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🌐 Maintaining and propagating the shipworms *Lyrodus pedicellatus* and *Teredo bartschi* (Teredinidae: Bivalvia) in laboratory culture



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Symbiosis Model Systems

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We use this protocol and it's working

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Abstract

While many publications report the captive growth and reproduction of shipworm species (Eckelbarger and Reish 1972a; Coe 1941; Willer et al. 2023; Gallager, Turner, and Berg 1981; Mann and Gallager 1985a; Mann and Gallager 1985b), none describe their culture, maintenance, and propagation in detail. Here, we describe methods for cultivating, propagating, and maintaining two shipworm species, *Lyrodus pedicellatus* and *Teredo bartschi*, in a controlled laboratory setting. Shipworms provide a compelling model for studying bacteria-animal symbiosis as both the hosts and their intracellular symbionts are cultivable. **However, shipworms are potentially invasive and highly destructive to property and ecosystems.** Therefore, the methods described herein, particularly those relating to biological containment, must be strictly followed. This protocol details specific conditions for aquarium maintenance, including salinity, temperature, feeding, and timing of reproductive cycles. Methods are also described for collecting and propagating shipworms and artificially inducing spawning.

Image Attribution

Marvin A Altamia



Guidelines

Shipworms are wormlike wood-boring marine bivalves of the family *Teredinidae*. This family includes approximately 70 species that differ dramatically in appearance, trophic and reproductive strategies, life histories, habitats, and geographic distribution. All species display an elongate wormlike appearance, are adapted to burrowing in hard substrates, and line their burrows with a shell-like calcareous material (Turner 1966). All species examined to date harbor bacterial symbionts in a specialized tissue within the gills called the Gland of Deshayes. Most burrow in and feed on wood and harbor cellulolytic intracellular bacterial symbionts (Altamia and Distel 2022). Their unusual morphology, development, trophic strategies, and symbiotic associations make them desirable subjects for research.

Here, we describe the cultivation of the shipworms *Lyrodus pedicellatus* and *Teredo bartschi*. While much information is available regarding the biology of *L. pedicellatus*, little has been published about *T. bartschi*. However, in our experience, the life history and growth requirements of *Teredo bartschi* are substantially similar to *L. pedicellatus*. Therefore, the same cultivation protocols are successful for both species.

Materials

1. Materials and Equipment:

Each culture apparatus should be equipped with the following:

- One 5.5-gallon (20.8-liter) aquarium (e.g., Aqueon 5.5-gallon standard glass rectangle aquarium, PetSmart, Phoenix, AZ).

Equipment	
Aqueon Standard Glass Rectangle Aquarium	NAME
Standard aquarium	TYPE
Aqueon	BRAND
5345488	SKU
https://www.petsmart.com/fish/tanks-aquariums-and-nets/aquariums/aqueon-standard-glass-rectangle-aquarium-5345488.html	LINK

- One high-quality aquarium heater, preferably one with an accurate thermostat and digital display (e.g., Hygger pinpoint titanium heater with ic temp controller, Hygger Aquarium, Chino, CA).

Equipment	
hygger Titanium Aquarium Heater	NAME
Hygger	BRAND
HG802	SKU

- One aquarium air pump, with air tubing and air stone (e.g., Top Fin 10-gallon aquarium air pump, PetSmart, Phoenix, AZ).

Equipment	
Top Fin® Aquarium Air Pump	NAME
Aquarium Air Pump	TYPE
Top Fin	BRAND
5148676	SKU
https://www.petsmart.com/fish/filters-and-pumps/air-and-water-pumps/top-fin-aquarium-air-pump-16855.html?fmethod=Search	LIN K

- A lid should be made for each aquarium from corrugated plastic (e.g., 1/8-inch Coroplast, Home Depot, Atlanta, GA).
- Twenty liters of natural seawater. Artificial seawater may also be used (e.g., Instant Ocean Reef Crystals, Blacksburg, VA).
- An aquarium filter is not required.

Additional aquarium supplies:

- A fifty-micron pore size high flow filter bag (e.g., 50 Micron, Size 3, 20 GPM Max Flow, High Flow Bag Filter, 4.1 Inch Diameter, 8 Inch Long, 1/2 Sq. Ft., PENTAIR, Minneapolis, MN)-
- A Refractometer (e.g., Imagitarium Aquatic Refractometer, Petco, San Diego, CA).
- An aquarium cleaning siphon vacuum (e.g., Top Fin® Aquarium Gravel Vacuum, PetSmart, Phoenix, AZ).

Equipment

Top Fin® Aquarium Gravel Vacuum

NAME

Aquarium Gravel Vacuum

TYPE

Top Fin

BRAND

5162805

SKU

<https://www.petsmart.com/fish/maintenance-and-repair/vacuums/top-fin-aquarium-gravel-vacuum-5162805.html>

LINK

- Miscellaneous buckets and carboys to be used for preparing and decontaminating seawater.

Additional equipment

- A dissecting stereo microscope or equivalent magnification tool for visualizing larvae and early growth stages.
- A ground-fault interrupter (GFI) outlet bar and extension cord to safely connect electrical equipment, pumps, heaters, etc.
- A handheld electric drill with ¼ inch drill bit.

Settlement panels:

For each solid settlement panel:

- One 1" x ½" x 5" length of pine molding (available in any hardware or lumber store).
- One fishing float (e.g., Woozett float, hard ABS fishing bobber with push button 1.5", Amazon)
- Clear nylon fishing line
- One 4" cable tie with a write-on tag label (e.g., 4-inch nylon marker cable ties with write-on tag, XINGO, Zhejiang, China).

For each laminated settlement panel

- 3-5 Scotch pine laths (1.0" W, 0.04 " H, 3.25 " L)
- 2 rectangular clear acrylic (Plexiglass) strips (1.0" W, 0.25" H, 3.25" L) center drilled 0.5" from each end.
- 2 nylon hex head bolts (¼" outer diameter, 20 threads per inch)
- 2 nylon wingnuts (¼" inner diameter, 20 threads per inch)
- 1 fishing float (e.g., Woozett float, hard ABS fishing bobber with push button 1.5", Amazon)

- Clear nylon fishing line
- 1 four-inch cable ties with write-on tag labels (e.g., 4-inch nylon marker cable ties with write-on cable tag, XINGO, Zhejiang, China).

Assembly instructions:

1. Sandwich 3 Scotch pine laths between 2 acrylic strips and clamp together.
2. Using the holes in the acrylic strips as a guide, drill through the Scott's pine laths with a 1/4" drill bit to create matching holes in the laths.
3. To assemble the laminated settlement panel, loosely bolt the laths and acrylic strips together with 2 nylon bolts and 2 nylon wingnuts.
4. Soak the assembled laminated settlement panels in seawater overnight before hand-tightening the wingnuts.
5. Attach the fishing float, cable tie, and tag to the assembled laminated settlement panel
6. Label with a unique ID, the deployment date, and any additional information desired.

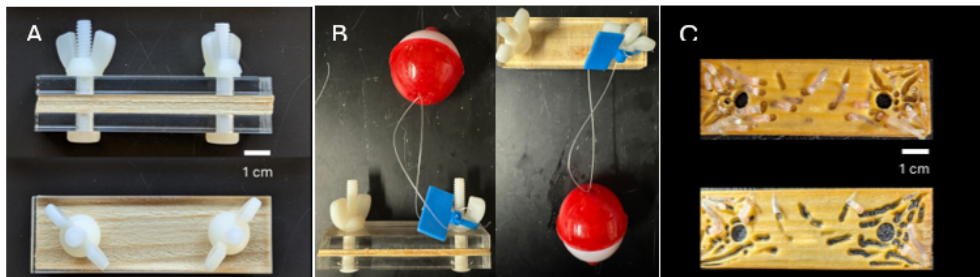


Figure 2: Laminated settlement panel. (A) Laminated settlement panels comprised of three to five Scotch pine laths, each 1 mm thick, sandwiched between two Plexiglas plates and held together with nylon bolts. (B) Laminated settlement panel with float, cable tie, and tag. Larvae settle along the exposed edges and burrow into the laminated wood. (C) Intact animals can easily be removed from the panels by removing the bolts and carefully separating the layers.

Safety warnings

! **SHIPWORMS ARE POTENTIALLY HIGHLY DESTRUCTIVE INVASIVE SPECIES!** The accidental release of shipworms into non-native marine environments may result in **EXTREME ECOLOGICAL AND ECONOMIC DAMAGE**. For example, the unintentional introduction of the shipworm *Teredo navalis* into San Francisco Bay in 1917 caused **~\$300 MILLION IN DAMAGE** to marine vessels and structures in just the following two years.

For this reason, shipworms, wood that contains shipworms, fecal and reproductive products of shipworms, and all water that comes into contact with shipworms must **NEVER BE ALLOWED TO ENTER ANY NATURAL WATER BODY**.

Following the correct disposal procedures to prevent the spread of shipworms is **CRUCIAL**. Before disposal, wood and all solid materials in contact with shipworms should be thoroughly dried in a drying oven to ensure that all contained specimens are dehydrated and dead. Shipworms may survive in wood for up to 2 months if the wood is not thoroughly dried. Dried material may be discarded in a regular terrestrial waste stream but **not in any natural water body**.

Any water that has contacted shipworms must be discarded as biological waste according to the policies of the recipient institution. Wastewater should be passed through a filter with < 50µm pore size to remove reproductive products, then decontaminated by heating to > 70°C for 30 minutes to kill any remaining eggs, sperm, or larvae. The treated wastewater may then be discarded in an appropriate wastewater stream, such as a municipal one, but should never be released into any natural water body.

To prevent environmental contamination, **shipworm culture systems must be closed and disconnected from the environment**, and all disposed materials must be managed according to local regulations.

Ethics statement

This study followed the guidelines recommended by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and Northeastern University's Institutional Animal Care and Use Committee policies. Although no live vertebrate animals were used in this study, the general principles of humane animal care were applied to the treatment of live invertebrate animals.

Before start

Shipworm culture can be maintained in a simple, inexpensive seawater aquarium system. The system described here can be used successfully to cultivate most long-term brooding shipworm species but can be altered to suit individual laboratory conditions.

Settlement panels:

2d 18h

1 Assembly instructions:

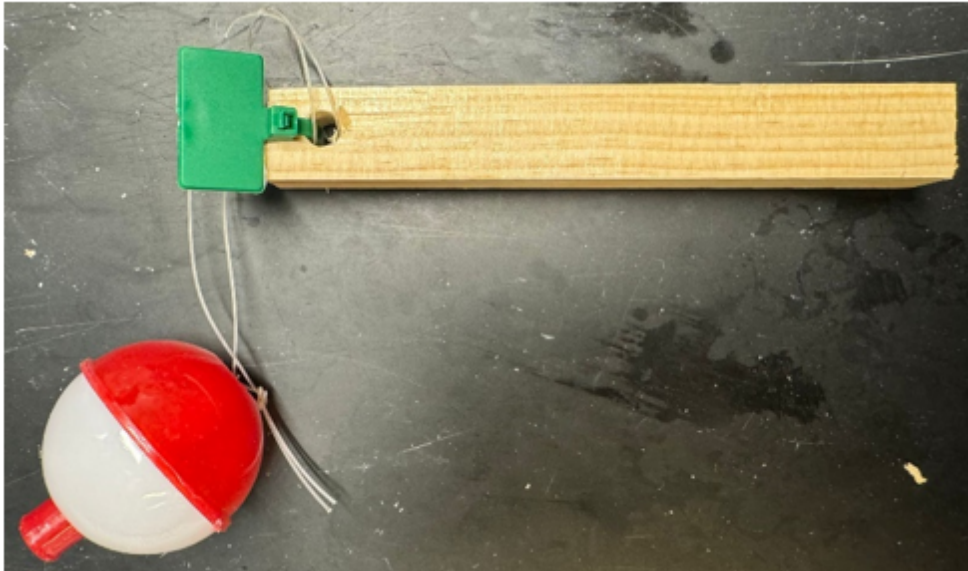


Figure 1: Solid settlement panel.

- 1.1 Drill a $\frac{1}{4}$ " hole $\frac{1}{4}$ " from the end of each 1" x $\frac{1}{2}$ " x 5" length of pine molding.
- 1.2 Thread a 4" cable tie through the hole and connect loosely.
- 1.3 Clip the fishing float to the cable tie.
- 1.4 Label the cable tie tag with a unique ID, the deployment date, and any additional information desired.
- 1.5 Pre-soak the settlement panels in seawater for 1-5 days before use.

2 Assembly instructions:

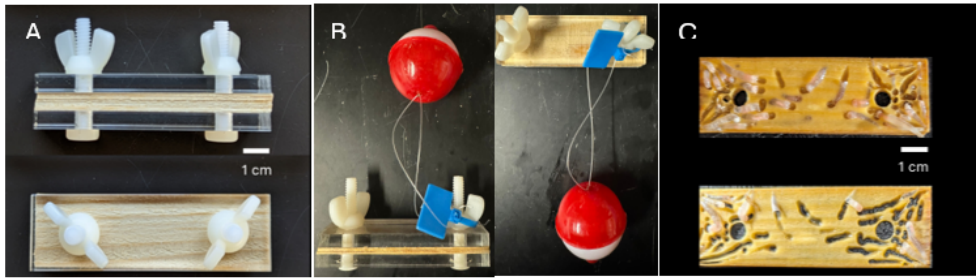


Figure 2: Laminated settlement panel. (A) Laminated settlement panels comprised of three to five Scotch pine laths, each 1 mm thick, sandwiched between two Plexiglas plates and held together with nylon bolts. (B) Laminated settlement panel with float, cable tie, and tag. Larvae settle along the exposed edges and burrow into the laminated wood. (C) Intact animals can easily be removed from the panels by removing the bolts and carefully separating the layers.

- 2.1 Sandwich 3 Scotch pine laths between 2 acrylic strips and clamp together.
- 2.2 Using the holes in the acrylic strips as a guide, drill through the Scott's pine laths with a 1/4" drill bit to create matching holes in the laths.
- 2.3 To assemble the laminated settlement panel, loosely bolt the laths and acrylic strips together with 2 nylon bolts and 2 nylon wingnuts.
- 2.4 Soak the assembled laminated settlement panels in seawater overnight before hand-tightening the wingnuts.
- 2.5 Attach the fishing float, cable tie, and tag to the assembled laminated settlement panel.
- 2.6 Label with a unique ID, the deployment date, and any additional information desired.

Obtaining shipworm specimens:

2d 18h

- 3 The colony described here was stocked with specimens of *Lyrodus pedicellatus* and *Teredo bartschi* found in deposited wood—mostly naturally occurring fallen mangrove branches—collected by hand in shallow water in a mangrove thicket on the western shore of the Banana River, Merritt Island, Florida just north of the Bennett Causeway on Florida Route 528 (Figure 3). The approximate GIS coordinates of the collection site are N 28.40605, W 80.66034.

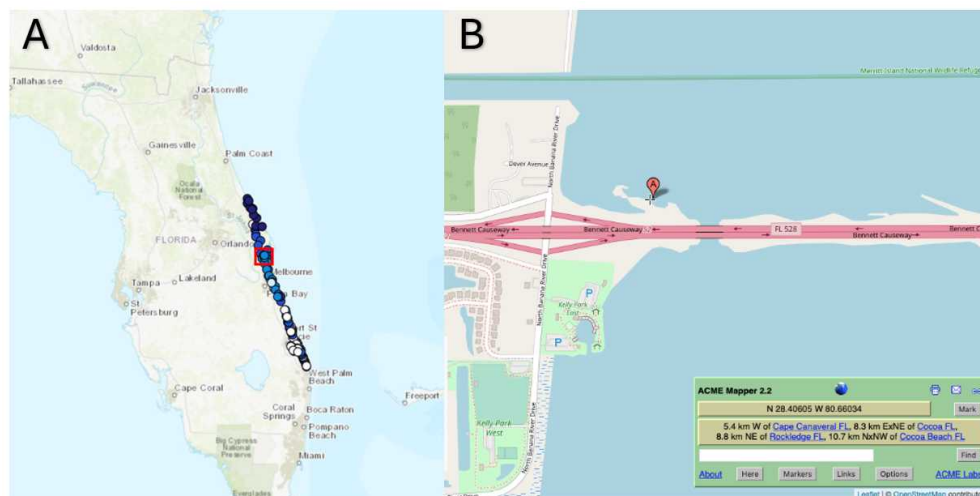


Figure 3: Collection location for *Lyrodus pedicellatus* and *Teredo Bartschi*. (A) The collection location is in the Banana River (part of the Indian River Lagoon) near the central Atlantic coast of Florida. The red box indicates the collection location. (B) Detail of red box in (A).

Transporting living specimens:

2d 18h

- 4 Collected wood containing shipworms should be wrapped in seawater-dampened paper towels and placed in a watertight plastic bag. Do not fill the bag with water. Instead, leave ample airspace to prevent asphyxiation. Place the bag in a Styrofoam shipping container to minimize temperature variation during transport. Specimens will survive for several days in this packaging and may be shipped by air freight.

Establishing culture:

2d 18h

- 5 Clean the surfaces of the source wood as well as possible to remove any living or dead organisms.
- 6 Place the source wood in a prefilled, prefiltered, and preheated 5.5-gallon seawater aquarium.
 - 6.1 Due to the shock of collection, handling, and temperature fluctuations, shipworms typically begin spawning immediately upon being placed in the aquarium.
- 7 Float up to 15 presoaked settlement panels on the surface of the seawater in the aquarium.

- 7.1 Shipworm larvae typically settle on the wood just below the air-water interface. The panel-float system is designed to float horizontally at the water surface while the larvae settle, then sink to a vertical position as the panel becomes waterlogged. Settlement will typically begin between 18 and 48 hours after spawning.
 - 8 When larvae settle on the panel, the panel may be transferred to a new aquarium to serve as breeding stock. Allow 10-20 larvae to settle per panel before transferring. Metamorphosis should occur 10-20 days after settlement.
 - 9 Repeat this process with each new generation to propagate shipworms.
-
- 9.1 Note that two species are present at the collection location described here. Larvae are indistinguishable until the juvenile stage, when pallets form. The color of the pallets can then differentiate the two species.
L. pedicellatus has black-tipped pallets, and *T. bartchsi* has white-tipped pallets. A dissecting microscope is needed to see the pallets inside the tiny burrows. At this stage, monocultures can be obtained by killing unwanted individuals with a dissecting needle. Alternatively, induced spawning can obtain new lineages from individual adults (See Induced spawning).

Colony Maintenance and Growth Conditions:

2d 6h

10 Salinity and Temperature:

- Adult specimens of *Lyrodus pedicellatus* have been reported to survive in salinities ranging from 7 to 35 ppt and temperatures from  10 °C to  30 °C . However, the minimum long-term survival requirements for adults are 22-25 ppt and  11 °C .
- Adult and larval survival and settlement rates increase toward the higher ends of these ranges, with maximal larval survival at  22 °C -  24 °C and 35ppt (Eckelbarger and Reish. 1972a).
- We routinely maintain both *L. pedicellatus* and *T. bartschi* at 27°C and 31 ppt.

11 Substrate requirements:

- *Lyrodus pedicellatus* is obligately xylophagous (wood-eating) and xylophagous (wood-boring).
- Individuals of *L. pedicellatus* must remain within their burrows to survive, except during the pediveliger larval stage, during which it swims freely in the water column.
- This species is known to settle on and burrow in many types of wood, including hardwoods (e.g., red oak) and softwoods (e.g., Scotch pine).

- In our experience, growth rates are higher in softwood.
- Animals cannot be transferred to a new wood substrate once burrowing commences.

12 **Reproduction:**

- Most shipworm species are broadcast spawners, i.e., eggs and sperm are released into the water column where fertilization occurs.
- However, species in the genera *Lyrodus* and *Teredo* are larviparous (Figure 2). In these species, fertilization occurs internally within the mantle cavity (Turner 1966).
- Larvae are retained within brood pouches on the dorsal side of the gills either until they reach the straight hinge stage, as in *Teredo navalis* (short-term brooding), or the pediveliger stage, as in *Teredo bartschi* and *Lyrodus pedicellatus* (long-term brooding).
- Both species develop as simultaneous hermaphrodites. *L. pedicellatus* has been shown to self-fertilize (Eckelbarger and Reish 1972b).

Note

Our observations suggest that *T. bartschi* can also self-fertilize. Self-fertilization simplifies culture, as it is unnecessary to ensure that both males and females are present in a breeding colony. Self-fertilization is also advantageous for breeding experiments, as a single individual can create a new population.

13 **Development:**

2d 6h

The early developmental stages of *L. pedicellatus* are depicted in Figure 4 and are similar to those of *T. bartschi*. For both *L. pedicellatus* and *T. bartschi*, larvae develop within the brood pouches until the pediveliger stage, the last stage of larval development, and so can settle almost immediately after spawning.

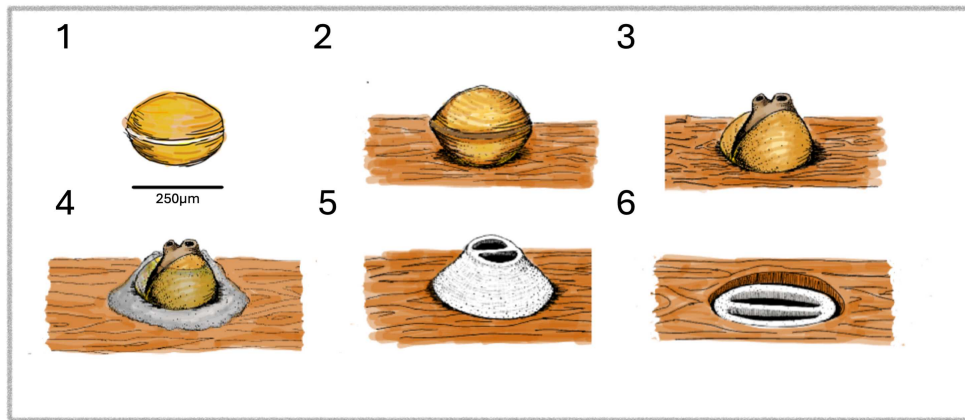


Figure 4. Early developmental stages of *Lyrodus pedicellatus*. Free swimming larvae (1) settle on wood and explore the surface briefly (2) before beginning to burrow (3). A conical ring of tissue forms around the larva at the start of metamorphosis (4).

- Pediveliger larvae typically settle on wood within 18:00:00 to 36:00:00 after spawning (Eckelbarger and Reish 1972a) and do not feed while in the plankton (Pechenik, Perron, and Turner 1979). This makes long-term brooders easy to maintain in pure culture, as the larval period is brief, and the larvae require no special care or feeding.

Note

Depending on the temperature and salinity, pediveliger larvae can survive in the plankton for five to 13 days before finding and settling on a suitable wood substrate.

13.1 After settlement, larvae crawl on the wood surface briefly before burrowing. When the burrow depth reaches about one-half the diameter of the valves, the velum is resorbed, and a clear cone-shaped ring of mantle tissue forms around the shell.

- Within days, the surface of this tissue becomes calcified and forms a delicate white calcareous cap. As the burrow deepens, this calcification extends to form the lining of the burrow.
- In a week to 14 days, the paired siphons may be seen protruding from the burrow entrances, and the production of fecal material becomes apparent.
- After several weeks, the pallets become visible within the burrows. Pallets are best observed under a dissecting microscope.

- To visualize the pallets, use a jet of water to disturb the shipworms, causing them to withdraw their siphons and expose their pallets. The appearance of pallets marks the beginning of the juvenile stage.

13.2 After 4-8 weeks, larvae appear within the brood pouches. Spawning begins shortly after that, marking the start of the adult stage.

- Individuals may reach the adult stage at approximately 5 mm in length. *L. pedicellatus* displays indeterminate growth and will continue to grow and elongate the burrow as long as wood is available.
- Individuals reproduce episodically throughout their lifespan, which can last two years or more in culture.
- Individuals may grow to the entire length of the settlement panel (5 inches or 12.7 centimeters) but will die when the wood supply is exhausted. Overcrowding will severely stunt the growth of Individuals, limiting most individuals to one centimeter or less in length.

14 Feeding:

Note

Phytoplankton supplementation is optional.

Individuals of *L. pedicellatus* do not feed as swimming larvae but can obtain nutrients from phytoplankton as adults (Pechenik, Perron, and Turner 1979). However, wood alone can support growth and reproduction. No differences in burrow length, tissue dry weight, percent carbon and nitrogen, total wood consumption, larval production, biodeposition, respiration, and ammonia excretion were observed when *L. pedicellatus* was reared on wood-only compared to phytoplankton-supplemented diets (Gallager, Turner, and Berg 1981). However, phytoplankton supplementation increased the growth rate of *Bankia gouldi*, another shipworm species (Mann and Gallager 1985a).

15 Routine colony maintenance (weekly):

15.1 Check salinity using a refractometer before cleaning each aquarium.

15.2 Check and adjust the temperature as necessary.

- 15.3 Use a siphon to vacuum up fecal material that accumulates at the bottom of the tank.
- 15.4 Replace the seawater removed by vacuuming. This should amount to 10-20% of the total tank volume. This volume of seawater replacement is sufficient to maintain water quality. It is not necessary to use an aquarium filter unit in the tanks.
- 15.5 (Optional) Add commercially prepared cultured phytoplankton (e.g., Kent Marine Phytoplex, Coralife, Central Garden and Pet, Walnut Creek, CA.) according to the manufacturers' recommended amount (1 ml per 16 liters of seawater).
- 15.6 Measure salinity again after refilling the aquarium. Adjust as necessary.
- 15.7 Inspect settlement panels, adding new settlement panels as needed to maintain sufficient shipworm production and removing panels that have become too old.
- 16 **Induced spawning:** Mild stress, such as temperature shock, induces spawning in sexually mature adults.
- To induce spawning of all individuals in a settlement panel, remove the panel from the aquarium and place it in a refrigerator at  4 °C for several hours.
 - Adults will spawn upon being returned to seawater at ambient temperature.
- To induce spawning in individuals,
- 16.1 Grow the shipworms in a laminated settlement panel (Fig 2A) by placing the panel in an aquarium containing sexually mature adult shipworms.
- 16.2 After several days, monitor the panel for larval settlement using a stereoscope.
- 16.3 When 10-20 larvae have settled, transfer the panel to a new aquarium that contains no other shipworms.
- 16.4 Allow shipworms to become sexually mature and start to spawn (1-3 months). remove the panel from the aquarium and carefully disassemble it.
- 16.5 By removing individual wood laths, the animals can be exposed and easily removed from the wood with their tubes intact (Fig 2B).

- 16.6 Place individual shipworms in 1.5 ml microcentrifuge tubes with 1 ml of sterile-filtered seawater (0.22 μm) and incubate for  12:00:00 at  4 °C .
- 16.7 Replace the filtered seawater (0.22 μm) with preheated filter seawater at  27 °C . This will induce spawning immediately.
- 16.8 Allow two hours for spawning to proceed.

12h



Video 1: Shipworm *Teredo bartschi* spawning. Larvae are released from the exhalant siphon.

- 16.9 Using a stereoscope, or by eye, check the tube for larvae.



Video 2: Newly spawned actively swimming pediveliger larvae of *Teredo bartschi*.

- 16.10 Stress causes the release of both mature and immature larvae. Transfer the larvae from the tube to a petri dish and select only actively swimming larvae (pediveligers) for subsequent propagation using a stereoscope.

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