Colonial Tunicates

Tunicates are small marine filter feeder animals that have an **inhalant siphon**, which takes in water, and an **exhalant siphon** that expels water once it has trapped food particles. Tunicates get their name from the tough, nonliving **tunic** formed from a cellulose-like material of carbohydrates and proteins that surrounds their bodies. Their other name, **sea squirts**, comes from the fact that many species will shoot water out of their bodies when disturbed.

A colony of tunicates is comprised of many tiny sea squirts called **zooids**. These individuals are arranged in groups called **systems**, which form interconnected colonies. Systems of these filter feeders share a common area for expelling water instead of having individual **excurrent siphons**. Individuals and systems are all encased in a matrix that is often clear and full of blood vessels. All ascidian tunicates have a tadpole-like larva that swims for less than a day before attaching itself to a substrate and metamorphosing. They become stationary filter feeders, trapping food particles in their pharynx. They are hermaphroditic, which allows them to reproduce and spread to new locations at an alarming rate.

The first tunicates that evolved are believed to be solitary such as the invasive club tunicate (*Styela clava*) and the transparent ciona tunicate (*Ciona savignyi*). Tunicates later evolved into complex, interconnected colonial species like the star sea squirt (*Botryllus schlosseri*), chain sea squirt (*Botryloides violaceus*), and *Didemnum* (*Didemnum* spp.), which have become a nuisance in areas of the West Coast.

Gretchen Lambert

Massively lobate colony of Didemnum sp. A growing on a rope in Sausalito, in San Francisco Bay.

INVASIVE SEA SQUIRTS

Star sea squirts (*Botryllus schlosseri*) are so named because the systems arrange themselves in a star. Zooids are shaped like ovals or teardrops and then group together in small circles of about 20 individuals. This species occurs in a wide variety of colors: orange, yellow, red, white, purple, grayish green, or black. The larvae each have eight **papillae**, or fleshy projections that help them attach to a substrate.

Chain sea squirts (*Botryloides violaceus*) have elongated, circular systems. Each system can have dozens of zooids. They occur in orange, yellow, red, purple, and tan (or, rarely, lavender or brown). Like star sea squirts, individual zooids are also ovals or teardrops. The best way to tell the difference between *Botryllus schlosseri* and *Botryloides violaceus* is by looking at the shapes of the systems. Larvae of this species have more papillae than star sea squirts, and an individual can have between 24 and 25 papillae.

Sea vomit (*Didemnum* spp.) are very different from the first two tunicates. A colony's growth can be very bulbous and lobate, or it can grow in a sheet-like manner, folding over upon and fusing with itself. This shape and slimy texture of *Didemnum vexillum* has led to its colloquial name "sea vomit." Lobes can grow up to a meter long and look like rope. Colonies are usually tan in color, but they can also be cream, yellow, orange, or slightly pink. Embedded in the matrix are very small and spiny balls of calcium, which can only be seen with a microscope.

Luis A. Solarzano

Andrew Cohen

Above: A Botryllus schlosseri *(star sea squirt) colony with dark orange zooids, growing on a bay mussel (*Mytilus trossulus/galloprovincialis *complex) in San Francisco Bay.*

Right: Detail of the colony.

NATIVE AND INVASIVE RANGE

Star sea squirts are native to Europe and have successfully invaded areas in Argentina, Japan, Hong Kong, Australia, and New Zealand. They have also established themselves on the East Coast of the United States, stretching from southern Maine to Florida and the Gulf of Mexico.

COOL FACTS

There is a feature of the tunicate circulatory system that is unique in the phylum Chordata: their blood will flow in one direction for a few heartbeats, and the heart will pump it in the other direction for a few beats.

At first glance, they appear to be simple creatures, but they're actually quite complex.. Did you know primitive tunicates in the phylum Chordata, especially their swimming larval stage, have played an important role in the evolution of organisms with a spinal cord and nerve, including humans?

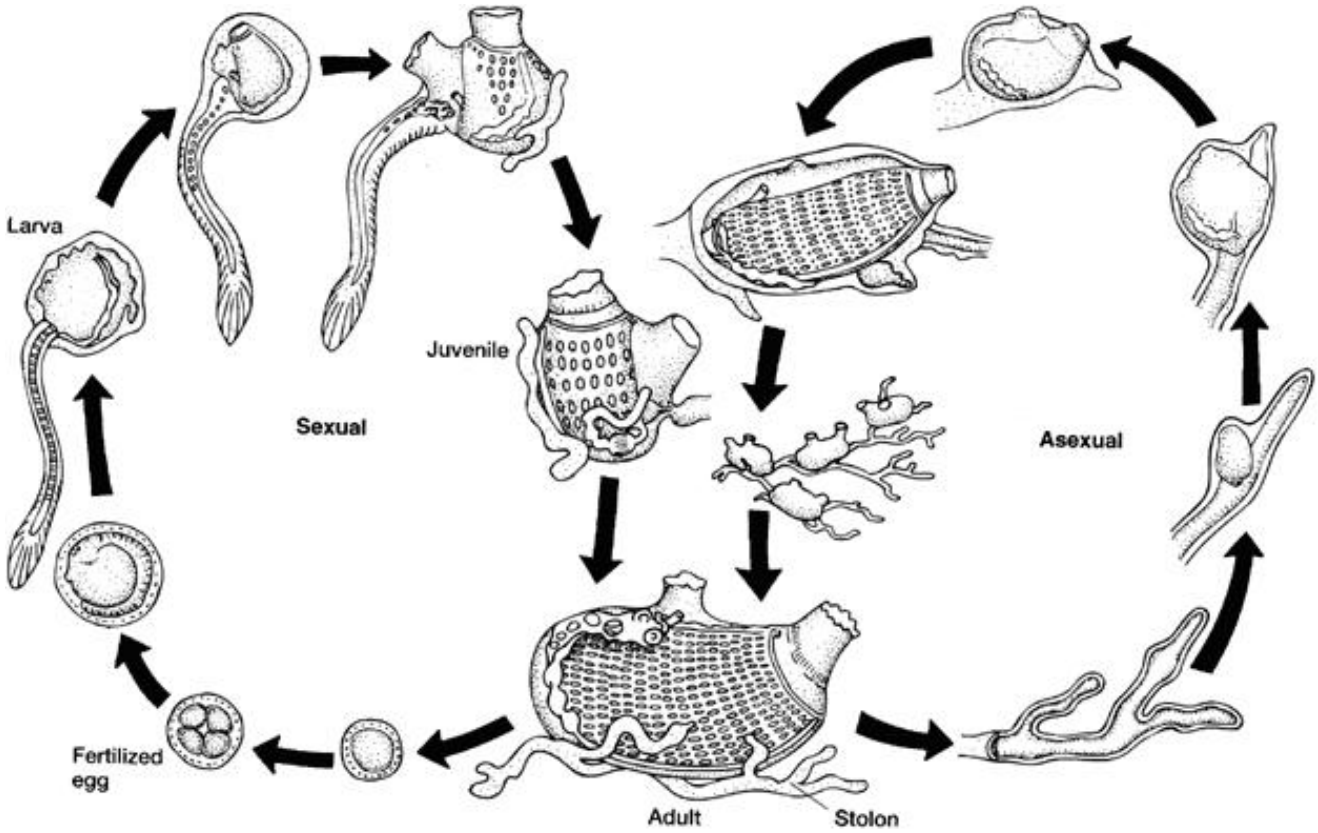
Many species of tunicates have bioactive chemicals, which are produced by cyanobacteria that grow in a mutual relationship, that they use as a defense from predators. Some of these chemicals might have medicinal value to humans. For example, a drug called Aplidin® is currently being tested as a drug to fight cancer.

Chain sea squirts originated from Japan and the Asian-Pacific Coast from Southern Siberia to Southern China. It has been spotted on the East Coast of the United States; the Netherlands; Queensland, Australia; and a lagoon in Venice, Italy.

The native range of *Didemnum* sp. is largely unknown because its taxonomy is still debated. It is known, however, that tunicates from the genus *Didemnum* have invaded locations across the globe. This includes the East and West Coasts of the United States, Europe, New Zealand, and possibly Japan.

Luis A. Solarzano

The ascidian B. violaceus was found growing on a mussel at the Richmond Marina in San Francisco Bay. Specimen identified by Gretchen Lambert.



Life cycle of the tunicate. From *Vertebrates: Comparative Anatomy, Function, Evolution* by Kenneth Kardong. Copyright 2006 McGraw-Hill. Used with permission of The McGraw-Hill Companies.

WEST COAST DISTRIBUTION

Star sea squirts can be found in bays and harbors throughout the entire Pacific Coast of North America, from British Columbia to Baja. Chain sea squirts have a similar range, except they are found farther north in Prince William Sound, Alaska.

The first appearance of *Didemnum* spp. on the West Coast occurred in the San Francisco Bay in 1993. In 2004 it was observed in the cooler waters of Puget Sound, Washington, and the Strait of Georgia, British Columbia, Canada. In Oregon, colonies of *D. vexillum* were first observed within the Umpqua Triangle of Winchester Bay in 2010, and they have since been documented within the Charleston Boat Basin. Since their first discovery on the coast of Oregon, tunicates are still present within the Charleston and Winchester Bays. On the West Coast, it appears to be predominantly a fouling organism.

ECOLOGY

Life cycles and migration patterns

Most tunicate species are **hermaphroditic**, containing both male and female reproductive organs capable of producing eggs and sperm. Sexual reproduction normally

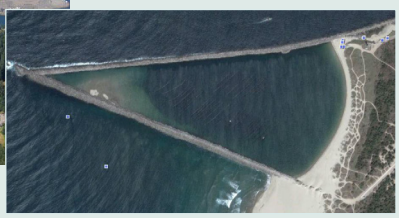
SOLITARY TUNICATES

Not all tunicates live in systems like star sea squirts, chain sea squirts, and *Didemnum*. Sometimes they are solitary. While individuals live near each other, they do not share a common matrix or outflow siphon. They can also be invasive, like the club sea squirt (*Styela clava*) and transparent *Ciona* sea squirt (*Ciona savignyi*). Club sea squirts from Asia are common in the San Francisco Bay and can be found along the entire West Coast. *Ciona savignyi* is found in large numbers in Washington State.

Case Study:
Invasive Tunicate *Didemnum vexillum* Found in Oregon

One of Oregon's 100 Worst Invaders, *Didemnum vexillum*, or sea squirt, was detected in the Charleston Boat Basin and Winchester Bay, Oregon. It was discovered in 2010 by citizen scientists in an oyster bed, where the species was outcompeting other organisms for resources. A coordinated response, led by Oregon Sea Grant, included education, monitoring, and control.

Above: Winchester Bay, Oregon. Note the triangle jetty at the entrance to the bay.



Google Maps

Pie-shaped wedge of the Triangle in Winchester Bay. Tunicates are found on the middle of the inside wall of the north jetty.

Didemnum infested jetty on the right, state beach in foreground, and oyster farm in center.

Clean, Drain, & Dry!

Left: *Didemnum*-infested tire pulled from Charleston Boat Basin. Above: Portion of *Didemnum vexillum* colony.

Actions:
Educate, Collaborate, Monitor, Decontaminate, & Prevent Spread

EDUCATE the public on how they can prevent spread. Sam Chan, Oregon Sea Grant, points out identification of tunicates at a teacher training workshop in Coos Bay, Oregon.

MONITOR: Volunteer divers inspect hulls, docks, and other structures for invasive species.

COLLABORATE: Public and private agencies and businesses work together to mitigate the effects of *Didemnum* infestation. Here, Construction barges are wrapped through a collaborative effort by Port of Newport, Anderson Construction, and West Coast Contractors.

DECONTAMINATE AND PREVENT SPREAD: Docks and other structures are wrapped with tarps to "suffocate" tunicates, which kills the invasive population in about two weeks. In 2016, the tunicates could no longer be detected in the Charleston Boat Basin. However, they do still persist in Winchester Bay. It is important to clean, drain, and dry all equipment and boats as they travel between harbors.

occurs in summer and results in free-swimming larvae called tadpoles, which are dispersed by ocean currents before using their ampullae to attach to hard substrates within 24 hours. Once attached, they metamorphose into an adult tunicate and start a new colony. *Didemnum* spp. can also spread when fragments break from long-lived colonies and re-establish elsewhere. Colonies reproduce asexually by budding. Tunicates tend to have a life span of a few months, but easily replace themselves. They don't have any natural migratory tendencies—just a knack for growing on ships and boats.

Habitat modification and food webs

Star sea squirts are very tolerant of extreme conditions, often doing well in polluted water. However, they cannot survive in water below 3°C or with salinity below 16 parts per thousand (ppt). Chain sea squirts prefer water at 8° to 25°C, with a salinity range of 26 to 34 ppt. Both species occupy similar habitats, including boats, marine equipment, aquaculture equipment, and other organisms.

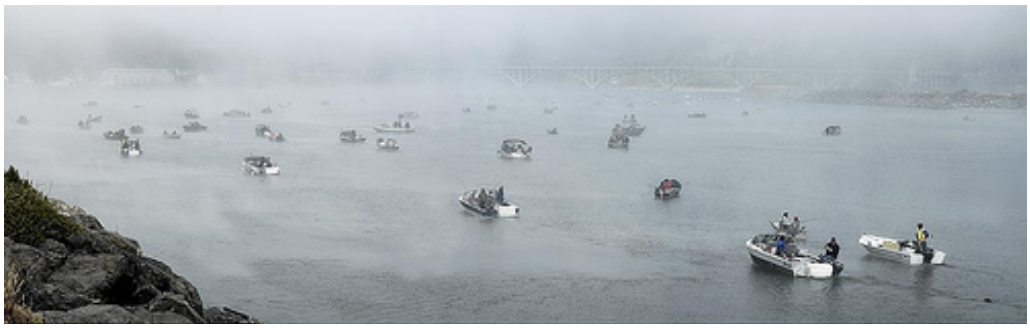
The tunicate *Didemnum* spp. is strictly marine, but otherwise possesses broad environmental tolerances. It is found at depths ranging from intertidal to 65 m, and temperatures from −2° to 24°C. However, its growth rate in deep, open coast habitat is faster than in shallow protected embayments, harbors, and marinas. *Didemnum* colonies that fall off ships die if they land on a soft substrate like mud or sand, but they will attach and continue to grow if they land on a hard surface.

HOW THESE SPECIES GOT HERE

It is most likely that colonial tunicates traveled from their native region attached to the bottoms of boats and ships. This mode of transport is called vessel fouling and can be very harmful to the harbors or bays that the organism is introduced to. Another major vector of introduction is aquaculture. Farmers of shellfish species often import the most profitable or delicious species from other countries, and invasive tunicates can arrive as stowaways on the shells of these organisms. It is unlikely that most colonial tunicates travel in ballast water like other invasive marine species because the larvae are so short-lived. However, it is possible that fragments of adult *Didemnum* spp. colonies will survive an ocean voyage in ballast water.

HOW THESE SPECIES SPREAD

Colonial tunicates can spread within the United States on the bottoms of recreational boats and other marine equipment, aquaculture equipment, and oyster or shellfish stock. Small pieces removed from *Didemnum* spp. colonies show dramatic increases in size after a few weeks due to their ability to reproduce asexually.



Tunicates are spread on ships and recreational boats that carry live colonies from one port to the next. This mode of transport is called "vessel fouling."

Consequently, if removal operations are not carried out carefully, these efforts can actually make the infestation worse. Also, **pelagic** movement of larvae promotes the local spread of the species.

ECOLOGICAL IMPACTS

Colonies of sea squirts outcompete native species for available substrate and block settlement of native larvae. They may also compete with native filter feeders (scallops, oysters, and mussels) for food within the water column. Sea squirt colonies spread quickly, overgrowing and smothering other sessile marine organisms. It is also very difficult for other organisms to grow on tunicates. Such transformation of the native community can alter the food supply for animals in higher trophic levels (like fish and birds) adapted to native prey.

There are many documented impacts that are specific to *Didemnum* sp. Their success in deep, open waters appears to be a consequence of the low recruitment rates of competing species in these habitats. The cellulose matrix cover protects this tunicate from most predators, except the invasive periwinkle snail (*Littorina littorea*). On the East Coast, these snails graze on *Didemnum* colonies weakened by cold water temperatures or stressed by desiccation. For example, at the Georges Bank in the Atlantic, *Didemnum* spp. had colonized a total of 88 square miles of the sea floor by 2005. The question of whether it has directly contributed to the decline in the fishery is still being investigated.

ECONOMIC IMPACTS

The primary economic impact of colonial tunicates is fouling. Aquaculture, recreational boating, and shipping industries must spend time and money removing adult colonies from structures in the water. Shellfish farms must also remove tunicates from the shells of their harvest, because an uncontrolled tunicate population could smother and kill farmed species. The colonies that take over the sea floor may also cause economic damage if they play a role in degrading a fishery through preventing the growth of marine plants.

The costs of tunicate removal are not confined to commercial industries; public funds have been used as well. In January 2006, Washington State provided $250,000 in emergency and supplemental funds to respond to and prevent invasive tunicates from spreading in Puget Sound. Another $500,000 was allocated by the legislature in 2007, and it is expected that tunicate control in Puget Sound will cost well over $1 million.

Marine species such as tunicates are known to emit a wide array of chemicals to stay alive in their underwater habitats. These chemicals are often studied for the potential of new life-saving drugs. One such drug referred to as ecteinascidin, or by the brand name Yondelis®, is currently undergoing clinical trials to determine if it can be used to safely kill cancer cells in humans. This drug contains sources of a chemical emitted by the *Ecteinascidia turbinata*, a species of tunicate found in the coral reefs and mangrove swamps of the West Indies.

Report your sightings of these species!

In **Oregon,** call 1-866-INVADER; visit www.OregonInvasiveHotline.org

In **Washington**, call 1-888-WDFW-AIS; visitwww.invasivespecies.wa.gov/report.shtml

In **California,** call 1- 916-651-8797; email invasives@dfg.ca.gov;
or visit https://www.wildlife.ca.gov/Conservation/Invasives/report

In **other states**, contact the National Invasive Species Hotline: 1-877-STOP-ANS

Help spread the word by informing policy makers about invasive species and actions they can take to protect our waters. Increased awareness and understanding of invasive species is critical to their prevention and management.

What you can do!

Clean, Drain, and Dry! Keep your boat clean at all times, especially when moving it to a new body of water. If you own a boat, you can help stop the spread of colonial tunicates by cleaning your boat often and carefully examining it for hitchhikers before you move it to a new body of water.

Stay alert. The Puget Sound Action Team and other government agencies are encouraging recreational divers to learn to identify and report invasive tunicates. Early detection is important and prompt action is critical. Oregon Sea Grant has produced an identification guide for tunicates that can support early detection identification and reporting by divers, which is available at: http://seagrant.oregonstate.edu/

U.S. Department of Energy Joint Genome Institute.

Adult sea squirt.

CULTURAL SIGNIFICANCE

Colonial tunicates have a significant impact on recreational boaters. As they colonize boat hulls and other equipment, they become a visual blight and create drag when the boat travels. Some find the patterns and colors found in close-up views of *Botrylloides violaceus* and *Botryllus schlosseri* to be visually appealing. However, their overall aesthetic impact on a structure or large area can be negative. Also called "sea vomit," *Didemnum* spp. are almost universally described as an ugly blob. In Korea and Japan, a species of tunicate called *Halocynthia roretzi* is farmed for food and considered quite tasty.

LAWS CURRENTLY IN PLACE

Didemnum vexillum is mentioned as one of the 100 worst invasive species of Oregon. However, this listing does not carry regulatory or legal status. In Washington, nonnative tunicates are classified as a Regulated Type C species, prohibiting intended and unintended introduction. Possession is currently not prohibited unless attached to a boat being transported overland under the Clean, Drain, and Dry provisions. Ballast water regulations by Washington State Department of Fish and Wildlife and the International Maritime Organization may help regulate spread of *Didemnum*. Resources have been allocated by the Washington legislature for control and eradication of tunicates if found in state waters, but they are currently unlisted in state regulations, which means they are treated as prohibited species. California law does not yet regulate colonial tunicates specifically, as none of the species are listed as restricted species; however, there is a general prohibition against relocating any saltwater organisms into the waters of California.

HEALTH HAZARDS

Colonial tunicates pose no immediate health risk to humans. However, it is believed that tunicates accumulate toxic metals in their bodies from the water that they filter feed. This may have negative effects on people who regularly eat tunicates.

MANAGEMENT STRATEGIES

Few reliable control methods have been developed for these species; thus, monitoring, rapid response to infestations, and educational outreach to the public and scientific community will be critical to prevent their spread. A successful control method conducted in 2012 in the Charleston Marina of Oregon was the act of wrapping docks with tarps to "suffocate" the tunicates. This was found to kill the invasive population on the docks after about two weeks. Volunteer divers regularly check on this location and others to monitor for tunicates.

When colonies are small, physical removal may also be an option. However, physical removal of living *Didemnum* spp. must avoid the release of colony fragments to prevent further spread. In New Zealand, a diving and salvage company designed a suction cutter to remove tunicate growth from fouled vessels. Filtered tunicate material was then disposed of at a landfill.

In Puget Sound, Washington, *Didemnum* spp. have been found to die back when covered with plastic film and subjected to increased salinity. In field studies, manipulating environmental conditions (e.g., exposure to air or very high temperatures) just long enough to kill *Didemnum* without adversely affecting native or cultured species, has promise in controlling *Didemnum* spp. Stressing colonies with environmental extremes may also make it more susceptible to natural predation by the common periwinkle snail. To date, there are no examples of a successful application of this strategy in the field.

During the summer of 2006, divers contracted through Washington Department of Fish and Wildlife removed more than 90 lb of tunicates from 150 recreational boats, and they removed about 2,000 lb from docks in infested marinas in Washington. In Edmund, Washington, tunicate eradication cost well over $1 million. This is yet another example that, in the end, it is much cheaper to prevent invasions than to remove them.

INFORMATION GAPS

For the three species discussed in this resource guide, studies to determine precise ecological impacts and effective control methods are needed. Genetic analysis and molecular studies are needed to confirm the number, identity, and origin of *Didemnum* spp. Studies of this type will also confirm if there was a single invasion or repeated events. Distinguishing between primary invasion (source population from native range) and secondary invasion (source population from a previously invaded site) is fundamental to identifying pathways of invasion.

Identifying sources, vectors, and rates of spread for all three species of invasive tunicate could lead to more control and eradication methods.

REFERENCES

Anderson, K., K.R. Shultz, R.P. Meacham, W. Brown, and J. Schultz (Eds.). 2007. Washington State's Response To An Invasion Of Non-Native Tunicates, Report to the Legislature. Available from Washington Dept. of Fish and Wildlife Publications: http://wdfw.wa.gov/publications/00807/

Agius, B.P. 2005. Facilitating invasions: The effects of temperature on the role of non-indigenous ascidians in fouling communities. Presentation to the 2005 International Invasive Sea Squirt Conference, Woods Hole, Massachusetts, April 21–22, 2005.

Byrnes, J.E. and J.J. Stachowicz. 2005. Diversity in fouling communities and resistance to invasive ascidians: The role of settlement complementarity and species interactions. Presentation to Sea Squirt Conference, Woods Hole, Massachusetts, April 21–22, 2005.

Carlton., J.T. 2005. Setting ascidian invasions on the global stage. Presentation to Sea Squirt Conference, Woods Hole, Massachusetts, April 21–22, 2005.

Carlton, J.T. and M.H. Ruckelshaus. 1996. Non-indigenous marine invertebrates and algae (pp. 187–201). In D. Simberloff, D.C. Schmitz, and T.C. Brown (Eds.), *Strangers in Paradise: Impact and Management of Nonindigenous Species in Florida*. Washington, DC: Island Press.

Carman, M.R., S.G. Bullard, and J.P. Donnelly. 2005. Water quality, nitrogen pollution and ascidian diversity in northwestern Atlantic coastal waters of Massachusetts, USA. Presentation to Sea Squirt Conference, Woods Hole, Massachusetts, April 21–22, 2005.

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

Cohen, A.N. 2005. *Guide to the Exotic Species of San Francisco Bay*. Oakland, CA: San Francisco Estuary Institute. Available from: www.exoticsguide.org

Coutts, A.D.M. 2002. A Biosecurity Investigation of a Barge in the Marlborough Sounds (New Zealand). Cawthron Institute Report 744. Nelson, NZ: Cawthron Institute. Available from: http://www.slc.ca.gov/Laws-Regs/Article4.8/Coutts%202002.pdf

Davis, A. 2006. Medicines by Design. Chapter 3: Drugs from Nature, Then and Now, pp. 30. U.S. Department of Health and Human Services, NIH Publication No. 06-474. Available from http://publications.nigms.nih.gov/medbydesign/index.html

Hickman, C.P., L.S. Roberts, and A. Larson. 2005. *Integrated Principles of Zoology* (13th ed.), Chapter 23: Chordates (pp. 486–488). New York, NY: McGraw-Hill.

Kott, P. 2004. A new species of *Didemnum* (Ascidiacea, Tunicata) from the Atlantic coast of North America. *Zootaxa* 732:1–10. Available from http://woodshole.er.usgs.gov/project-pages/stellwagen/didemnum/images/pdf/kott_2004.pdf

McCarthy, A. 2005. Effects of temperature on growth rates of colonial ascidians: A comparison of *Didemnum* sp. to *Botryllus schlosseri* and *Botrylloides violaceus*. Presentation to Sea Squirt Conference, Woods Hole, Massachusetts, April 21–22, 2005.

Osman, R.W. and R.B. Whitlatch. 2005. Habitat variability in the invasion of Long Island Sound by *Didemnum* sp. and its interaction with the resident community. Presentation to Sea Squirt Conference, Woods Hole, Massachusetts, April 21–22, 2005.

Stachowicz, J.J., R. Terwin, R.B. Whitlatch, and R.W. Osman. 2002. Linking climate change and biological invasions: Ocean warming facilitates nonindigenous species invasions. *Proceedings of the National Academy of Sciences, U.S.A., 99*(24):15497–15500.

Tujula, N., J. Radford, S.V. Nair, and D.A. Raftos. 2001. Effects of tributyltin and other metals on the phenoloxidase activating system of the tunicate, *Styela plicata*. *Aquatic Toxicology 55*(3–4):191–201.

Valentine, P.C., M.R. Carman, D.S. Blackwood and E.J. Heffron. 2005. Ecological observations of the colonial tunicate *Didemnum* sp. in a New England tide pool habitat and strategies for managing invasive colonial ascidian species. Presentation to Sea Squirt Conference, Woods Hole, Massachusetts, April 21–22, 2005.

Whitlatch, R. and R. Osman. 2002. Observations posted to the U.S. Geological Survey's Marine Invasive Species website. Available from: http://woodshole.er.usgs.gov/project-pages/stellwagen/didemnum/

Zack, J. 2006. Introduced Species Summary Project: Sea Squirt (*Didemnum* sp.). Available from http://www.columbia.edu/itc/cerc/danoff-burg/invasion_bio/inv_spp_summ/Didemnum_sp.html (last updated November 14, 2006).

ADDITIONAL RESOURCES

***Didemnum* Video Clips**

USGS Woods Hole Coastal and Marine Science Center.

http://woodshole.er.usgs.gov/project-pages/stellwagen/didemnum/htm/video.htm

***Didemnum vexillum*—Georges Bank Images**

USGS Woods Hole Coastal and Marine Science Center.

http://woodshole.er.usgs.gov/project-pages/stellwagen/didemnum/htm/page7.htm

Korea–U.S. Aquaculture

NOAA Central Library website with information about bilateral efforts to improve aquaculture information and data exchange between the two countries.

http://www.lib.noaa.gov/retiredsites/korea/

Lab-Cultured Chain Sea Squirts

University of California–Davis and Bodega Marine Laboratory, Bodega Bay, California. Gallery of close-up photos of chain sea squirts cultured in a lab.

http://convoluta.ucdavis.edu/gallery/view_album.php?set_albumName=Botrylloides_violaceous

Marine Biotechnology

Consortium of European agencies with information on bioactive chemicals with potential uses for humans.

http://www.marinebiotech.eu/resources

Marine Nuisance Species

USGS Woods Hole Coastal and Marine Science Center website; includes video clips.

http://woodshole.er.usgs.gov/project-pages/stellwagen/didemnum/

Sea Squirt DNA Sheds Light on Evolution

U.S. Department of Energy, Joint Genome Institute.

http://jgi.doe.gov/news_12_12_02/

Sea Squirts, Our Distant Cousins

Website by W. von Egmond and J. Parmentier, published in Micscape Magazine*, 1998.*

http://www.microscopy-uk.org.uk/mag/indexmag.html?http://www.microscopy-uk.org.uk/mag/artaug98/tuni1.html

Wildscreen Arkive

Underwater video footage of a star sea squirt (no narration).

http://www.arkive.org/species/ARK/invertebrates_marine/Botryllus_schlosseri/Botryllus_schlosseri_00.html?movietype=wmMedProg