

# **Biological Synopsis of the European Sea Squirt (*Ascidella aspersa*)**

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BIOLOGICAL SYNOPSIS OF THE  
EUROPEAN SEA SQUIRT (*ASCIDIELLA ASPERSA*)

by

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## ABSTRACT

Mackenzie, A.B. 2011. Biological synopsis of the European sea squirt (*Ascidella aspersa*). Can. Manuscr. Rep. Fish. Aquat. Sci. 2968: iv + 15 p.

*Ascidella aspersa*, commonly known as the European sea squirt, is native to the Mediterranean Sea and Europe. The species has invaded far-off regions including Australia and the United States, and has been flagged as a possible invader to Atlantic Canadian waters. The main vector for its introduction is thought to be through ballast water or hull fouling. This solitary ascidian, which propagates through asexual and sexual reproduction, is a successful invader because of its rapid growth rate and tolerance of a wide variety of environmental conditions. Following its introduction to new habitats, this ascidian rapidly dominates the biological community, resulting in reduced biodiversity. The main control method is treating nets with copper, which has many negative effects on non-target species and the environment. This biological synopsis was prepared as a comprehensive collection of information.

## RÉSUMÉ

Mackenzie, A.B. 2011. Biological synopsis of the European sea squirt (*Ascidella aspersa*). Can. Manuscr. Rep. Fish. Aquat. Sci. 2968: iv + 15 p.

*Ascidella aspersa*, communément connue sous l'appellation d'ascidie sale, est indigène à la mer Méditerranée et l'Europe. Cette espèce a envahi des territoires en dehors de sa distribution normale tels l'Australie et les États-Unis, et est maintenant considérée comme envahisseur potentiel des eaux canadiennes de l'Atlantique. Le principal vecteur d'introduction semble être les eaux de ballast ou l'encrassement biologique des coques de navire. Ce tunicier solitaire, qui se propage autant par la reproduction sexuée qu'asexuée, constitue un envahisseur efficace en raison de son taux de croissance rapide et sa capacité à tolérer une grande variété de conditions environnementales. Suivant son introduction dans de nouveaux habitats, ce tunicier domine rapidement la communauté envahie et provoque une perte de la biodiversité. *A. aspersa* est la principale espèce nuisible encrassant les sites d'aquaculture en eaux tempérées, et pourrait potentiellement mener à l'augmentation des coûts opérationnels et à la réduction des rendements de par sa capacité à couvrir les mollusques cultivés. La principale méthode de contrôle est le traitement des filets au cuivre, une méthode engendrant de nombreux effets néfastes sur les espèces non-ciblées ainsi que sur l'environnement. Cette synthèse de la biologie a été conçue afin de servir de source d'informations détaillées pour aider les efforts d'atténuation ainsi que les prises de décision concernant la gestion de cette espèce.

# 1 INTRODUCTION

Aquatic invasive species (AIS) pose a great risk to native biodiversity. The European sea squirt, *Ascidella aspersa* (Müller, 1776), is a solitary ascidian with attributes that make it an ideal candidate for invasion and establishment in non-indigenous habitats (Altman and Whitlatch 2007). *A. aspersa* has become a biofouling pest in Tasmania (Hodson et al. 2000; Braithwaite et al. 2007) and the United States (Phillippi et al. 2001). The European sea squirt has been put on a watch list for possible invasions into Atlantic Canadian waters (Locke 2009).

The purpose of this biological synopsis is to summarize information about the native habitat of *A. aspersa* and impacts of its introduction on the environment. A discussion of its life history characteristics and potential impacts is included.

## 1.1 NAME AND CLASSIFICATION

Kingdom: Animalia  
Phylum: Chordata  
Subphylum: Tunicata  
Class: Ascidiacea  
Order: Enterogona  
Suborder: Phlebobranchia  
Family: Ascidiidae  
Genus: *Ascidella*  
Species: *Ascidella aspersa* Müller, 1776 (ITIS 1996)

Common Name: European sea squirt

Synonyms: It should be noted that past publications have reported *Ascidella scabra* as *A. aspersa*.

## 1.2 DESCRIPTION

*Ascidella aspersa* is a tall solitary tunicate that is often found in dense unfused aggregations (Curtis 2005; Picton and Morrow 2010). The body is oval with two prominent siphons: a terminal fluted oral siphon, and an upwardly-directed fluted exhalent siphon, located 1/3 of the way down the side (Curtis 2005; Picton and Morrow 2010; Salem Sound Coastwatch 2011) (Figure 1).

The test (tunic) is firm with rough papillae and is grey-black to brown in colour with red pigment near the siphons (Berrill 1928; Curtis 2005; Salem Sound Coastwatch 2011). It is often covered with detritus and filamentous algae (Curtis 2005; Picton and Morrow 2010).

The European sea squirt will attach to any hard substrate such as stones, shells, and piers (Millar 1966). Individuals attach to the substratum with a short basal structure at the posterior end and are typically 5–10 cm in length, although they can reach 12–13

cm (Berrill 1928; Picton and Morrow 2010). Once attached, they feed on suspended particles in the water column.

*Asciidiella aspersa* may be mistaken for many other species. When covered in debris, it resembles the clubbed tunicate *Styela clava*, but this species has two siphons in close proximity, where those of *A. aspersa* are separated (Salem Sound Coastwatch 2011). This feature also distinguishes *A. aspersa* from *Molgula* spp. (Salem Sound Coastwatch 2011). It may also be misidentified as *Ciona intestinalis*, but *C. intestinalis* has yellow marks around its siphons, and a more slender, softer body (Salem Sound Coastwatch 2011). The European sea squirt may be mistaken for species of the genus *Ascidia*, but these have firmer tests, or tunics (Curtis 2005).

Sea squirt bodies are made of tunicin, a substance similar to cellulose (Millar 1970). *A. aspersa* is a solitary tunicate with two siphons, an inhalant or oral siphon, and an exhalant or atrial siphon. Large oral tentacles may be seen when the oral siphon is expanded (Picton and Morrow 2010). Water is pumped through the oral siphon into the branchial sac, where oxygen is absorbed and food particles are trapped and transported to the gut by cilia (Moen and Svensen 2004).

*A. aspersa* is a simultaneous hermaphrodite, meaning they contain both an ovary and testis; however, they are also protandric, meaning the testis develops first (Millar 1952). Diploid specimens have 18 chromosomes which are of uniform diameter (Colombera 1971).

Ascidians have a unique heart which reverses the direction of pumping blood in regular intervals, spanning minutes or hours (Barrington 1965). The nervous system of ascidians is poorly understood; however, a single nerve ganglion has been observed which keeps a close relationship with the neural gland that opens up to the pharynx (Barrington 1965). The function of the pharynx is known as taking in nutrients from the sea water (Barrington 1965). Two pairs of nerves arise from this ganglion: the anterior set supplying the oral siphon, and the posterior set supplying the atrial siphon (Barrington 1965). A third nerve (unpaired) goes from the ganglion to the organs (Barrington 1965).

## **2 DISTRIBUTION**

### **2.1 NATIVE DISTRIBUTION**

*Asciidiella aspersa* (Müller, 1776) was originally described from the Christiania fjord (Berrill 1928). It is native to the Mediterranean Sea and occurs throughout Europe (Cohen et al. 2000), including the west and south coast of Norway, the west coast of Sweden, the east coast of the British Isles, and the Shetland Islands (Millar 1966).

### **2.2 NON-NATIVE DISTRIBUTION (EXCLUDING CANADA)**

The European sea squirt was first recorded off New England in the 1980s and has spread over the Gulf of Maine from Massachusetts to Connecticut (Salem Sound Coastwatch 2011).

Specimens have been found in the English Channel (Cohen et al. 2000), the Irish Sea (Cohen et al. 2000), France (Hily 1991), Ayrshire (Millar 1952), Scotland (Pirie and Bell 1984), Britain (Curtis 2005), and Australia (Cohen et al. 2000). Within Australia, populations are often found in sheltered estuaries from Western Australia to Victoria and Tasmania (Cohen et al. 2000).

### **2.3 DISTRIBUTION IN CANADA**

This species has not yet been reported in Canadian waters, though Locke (2009) has included it in a list of 17 possible invaders of Atlantic Canada by examining shipping routes and climate zones.

### **2.4 POTENTIAL VECTORS FOR INTRODUCTION**

It is not possible for *Ascidella aspersa* to have reached its wide distribution naturally since mobile capability only exists in the short-lived larval stage. *A. aspersa* was most likely introduced to the United States through hull fouling and/or ballast water (Salem Sound Coastwatch 2011). Millar (1982) suggests that this species may have been introduced to New Zealand during fisheries experiments.

## **3 BIOLOGY AND NATURAL HISTORY**

### **3.1 FEEDING AND RESPIRATION**

European sea squirt larvae are unable to feed during the free-swimming stage since the intestinal system does not develop until after metamorphosis (Niermann-Kerkenberg and Hofmann 1989). However, adult forms of *Ascidella aspersa* are sessile filter feeders. Water is pumped into the inhalant siphon, food particles are filtered out and passed down to the oesophagus (Provincetown Center for Coastal Studies 2011). The water is then moved by cilia to the gill clefts where respiration takes place, and finally exits via the exhalant siphon (Provincetown Center for Coastal Studies 2011).

Pascoe et al. (2007) examined this species' feeding behaviour, and found that ingestion rate increased linearly with food concentration. The authors also found that feeding rates did not change when inorganic silt was added to the food source (*Isochrysis galbana*). Measuring pumping rates at the cloacal siphon using conical hot-film probes showed velocities at Reynold's number 300, meaning the siphon has laminar flow (Charriaud 1982).

It is estimated that ascidians filter 10–20 litres of water for every millilitre of oxygen consumed, and that each millilitre of oxygen can combust 0.8 mg of food (Barrington 1965). This means that an individual requires 0.05 mg of organic material per litre of seawater filtered to maintain its respiration rate (Barrington 1965). When taking into account other requirements, such as growth and reproductive investment, it is estimated that an individual requires 0.15 mg of food per litre of water (Barrington 1965). This is not an issue for many temperate waters. For example, the English Channel averages 1.6–1.8 mg per litre of particulate matter (Barrington 1965). Ascidians secrete ammonia

as a waste (Barrington 1965). Waste is discharged through the anus (Moen and Svensen 2004).

### 3.2 REPRODUCTION AND DEVELOPMENT

The European sea squirt employs sexual reproduction to propagate the species. Niermann-Kerkenberg and Hofmann (1989) studied the development of *Ascidiella aspersa* in depth as a model for chordate development and found that development from fertilization to metamorphosis takes about one day (24 hours) at 20°C.

Mature sperm are divided into a head and tail, with the head containing the nucleus surrounded by one large mitochondrion (Niermann-Kerkenberg and Hofmann 1989). *A. aspersa* eggs are unique among ascidians because they float in seawater with salinity between 30 and 35‰ (Berrill 1928). More information is needed regarding egg production. The eggs and sperm are released into the water where fertilization occurs (Niermann-Kerkenberg and Hofmann 1989). *A. aspersa* shows sperm chemotaxis, the directional orientation and swimming of sperm toward compounds originating from eggs (Bolton and Havenhand 1996). This adaptation may increase the probability of gamete contact even when concentrations are low, as well as conserve sperm swimming energy when eggs are not present (Bolton and Havenhand 1996). This behaviour is widespread in solitary ascidians, and also occurs between gametes of different ascidian species (Bolton and Havenhand 1996). Bolton and Havenhand (1996) examined the cross-reactivity of this response and found that *A. aspersa* sperm show a higher degree of specificity than *C. intestinalis*, as sperm of *A. aspersa* did not respond to *C. intestinalis* egg cues, while sperm of *C. intestinalis* did respond to the presence of *A. ascidia* eggs in the water. The response is also associated with declines in sperm activity and longevity. After 1.5 hours of induced activity, *C. intestinalis* sperm were no longer capable of fertilizing eggs; however, *A. aspersa* sperm tolerated twice the time of induced activity before longevity ceased (Bolton and Havenhand 1996).

The presence of an acrosome in ascidian sperm has been debated, but it is now known to exist; however, its function in fertilization is still unclear (Fukumoto and Zarnescu 2003). Due to its small size, it may participate in the fusion of the plasma membranes upon gamete contact (Fukumoto and Zarnescu 2003). Multiple sperm bind to the chorion of the egg upon contact, and if a sperm cell passes through, its mitochondrion shifts to the tail (Niermann-Kerkenberg and Hofmann 1989).

Fertilization causes the egg to change shape, with the first meiotic division occurring 15 minutes after insemination (Niermann-Kerkenberg and Hofmann 1989). After multiple cleavages, the spherical embryo reaches the blastula stage at 64 cells, and becomes a gastrula after an invagination is formed (Niermann-Kerkenberg and Hofmann 1989). This stage is followed by neurulation, where the embryo lengthens and a section flattens to form the neural plate (Niermann-Kerkenberg and Hofmann 1989). A groove forms along the midline of this plate, which then folds and fuses together to form the neural tube (Niermann-Kerkenberg and Hofmann 1989). Next, the embryo develops to the tail-bud stage, where three adhesive papillae develop along with a pre-gut and sensory vesicle along the neural tube (Niermann-Kerkenberg and Hofmann 1989). The

sensory vesicle contains two pigment spots: the anterior otolith forming the larval static sense organ, and the posterior ocellus forming the larval eye (Niermann-Kerkenberg and Hofmann 1989).

Ascidian embryos hatch by either enzymatic digestion or rupturing of the egg membrane (Berrill 1930). The former is the more primitive method, and use of a hatching enzyme was first seen in *Asciella aspersa* (Figure 2). Hatching of the developed larva is also aided by mechanical twitching (Lübbering and Hofmann 1995; Niermann-Kerkenberg and Hofmann 1989). Following hatching, the tail straightens out, allowing the larva to swim freely for an average of 5 hours (Niermann-Kerkenberg and Hofmann 1989). Young tunicates originally show positive phototaxis and negative geotaxis, but this later reverses (Niermann-Kerkenberg and Hofmann 1989). When the larva finds a suitable substrate it attaches using its adhesive papillae. The adhesive papillae also function as temporary respiration organs until the heart is fully developed (Berrill 1930).

Cloney (1982) divided the larval structures into three groups: transitory larval organs (TLO), prospective juvenile organs (PJO), and larval-juvenile organs (LJO). The TLO group includes organs which are phagocytized at metamorphosis, while all other organs are kept for juvenile development. The TLO group includes structures needed for larval locomotion, sensory input, and settlement (Cloney 1982). The PJO group includes organs such as branchial siphons and ampullae that do not develop until metamorphosis, and the LJO group develops cells for blood and muscles (Cloney 1982). Ascidian blood cells have many different functions, such as excretion, tunic synthesis, immune response, metal accumulation, and asexual reproduction (Pirie and Bell 1984). Pirie and Bell (1984) found that blood cells may contain vanadium and sulphuric acid, or just sulphuric acid alone. The function of vanadium concentrations in ascidians is unknown, though the function of respiration has been explored (Barrington 1965).

Metamorphosis consists of the following steps: phagocytosis of the tail and larval nervous system, growth of ampullae, and rotation of the mouth and atrial sacs to the distal part of body (Berrill 1930). Three days after the onset of metamorphosis, the young adult will have one oral siphon, two atrial siphons, a branchial basket with 3 or 4 branchial clefts, an endostyle, a stomach, and a heart (which changes the direction of blood flow occasionally) (Niermann-Kerkenberg and Hofmann 1989). As an adult, the two atrial siphons are fused into one, and branchial clefts are absent (Niermann-Kerkenberg and Hofmann 1989).

The European sea squirt has the ability to delay metamorphosis when conditions are unfavourable, but prolonged swimming activity depletes energy reserves for post-metamorphic growth (Berrill 1928). Berrill (1928) found that when metamorphosis is delayed, the developing adult organ systems in the larval body may increase the survival success of energy-depleted larvae by allowing for more rapid metamorphosis when conditions are favourable. Delayed *A. aspersa* developed a branchial siphon, branchial basket, endostyle, and gut (Berrill 1928).

### **3.3 LIFE CYCLE: GROWTH, GENERATION TIME, AND LONGEVITY**

*Ascidrella aspersa* is an annual species, growing from larva to sexually reproductive adult and deteriorating to senescence within 18 months (Millar 1952). Larvae settle in the summer (July) and grow until the end of September when growth is halted by colder winter temperatures (Millar 1952). Sexual reproduction takes place the summer after establishment, followed by death in the winter or shortly after (January to April) (Millar 1952).

The European sea squirt is hermaphroditic and slightly protandrous since the sperm develop before the oocyte (Millar 1952). Sexual maturity in this species is dependent on size; sperm development occurs when animals are about 25 mm long, while eggs develop in the oviduct when animals are about 30 mm long (Millar 1952).

Growth of this simple ascidian is best measured by increasing body length (Millar 1952), with tunicates reaching a maximum length of 13 cm (Berrill 1928).

### **3.4 HABITAT AND ENVIRONMENTAL TOLERANCES**

The European sea squirt can be found in the lower intertidal and in the sublittoral to 80 m (Curtis 2005). It is often abundant in eutrophic habitats with high densities of plankton and organic matter (Mastrototaro et al. 2008). This ascidian prefers calm water with a steady current and is often found in estuaries (Curtis 2005; Salem Sound Coastwatch 2011). Mastrototaro et al. (2008) reported that they were almost exclusively found on natural bottoms, and Cohen et al. (2000) noticed a preference for deep muddy sediments.

Many ascidians can tolerate heavy environmental stressors (Mastrototaro et al. 2008). *Ascidrella aspersa* takes advantage of algal biomass blooms during the spring by using them as larval settling grounds (Mastrototaro et al. 2008). *A. aspersa* also tolerates low salinities (18‰), making estuaries suitable habitat (Curtis 2005).

The hatching enzyme used by the developed larvae has an optimum temperature tolerance of 3–7°C and is active between pH 7 to 10 (Berrill 1930). Hatching itself is inhibited below pH 6–8, but can continue if transferred to water of pH 8 (Berrill 1930). Once hatched, free-swimming larvae can be induced to swim longer when pH is 9 or higher (Berrill 1930). Berrill (1930) also found that metamorphosis could be induced if larvae are moved from high to low pH.

### **3.5 ECOLOGY**

*Ascidrella aspersa* grows on hard substrates which are abundant at aquaculture sites. Competition for space in benthic communities is high, with the advantage going to fast-growing organisms. The artificial substrates provided by fish farms are prime real estate for settlement and growth for the compound sea squirt, with minimal competition or predation risk (Carman et al. 2010).

*A. aspersa* also stands out on natural substrates due to its high growth and reproductive rates, making it dominant in species assemblages of invaded areas. These characteristics have the potential to significantly affect species composition, often reducing overall biodiversity.

The success of a species invasion in a new habitat often depends on the species composition of the resident community, as changes to the environment may aid successional steps. Schmidt (1983) found this to be the case when the hydroid *Tubularia larynx* facilitated the introduction of *Ascidella aspersa*. This hydroid may have reduced current and attenuated light, making the substratum better suited to the European sea squirt, which continued its growth and monopolized the substratum (Schmidt 1983).

### **3.6 DISEASES AND PARASITES**

There is little known about tunicate diseases, but there are reports of parasites within solitary ascidians such as the copepod *Leucothoe ascidicola*, found within the solitary ascidian, *Microcosmos exasperatus* (Thiel 2000). Also, fouling communities may facilitate the introduction of diseases and parasites, such as netpen liver disease, amoebic gill disease, the nematode *Hysterothylacium aduncum*, and the sea louse *Lepeophtheirus salmonis* (Braithwaite et al. 2007).

## **4 HUMAN USES**

Niermann-Kerkenberg and Hofmann (1989) express the usefulness of *Ascidella aspersa* as a rapidly developing model organism for the study of chordate development. Davidson and Swalla (2002) agree with this statement, stating that solitary ascidians are useful for examining developmental mechanisms because of their simplicity and phylogenetic position. Also, most ascidians have been found to be cytotoxic, possessing anti-leukemic properties (Teo and Ryland 1994).

## **5 IMPACTS ASSOCIATED WITH INTRODUCTIONS**

### **5.1 IMPACTS ON THE ENVIRONMENT**

The presence of the European sea squirt may affect the environment by creating anoxic sediments through the deposition of faeces and ammonia; however, the major threat to environmental health in invaded regions comes from the indirect consequences of biofouling. On fish farms, biofouling by the European sea squirt has substantial economic impacts. The aquaculture industry uses copper-treated nets to deter ascidian settlement and growth; however, this practice has broad ecological impacts. While copper is an essential nutrient for plant and animal growth, it is toxic in high concentrations (Hall and Anderson 1999). There are many sources of copper to the aquatic environment, including mineral deposits from soil and dead organic material (plants and animals), as well as atmospheric deposition and anthropogenic inputs (Hall and Anderson 1999). Anthropogenic inputs derive from pesticides and manure, but copper is often introduced through industries using antifouling paint on aquaculture gear

and ship hulls (Hall and Anderson 1999). Copper accumulates in the aquatic ecosystem, with highest concentrations in marinas and harbours, followed by estuaries and finally open seas where concentrations are lowest (Hall and Anderson 1999). Seasonally, copper concentrations increase during the summer months of June to August in marinas and harbours. Matthiessen et al. (1999) took similar readings in Essex and Suffolk, and found the ambient copper levels in coastal waters exceeded safety levels 1992–1996.

## 5.2 IMPACTS ON OTHER SPECIES

*Asciidiella aspersa* is a strong competitor capable of shifting species composition and reducing biodiversity; however, it is the biofouling potential of this species that indirectly poses the greatest threat to other species. Copper used to deter biofouling species from attaching to fish farm nets and structures has effects that are not exclusive to the target species.

Since copper concentrations are highest in smaller bodies of water, Matthiessen et al. (1999) predicted that species which spend all or part of their life cycle in estuarine waters would be most vulnerable to toxic effects, and that species which live in open waters would be at lesser risk. Bellas (2006) noted that most invertebrate embryos and larvae are much more sensitive to toxins than adults. Atlantic cod (*Gadus morhua*) larvae experienced increased mortality rates when exposed to 11.5 µg/litre of copper in combination with 5 µg/litre of tributyltin (TBTO) (Granmo et al. 2002). TBTO was the primary antifoulant used historically, before it was banned on most vessels due to environmental health concerns (Granmo et al. 2002). These chemicals were tested together in order to see the effects of multiple antifoulants that may be present in the waters (Granmo et al. 2002).

Antifouling paint on recreational boats is the major single source of copper pollution in Swedish waters (Andersson and Kautsky 1996). They found that 20 µg/litre seawater affected the germination frequency of *Fucus vesiculosus*. Marine plants are important as nurseries for young fishes and as attachment sites for epiphytes (Andersson and Kautsky 1996). Newly painted boats enter the water in May and June, which is just before and during gamete release of *F. vesiculosus* in the Baltic Sea (Andersson and Kautsky 1996). Concentrations of 10–50 µg/litre were found to inhibit development of *Laminaria saccharina*, while *Fucus spiralis* and *Fucus serratus* were more sensitive and *Ascophyllum nodosum* was more resistant (Andersson and Kautsky 1996).

Douglas-Helders et al. (2003) found that copper-treated cages had higher numbers of *Neoparamoeba pemaquidensis*, the organism responsible for amoebic gill disease. This disease is a major issue among the salmon industry in Australia (Douglas-Helders et al. 2003).

Some aquaculture species, however, appear to be equipped for detoxification. Blue mussels (*Mytilus edulis*) appear to be adept at regulating copper concentrations within their tissues so even high concentrations in the water column do not pose a threat to human health through consumption (Solberg et al. 2002). Atlantic salmon, through

natural detoxification processes, also seems to be able to cope with small amounts of copper leached into the water from the treated pens (Solberg et al. 2002).

### **5.3 IMPACT SUMMARY**

Potential impacts of an *Ascidella aspersa* invasion are widespread. The species grows rapidly and can tolerate a wide variety of conditions, making it a major biofouling pest. This tunicate also has the potential to reduce biodiversity and threaten species composition shifts which could affect commercially important species. More information on its life history and environmental tolerances in marine environments similar to those in Atlantic Canada and timely control methods will improve the ability to control its potential impacts.

## **6 CONSERVATION STATUS**

*Ascidella aspersa* is not listed under the International Union for Conservation of Nature (IUCN) list of threatened species and is therefore considered to be without conservation status (IUCN 2011).

## **7 SUMMARY**

The European sea squirt is a solitary ascidian with an annual life cycle. It grows rapidly and has invaded non-native habitats such as Australia and the United States. This species has been flagged as a possible invader to Atlantic Canadian waters. Copper-treated nets are currently the main method used to eradicate biofouling pests, but non-toxic substitutes are being explored. This biological synopsis was prepared as a collection of information.

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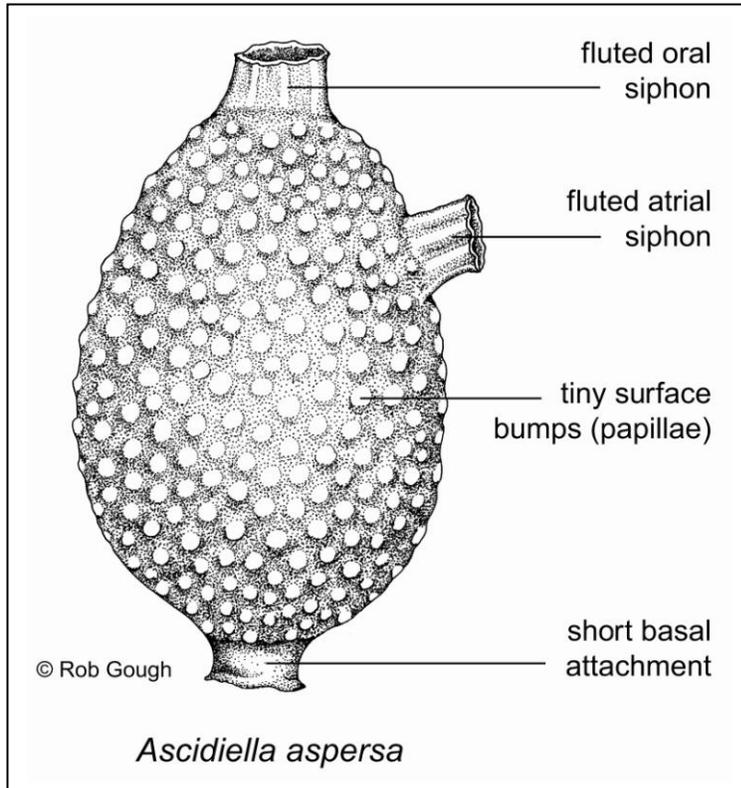
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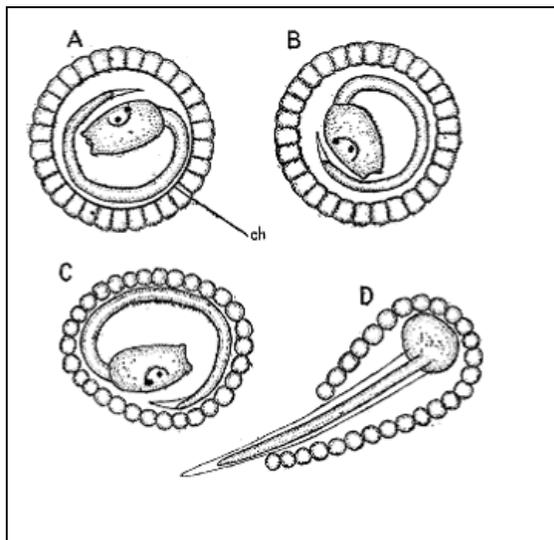
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**Figure 1. *Ascidiella aspersa***  
 (Salem Sound Coastwatch 2011)  
 (Reproduced with permission from Rob Gough and the Salem Sound Coastwatch)



**Figure 2. Hatching of *Ascidiella aspersa* through digestion of the egg membrane**  
 A. Fully formed larvae before hatching; B. Digestion beginning; C. Cells collapsing onto larvae after disappearance of chorion; D. Larvae twitching to get free of cells (Berrill 1930)  
 (Figure reproduced with permission from the Royal Society of London)