia. Furrow runs along one side of larva accounting for approximately 1/8th of larva's circumference. Semi-triangular darker color bands present on sides of larva.

One Day Old Juvenile: Small purple, bubble-like test with few distinct features. Size = 0.29 mm. Two darker purple lines run across top of zooecium. Appears shiny.

Week Old Juvenile: Purple colored branching colony standing upright on substrata. Height = 2.00 mm. Growth rates variable, although 4 feeding zooids and several additional budding zooids are often found in one week old colonies. Somewhat more robust and not as highly branched as *B. simplex* or *B. turrita*. Overall coloration purple, although some areas transparent.

Spawning: Spawning was carried out according to the "light shocking without cycles" protocol (Page 17). Adult colonies were collected from floating docks at Salem, MA on August 18, 2003. Spawning techniques were nearly identical to *B. turrita*.

Electra crustulenta (Pallas, 1766) (Fig. 4)

Larvae: Paraphrased from Ryland (1965). Oval shaped cyphonautes larva. Size = approximately 0.20 mm. Shell encrusted with granular particles. Brownish or grey in color.

One Day Old Juvenile: Single small circular or egg-shaped zooecium. Size = 0.27 mm. Test opaque white with grey highlights. Zooecium appears flush with substratum. Few distinguishing features.

Week Old Juvenile: Linear or clustered assemblage of zooecia. Overall length = 1.39 mm. Test opaque white. Long vertical spines near openings for zooids that rarely overlap with zooids. Zooids light orange with 12 tentacles. Newly developing zooids bud from previously formed zooids.

Spawning: Not determined.

Electra pilosa (Linnaeus, 1767) (Fig. 4)

Larvae: Paraphrased from Ryland (1965). Triangular cyphonautes larva. Up to 0.50 mm at metamorphosis. Basal points of triangle usually rounded. No spots along basal marginal.

One Day Old Juvenile: Single circular zooecium with spines surrounding operculum that point inward towards zooid. Size = 0.39 mm. Test opaque white. Small perforations at base of zooecium. Zooecium appears raised from substratum. Several indentations visible on side of zooecium which may represent newly forming zooids.

Week Old Juvenile: Linear arrangement of several zooecia. Overall length = 1.07 mm. Test white with grey highlights. Zooids white with brown accents. Zooecia have spines that fold inward and partially cover zooids. At this age often 3 distinct zooids, with additional newly developing zooids branching off established zooids. Ancestrula smallest and generally only feeding zooid.

Spawning: Not determined.

Membranipora membranacea (Linnaeus, 1767) (Fig. 4)

Larvae: Paraphrased from Ryland (1965). Large triangular cyphonautes larva. Size at settlement = 0.85 mm. Basal points of triangle usually pointed. Overall coloration transparent with brownish markings or spots present along basal margin.

One Day Old Juvenile: Two small twined (attached together) zooecia. Overall width = 0.29 mm. Test opaque white. Often has skull-like appearance.

Week Old Juvenile: Only one juvenile was collected during the 2003 field season and it died after 2 days. Hence, week old juveniles not available for descriptions.

Spawning: See Strathmann (1987).

Schizoporella errata (Waters, 1878) (Fig. 4)

Larvae: Not available.

One-Day Old Juvenile: Single shield-shaped zooecium with 8 spines surrounding operculum. Size = 0.40 mm. Few or no perforations on zooecium. If perforations present they are located between operculum and side of zooecium closest to operculum (this contrasts to *Cryptosula* were perforations are on side of zooid farthest from operculum). Light orange in color. Often with three dark orange spots; one spot on each side of zooecium and one at base.

Week-Old Juvenile: Cluster of shield-shaped zooecia. Size = 1.10 mm. Ancestrula has 8 spines around operculum. Additional zooecia lack spines. Ancestrula lacks perforations in zooecium; numerous perforations on zooecia of additional zooids. Coloration light to medium orange. Newly developing zooids branch off of established zooids.

Spawning: Not determined. S Bullard has previously spawned *Schizoporella unicornis* using "light shocking without cycles." *S. errata* can almost certainly be spawned the same way.

Note: *S. errata* can be easily confused with *S. unicornis*. *S. errata* is by far the most abundant species at our sites, but small numbers of *S. unicornis* may also be present. Because of the difficulty in distinguishing between these species (see Maturo & Schopf 1968), however, we consider all *Schizoprella* in our area to be *S. errata*.

Cryptosula pallasiana (Moll, 1803) (Figure 4)

Larvae: Light brown coronate larva. Size = 0.24 mm. Equatorial band of cilia encircles larva. Anterior end flattened, posterior end composed of two rounded lobes; similar to the shape of an air gun pellet.

One-Day Old Juvenile: Single shield-shaped zooecium. Size = 0.48 mm. Operculum shaped like a slice of bread. Zooecium with numerous (more or less) regularly spaced perforations between operculum and side of zooecium farthest from operculum. Coloration translucent orange-brown. No spines.

Recently settled *C. pallasiana* not as well developed. Appear as semi-amorphous light brown zooecium.

Size = 0.44 mm. Sometimes lack a regular outline, but often with 4 distinct lobes. At low magnification, developing zooid appears as two concentric circles inside zooecium; inner circle darker than the outer circle (Figure 4, left-hand illustration).

Week-Old Juvenile: Group of shield-shaped zooecia. Size = 1.10 mm. Operculums shaped like a slice of bread. At this age, two complete zooids usually present. Additional uncompleted zooids appear as wing-like extensions branching from anterior zooid. Semi-symmetrical perforations on anterior zooecium. Smaller, asymmetrical perforations on ancestrula. Ridges on ancestrula. Coloration light orange with darker orange highlights.

Spawning: Spawning was carried out according to the "light shocking without cycles" protocol (Page 17) from colonies collected at Avery Point docks August 30, 2003.

Bowerbankia gracilis Leidy, 1855 (Figure 4)

Larvae: Paraphrased from Reed (1980). Light yellow coronate larva. Size approximately 0.200 mm. Barrel shaped. Narrow slit on the median plane of the larva.

One Day Old Juvenile: Semi-erect tubular zooecium tapering to one side. Size = 0.54 mm. Zooecium mostly transparent with some translucent grey areas. Zooid opaque light brown or orange-brown. Exposed tentacles iridescent purple. As specimens age they develop lateral stolens that give rise to additional zooids.

Recently settled *B. gracilis* often smaller than 0.50 mm and appear as very small transparent or pinkish zooecium that tapers to one side (i.e., the juvenile in Color Plate 2 is newly settled).

Week Old Juvenile: Multiple tubular zooecia radiating from central attachment point. Size = 1.15 mm. Zooecia covered with sand grains and debris. Newly developing zooids appear as spheres near base of colony. Coloration translucent with medium brown highlights. Zooids with 8 tentacles. Lateral stolens often present.

Spawning: From Reed (1980). Spawning can be induced in *B. gracilis* using "light shocking without cycles" protocol (Page 17); however, larvae of *B. gracilis* var. *aggregata* do not respond to light shock. Larvae are positively phototactic and may swim for up to 10 h.

DISTINGUISHING BETWEEN MORPHOLOGICALLY-SIMILAR ASCIDIAN LARVAE

Distinguishing between *Ciona intestinalis* and *Styela clava*:

A major difference between *Ciona intestinalis* and *Styela clava* is that in *Styela* only an ocellus is visible while in *Ciona* both an ocellus and a statolith are usually visible. However, occasionally on a dissecting scope only the ocellus may be visible in *Ciona*, and rarely neither the ocellus or statolith; both structures should be visible at higher magnification on a compound scope. Additionally, *Ciona* have cells interspersed about the body and tail which make larvae appear to be decaying; this does not occur in *Styela*. Also, in *Ciona* the body and the tail look morphologically different. In contrast, in *Styela* the body and the tail look morphologically similar and appear to merge smoothly into each other.

Distinguishing between *Ciona intestinalis* and *Ascidiella aspersa*:

Ciona larvae are more heavily pigmented and darker than *Ascidiella*. Seen from the side, the body of *Ciona* is oval- or seed-shaped and has darkly colored structures ventral to the statolith and ocellus. In addition, the ocellus and statolith of *Ciona* are morphologically different from each other; the statolith (the more anterior spot) is usually smaller and often elongated. The ocellus (the posterior spot) is usually larger and often irregularly shaped. However, sometimes the two spots are approximately equal in size. One can safely identify a larva as *Ciona* if the spots are irregularly shaped and of different sizes. In some *Ciona* larvae (ca. 40%), only one spot can be seen when viewed at 50x magnification. Thus, it can be diagnostic of *Ciona* if only one or no spots are visible (but see "distinguishing between *Ciona intestinalis* and *Styela clava*"). The adhesive papillae of *Ciona* are visible, but are somewhat small, grouped together on the anterior ventral portion of the body, and are usually directed downward. *Ciona* almost always have numerous small cells interspersed about the body and tail giving larvae the appearance that they are decaying. *Ciona* larvae are relatively large, with a mean total length of 1.12 mm.

Ascidiella larvae are lighter in color than *Ciona* and appear somewhat transparent. Seen from the side, the body of *Ascidiella* is squarish and uniformly pigmented. No distinct structures are visible in the body, although at times the statolith and ocellus appear to be located inside a pouch-like region. The ocellus and statolith of *Ascidiella* are almost always of equal size and both are nearly spherical; both are always visible at 50x magnification. The adhesive papillae of *Ascidiella* are large and not confined to the ventral portion of the body; they may be anteriorly directed or slightly dorsal. In addition, the adhesive papillae may not be grouped together, but rather may be separate from each other. Although *Ascidiella* may have cells interspersed about the body, this is much less common than in *Ciona*. If the cells are present, there will be relatively few of them and they will be fairly large. *Ascidiella* larvae are smaller than *Ciona* with a mean total length of 0.85 mm.

SPAWNING TECHNIQUES

The following are general techniques that can be used to obtain larvae from different species of benthic marine invertebrates. Additional information about spawning techniques and larval culture may be found in Costello & Henley (1971) and Strathmann (1987).

Light Shocking With Cycles

This works well with ovigarous species. Species that may be spawned in this way include *Ciona intestinalis, Molgula manhattensis, Ascidiella aspersa*.

Step 1: Collect approximately 20 individuals and remove as much debris and epizootics from them as possible. Place organisms in a running sea water table and direct bright light onto them (halogen lights work well). Leave them in the light for 12-18 hours.

Step 2: Place organisms in a darkened container with running sea water for 6-8 hours.

Step 3: Remove organisms from the dark and direct a bright light onto them. Organisms should begin to release gametes within a few minutes. As the organisms spawn, collect the eggs and sperm and transfer the gametes to clean containers. Be sure to transfer eggs and sperm of several individuals to each container because some species may be self-sterile.

Spawning Techniques, continued:

Step 4: Keep the fertilized gametes in a location where temperature changes will be minimal and where they will not be disturbed. Because development proceeds at different rates at different temperatures it is not always possible to predict when larvae will be fully developed. Hence, containers should be checked periodically to determine whether larvae are present.

Light Shocking Without Cycles

This works well with viviparous species. Species that may be spawned this way include *Aplidium constellatum, Botrylloides violaceus, Botryllus schlosseri, Bugula turrita, Bugula simplex, Cryptosula pallasiana*.

Step 1: Collect approximately 20 individuals and remove as much debris and epizootics from them as possible. Place the organisms in running seawater table; alternatively, to ensure no larvae are lost, place organisms in a non-flow through container with an air stone. Completely cover the water table or container with black plastic sheeting. Allow the organisms to stand unmolested 10-12 hours.

Step 2: Remove the black plastic sheeting and direct a bright light onto the organisms. Depending on the species being examined, larvae will usually be released within 5-30 min after exposure to light.

Spawning via Gonad Removal

This works well with ovigarous species. Species that may be spawned this way include *Styela clava*

Step 1: Collect approximately 20 individuals and remove as much debris and epizootics from them as possible. Using a razor blade, cut open the organisms to expose the internal structures. Slice open the ovary and sperm duct and pipette the gametes into a container. Be sure to combined the gametes of several individuals in each container because some species may be self sterile.

Step 2: Keep the fertilized gametes under aeration in a location where temperature changes will be minimal and where they will not be disturbed. Because development proceeds at different rates at different temperatures it is not always possible to predict when larvae will be fully developed. Hence, containers should be checked periodically to determine whether larvae are present.

Special Techniques

Some species are difficult to spawn using conventional methods. For these species special techniques may be used to obtain larvae. For *Diplosoma listerianum*, mature larvae may be individually dissected out of adult colonies. For *Botryllus schlosseri*, larvae may be obtained by confining adult colonies in special reproductive chambers (see the *Diplosoma* and *Botryllus* sections for more detail on these techniques).

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APPENDIX I: SIZES OF LARVAL AND JUVENILE ASCIDIANS AND BRYOZOANS

Table 1. Size (mm) of larval (total length) and juvenile (width) ascidians from Long Island Sound.

Species	Larva	One Day Old Juvenile	Week Old Juvenile
Diplosoma listerianum	1.29	1.24	2.76
Didemnum sp.	1.56	0.93	1.73
Aplidium constellatum	1.27	1.10	1.32
Botrylloides violaceus	3.02	2.35	4.25
Botryllus schlosseri	1.14	0.52	2.93
Styela clava	0.85	0.29	0.54
Molgula manhattensis	0.48	0.22	0.88
Ascidiella aspersa	0.85	0.27	0.80
Ciona intestinalis	1.12	0.27	0.76

Table 2. Size (mm) of larval (total length) and juvenile (width) bryozoans from Long Island Sound.

Species	Larva	One Day Old Juvenile	Week Old Juvenile
Bugula simplex	0.20	0.17	2.00
Bugula turrita	0.24	0.17	2.53
Bugula neritina	0.29	0.29	2.00
Electra crustulenta	0.20	0.27	1.39
Electra pilosa	0.50	0.39	1.07
Membranipora membra.	0.85	0.29	NA
Schizoporella errata	NA	0.40	1.10
Cryptosula pallasiana	0.24	0.48	1.10
Bowerbankia gracilis	0.20	0.54	1.15

APPENDIX II: ABUNDANCE OF JUVENILE ASCIDIANS AND BRYOZOANS AT GROTON, CONNECTICUT

Table 3. Presence/absence of juvenile ascidians and bryozoans at Avery Point docks, Groton, Connecticut. Recruitment data collected between June 27, 2001 and Dec 10, 2003.

Species	Month											
Ascidians	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Diplosoma					X	X	X	X	X	X	X	X
Didemnum							X	X	X	X	X	
**Aplidium*												
Botrylloides					X	X	X	X	X	X	X	X
Botryllus				X	X	X	X	X	X	X	X	X
Styela					X		X	X	X	X		
Molgula						X	X	X	X	X	X	
Ascidiella				X	X	X	X	X	X	X	X	X
Ciona				X	X	X	X	X	X	X		
Bryozoans												
***Bugula spp.*						X	X	X	X	X	X	
Bugula neritina								X	X	X		
Electra crust.							X	X	X	X		
Electra pilosa						X	X	X	X		X	
Membranipora								X				
Schizoporella						X	X	X	X	X		
Cryptosula	X		X	X	X	X	X	X	X	X	X	X
Bowerbankia							X	X	X	X		

* *Aplidium* began to recruit to this site in very small numbers in 2003.
** Combination of *Bugula simplex* and *B. turrita.*

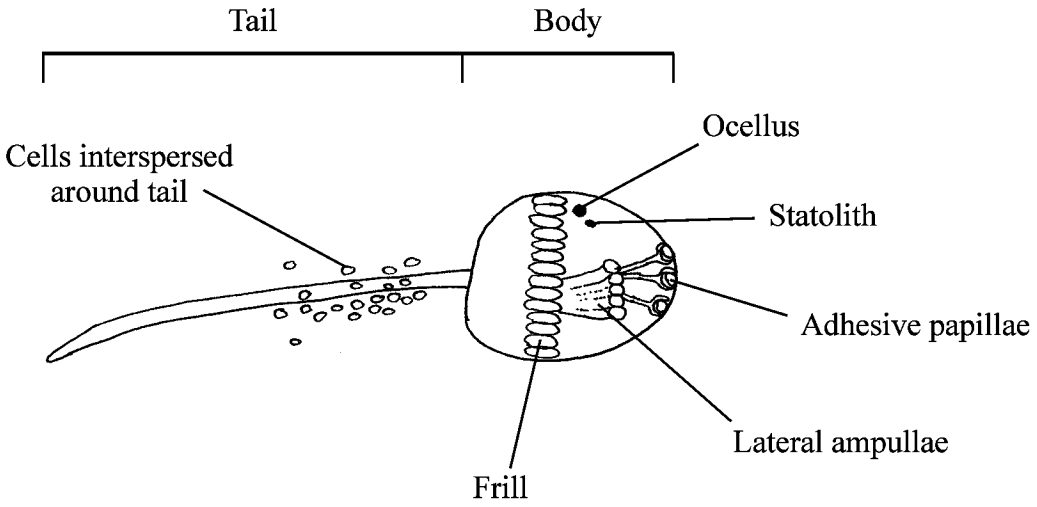
Table 4. Mean abundance of settled juveniles (#/100cm^2). Recruitment data collected between June 27, 2001 and Dec 10, 2003 from Avery Point docks, Groton, Connecticut.

Species	Month											
Ascidians	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
**Diplosoma*	0.0	0.0	0.0	0.0	0.2	5.3	23.6	118.8	151.4	25.2	4.5	0.3
**Didemnum*	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.6	0.7	0.6	0.1	0.0
**Aplidium*	--	--	--	--	--	--	--	--	--	--	--	--
Botrylloides	0.0	0.0	0.0	0.0	0.1	5.7	35.0	22.3	9.2	7.9	3.8	0.2
Botryllus	0.0	0.0	0.0	0.1	13.8	63.9	151.4	123.4	22.0	12.5	2.3	0.1
Styela	0.0	0.0	0.0	0.0	0.3	0.0	0.1	0.1	0.3	0.1	0.0	0.0
Molgula	0.0	0.0	0.0	0.0	0.0	3.0	32.4	162.9	55.3	1.7	0.1	0.0
Ascidiella	0.0	0.0	0.0	0.1	0.4	5.9	9.4	24.2	17.0	4.4	0.4	0.2
Ciona	0.0	0.0	0.0	0.2	0.8	0.6	1.3	1.2	1.7	0.4	0.0	0.0
Bryozoans												
***Bugula spp.*	0.0	0.0	0.0	0.0	0.0	8.5	116.7	215.7	57.9	12.1	0.5	0.0
Bugula neritina	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	1.3	0.2	0.0	0.0
Electra crust.	0.0	0.0	0.0	0.0	0.0	0.0	1.6	4.9	1.7	0.5	0.0	0.0
Electra pilosa	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.3	0.1	0.0	0.1	0.0
Membranipora	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
Schizoporella	0.0	0.0	0.0	0.0	0.0	0.3	11.2	4.2	0.2	0.1	0.0	0.0
Cryptosula	0.1	0.0	0.2	1.2	17.1	36.3	52.9	51.3	81.7	51.1	13.5	2.5
Bowerbankia	0.0	0.0	0.0	0.0	0.0	0.0	2.6	2.2	1.8	0.3	0.0	0.0

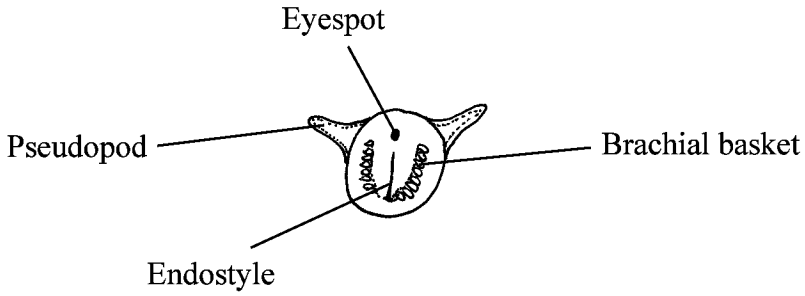
* *Diplosoma* and *Didemnum* were not present at this site all three years; *Diplosoma* was absent in 2003 due to a cold winter; *Didemnum* only began to recruit at this site in 2002. As a result, the mean abundance of juveniles for these species is relatively low. Additionally, *Aplidium* began to recruit to this site in very small numbers in 2003.
** Combination of *Bugula simplex* and *B. Turrita.*

Figure 1. Generalized diagrammatic views of ascidian and bryozoan larval and juvenile morphological characteristics.

A: Ascidian larva.



B: Ascidian juvenile.



C: Bryozoan zooecium shape.

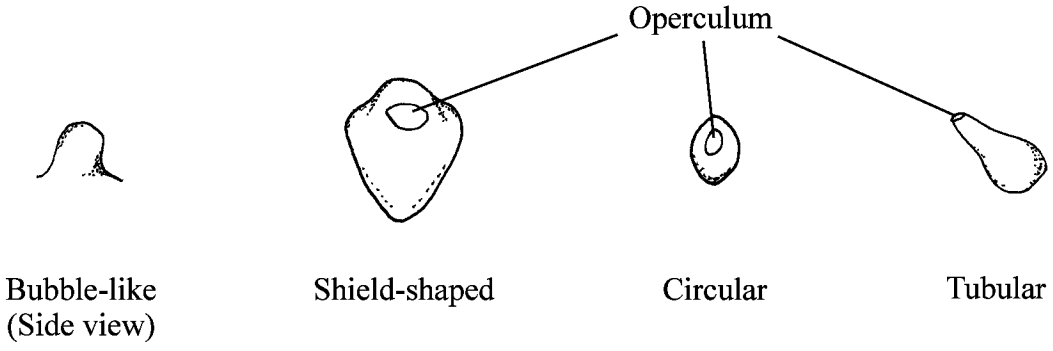


Figure 2. Colonial ascidians. Not to scale.

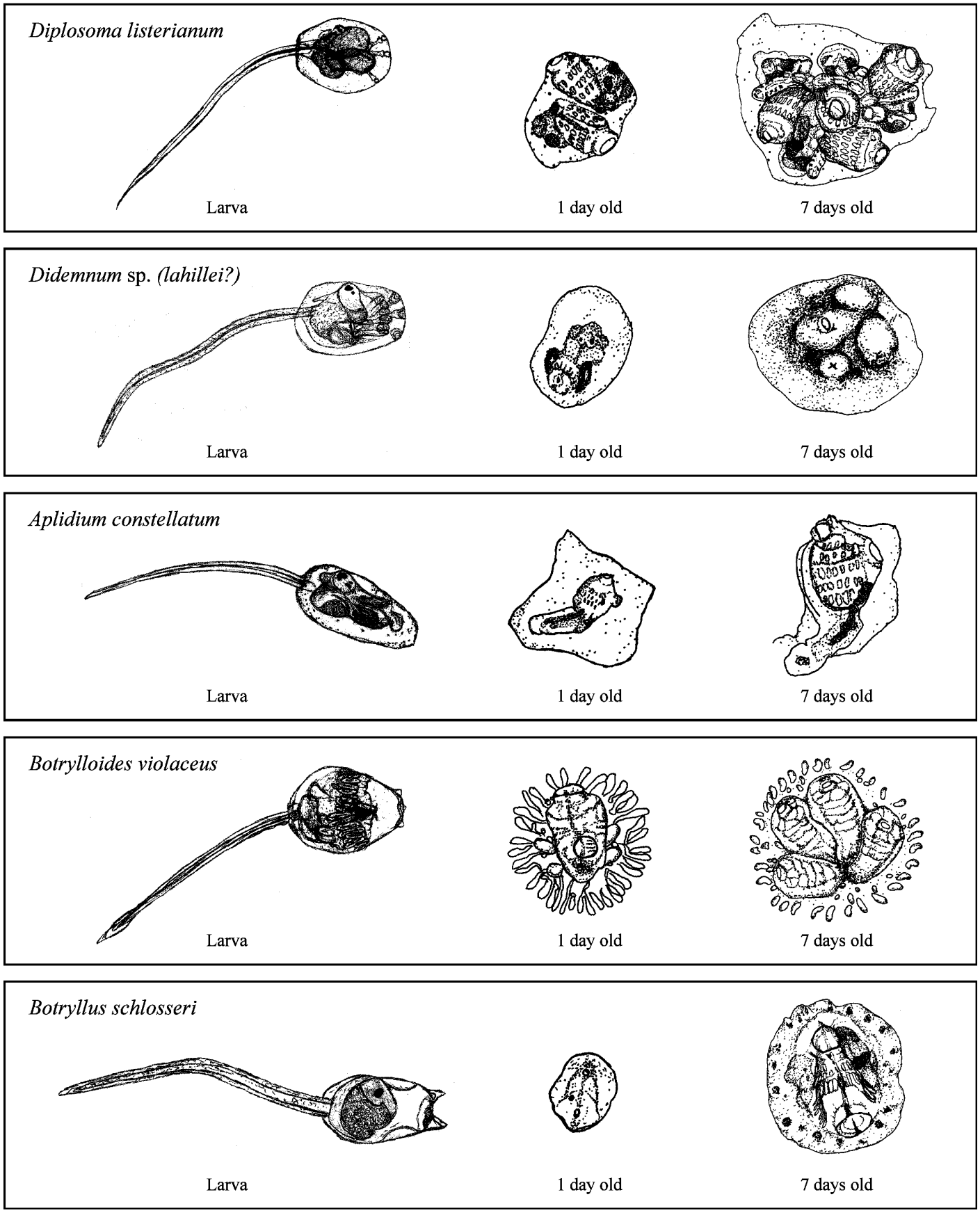


Figure 3. Solitary ascidians. Not to scale.

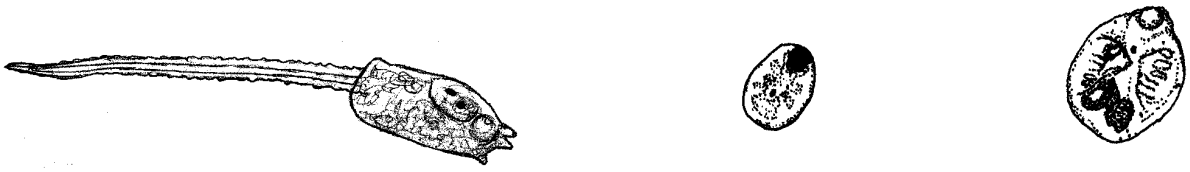
Styela clava

Larva 1 day old 7 days old

Molgula manhattensis

Larva 1 day old 7 days old

Ascidiella aspersa



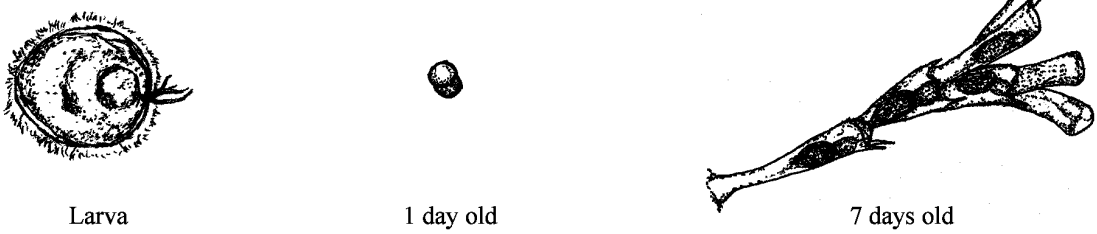
Larva 1 day old 7 days old

Ciona intestinalis

Larva 1 day old 7 days old

Figure 4. Bryozoans. Not to scale. *Redrawn from Ryland (1965).

Bugula simplex



Larva | 1 day old | 7 days old

Bugula turrita

Larva | 1 day old | 7 days old

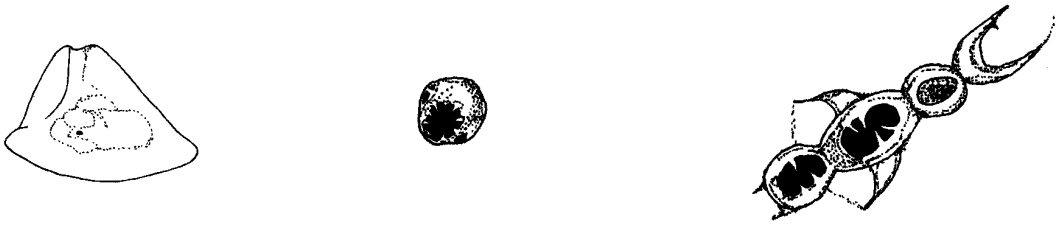
Bugula neritina

Larva | 1 day old | 7 days old

Electra crustulenta

Larva* | 1 day old | 7 days old

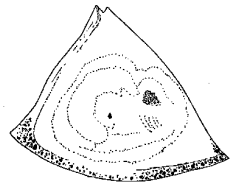
Electra pilosa



Larva* | 1 day old | 7 days old

Figure 4 continued. Bryozoans. Not to scale. *Redrawn from Ryland (1965). **Drawn from photograph in Reed & Cloney (1982).

Membranipora membranacea



Larva*

1 day old

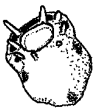
NA

7 days old

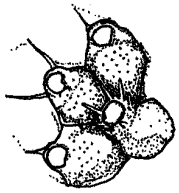
Schizoporella errata

NA

Larva



1 day old

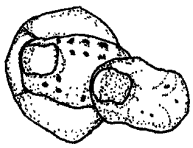


7 days old

Cryptosula pallasiana

Larva

1 day old



7 days old

Bowerbankia gracilis

Larva**

1 day old

7 days old

Figure 5. Larvae to scale.

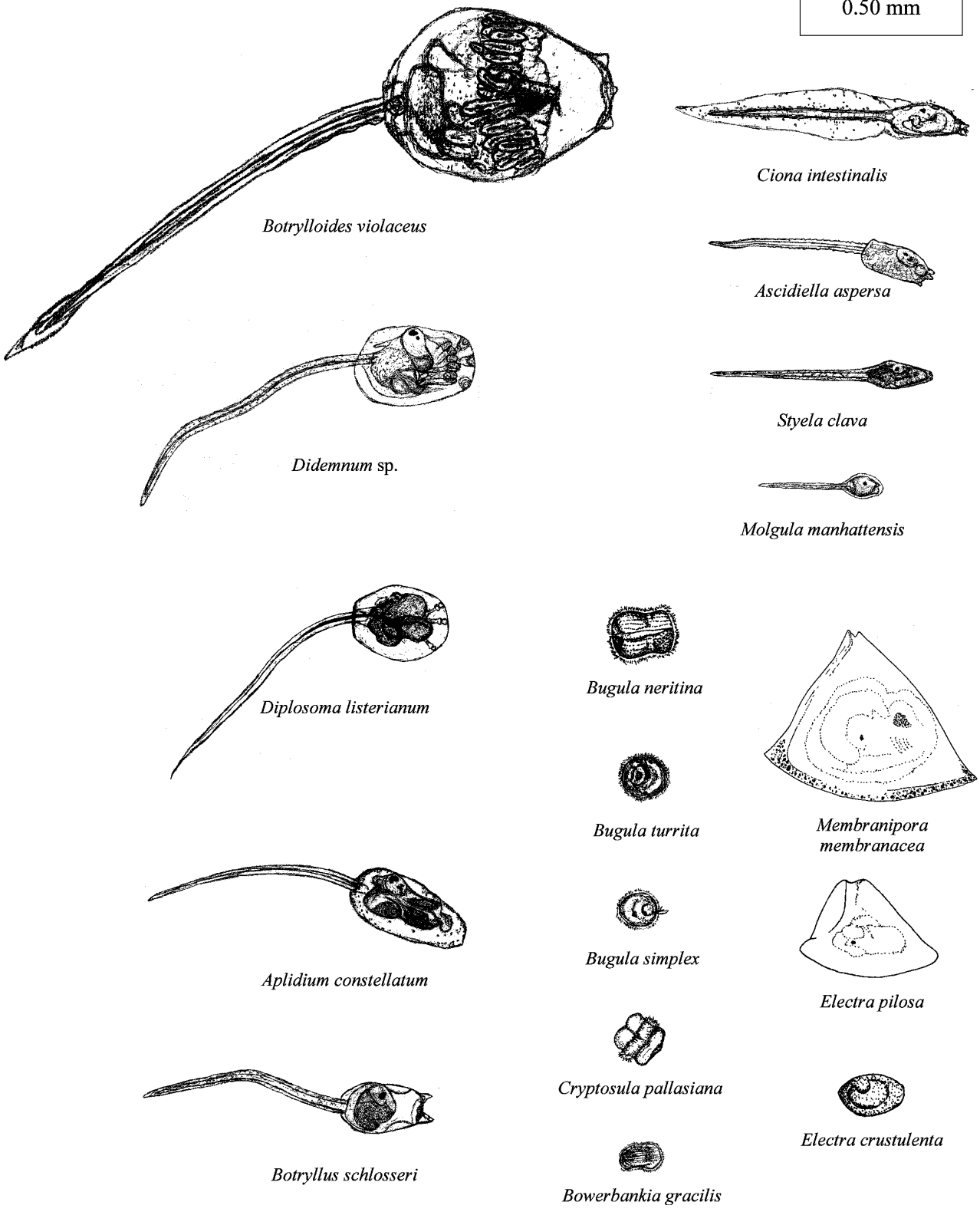
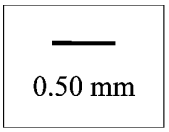
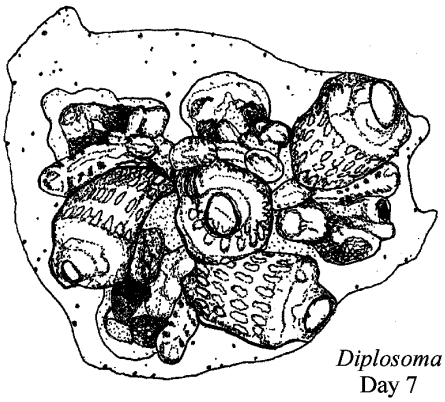
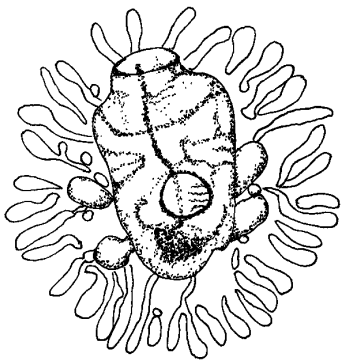


Figure 6. Juveniles to scale



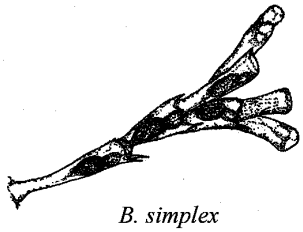


Diplosoma
Day 7

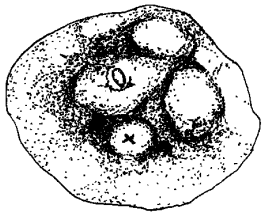


Botrylloides
Day 1

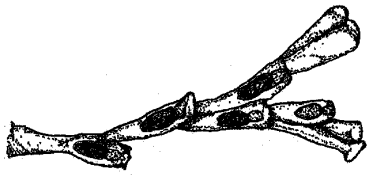
Botryllus
Day 7



B. simplex
Day 7

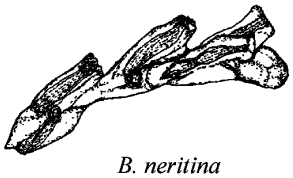


Didemnum
Day 7

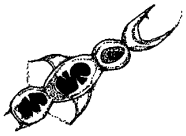


B. turrita
Day 7

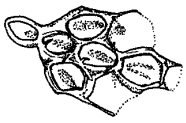
Aplidium
Day 7



B. neritina
Day 7

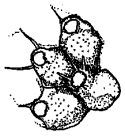


E. pilosa
Day 7



E. crustulenta
Day 7

Cryptosula
Day 7



Schizoporella
Day 7

Bowerbankia
Day 7

Molgula
Day 7

Ascidiella
Day 7

Ciona
Day 7

Styela
Day 7

Diplosoma
Day 1

Aplidium
Day 1

Didemnum
Day 1

Botryllus
Day 1

Molgula
Day 1

Ascidiella
Day 1

Styela
Day 1

Ciona
Day 1

Bowerbankia
Day 1

Cryptosula
Day 1

Schizoporella
Day 1

B. neritina
Day 1

B. turrita
&
B. simplex
Day 1

C. crustulenta
Day 1

C. Pilosa
Day 1

Membranipora
Day 1

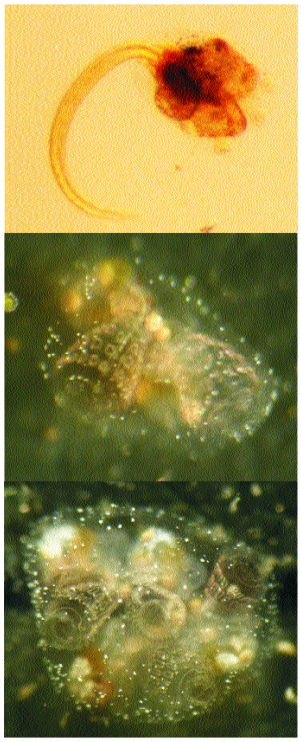
COLOR PLATES

Color Plate 1: Ascidians. Larva left, one day old juvenile middle, week old juvenile right. Alternatively, larva top, one day old juvenile middle, week old juvenile bottom.

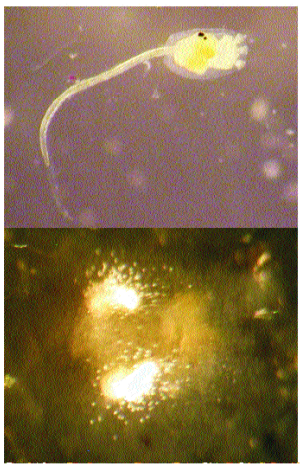
Color Plate 2: Bryozoans. Larva left, one day old juvenile middle, week old juvenile right. Alternatively, larva top, one day old juvenile middle, week old juvenile bottom. Photographs of larvae were not available for *Electra crustulenta, Electra pilosa*, *Membranipora membranacea*, *Schizoporella errata*, and *Bowerbankia gracilis*. Photographs of week old juveniles were not available for *Membranipora membranacea.*

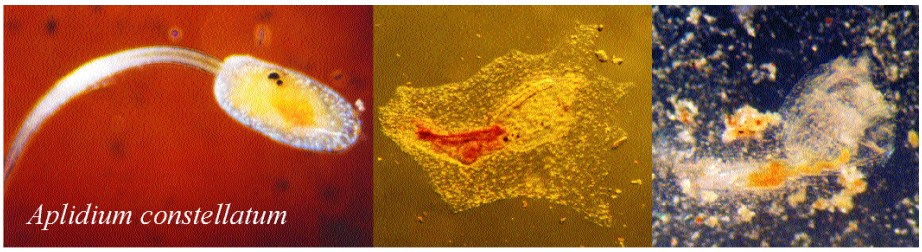
Color Plate 1: Ascidians

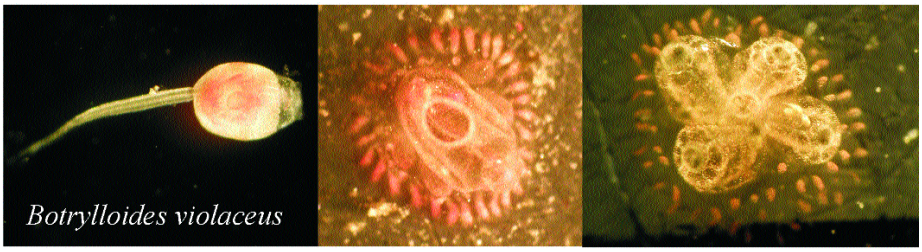
Diplosoma listerianum

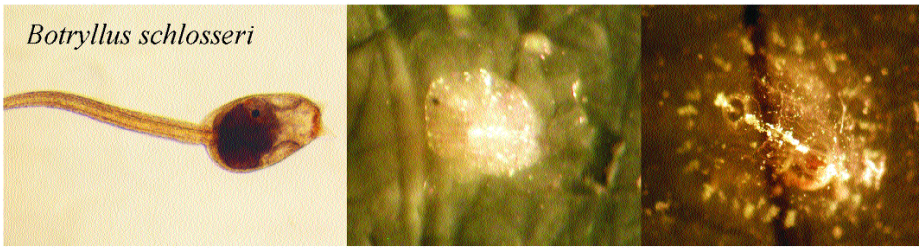


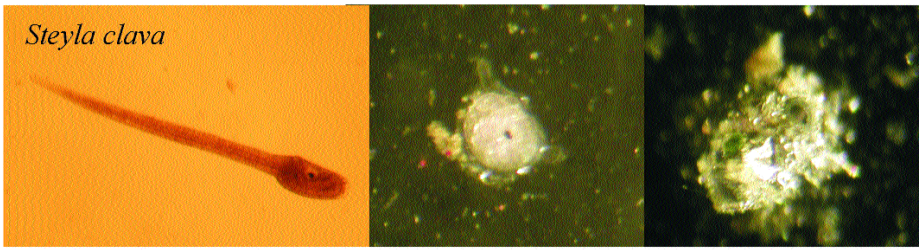
Didemnum sp.

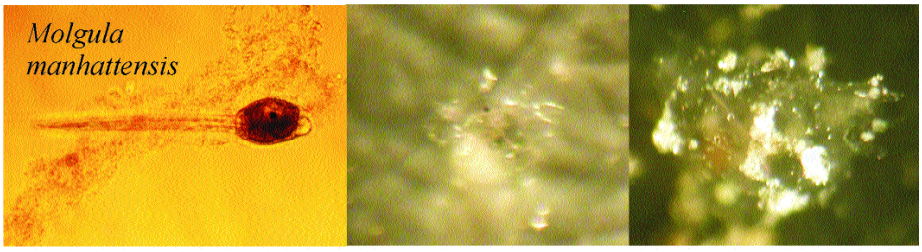


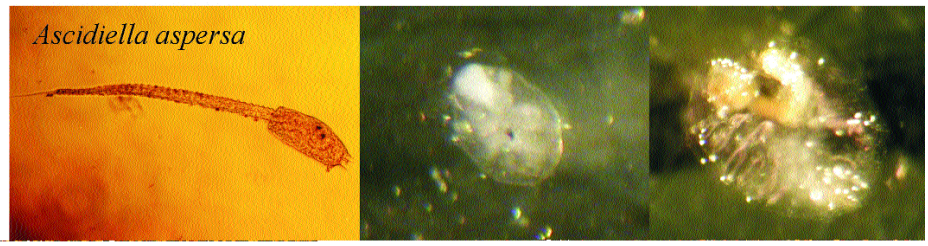




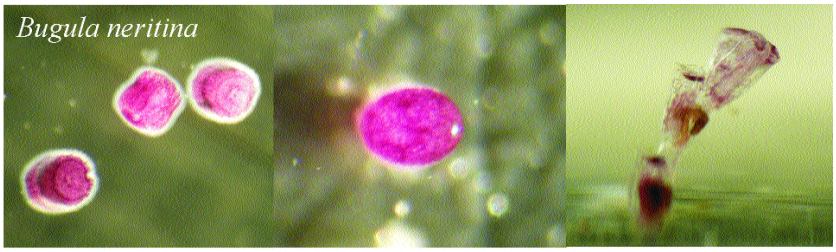




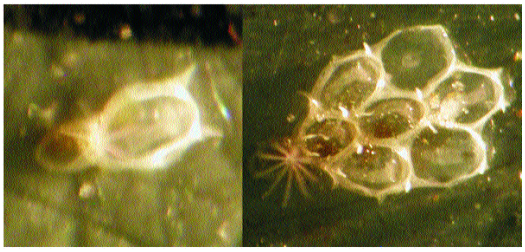




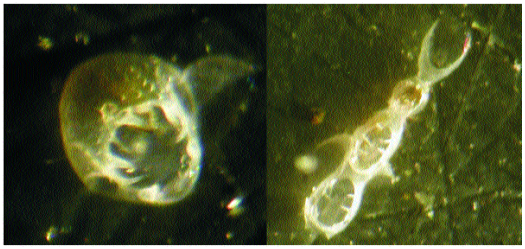
Color Plate 2: Bryozoans



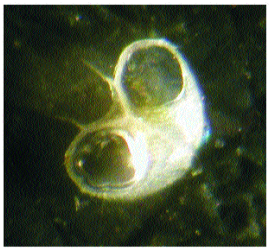
Electra crustulenta



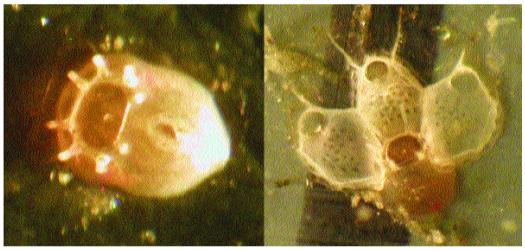
Electra pilosa

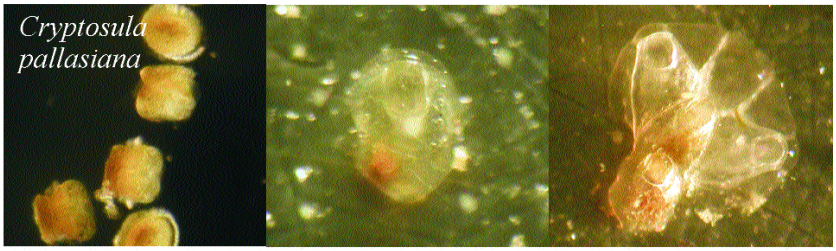


Membranipora membranacea



Schizoporella errata





Bowerbankia gracilis