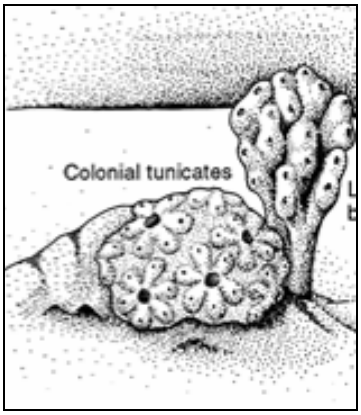
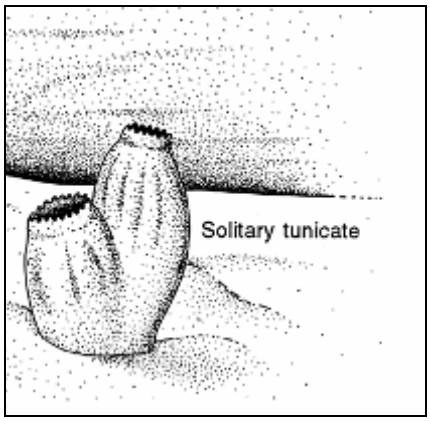
An introduction to tunicates

Tunicates are marine invertebrate animals (animals lacking a backbone) that are commonly found attached to rocks in the coastal ocean. If you spend time around harbors and marinas you are likely to have seen them attached to docks and pier pilings. Tunicates have a swimming larval stage, but during their adult stage most species are permanently attached to a substrate. Tunicates may be solitary (single animals, like humans) or colonial (living attached to one another, like corals).

Both solitary and colonial tunicates have sac-like bodies and feed by filtering water. Water is drawn in through an incurrent siphon and expelled through an outcurrent siphon. The siphons are visible on the larger solitary tunicates, which will sometimes expel water when touched, hence the nickname "sea squirts." Colonial tunicates are much smaller and live embedded in a common "tunic"; their siphons are difficult to see without a microscope. A colony is formed when a larva attaches to substrate and undergoes metamorphosis to become an adult tunicate, called a zooid. The first zooid then replicates itself asexually through a process called budding, creating additional zooids. All of the zooids are connected via the living tissue of tunic, which may be translucent and gelatinous or thick and leathery. While the individual zooids are small, colonies can be quite large. In some tunicate species, zooids are arranged in patterns, such as clusters in the shape of flowers or stars.





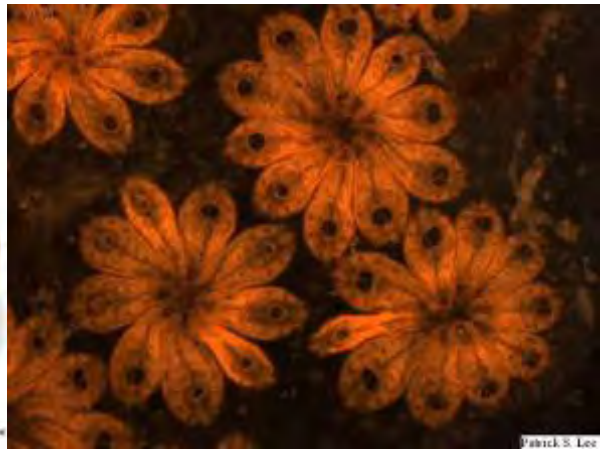
(picture from The Living Ocean, used with permission)

In the field, tunicates can sometimes be difficult to distinguish from sponges. In general, sponges feel "spongy" – compressing and then springing back when touched, while tunicates tend to resist compression. The siphons on solitary tunicates will usually retract if the animal is touched; sponges may have openings that resemble siphons, but these are not usually paired, and they do not retract. Colonial tunicates generally are slick and shiny while the surface of sponges is usually dull or porous.

Our focus: Botryllid tunicates

Colonial tunicates are highly variable in color, size, shape, and attachment substrate. Botryllids, the type of colonial tunicate in which we are particularly interested, may be any color including orange, yellow, black, cream-colored, red, blue, and others. They may have striking patterns with multiple colors.

Botryllid tunicates are commonly found on a variety of submerged and occasionally intertidal surfaces including docks, ropes, boats, mariculture pens, trays, nets, and pilings. They are also found on seagrass and rocky substrate in intertidal and subtidal habitats including boulders, walls, and even small cobble.

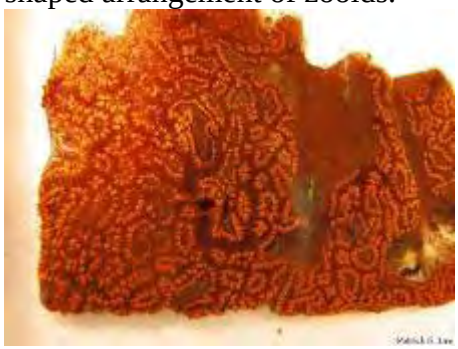


There are two general types of Botryllid tunicates: *Botryllus*. and *Botrylloides*. We would like to have samples of both.

In the photo on the left, there is a mussel with two *Botryllus* colonies: a large orange colony, and a small yellow/orange colony in upper left corner. In the same photo, there is also a small white/yellow *Distaplia* colony present in middle. *Distaplia* is a different type of colonial tunicate (non-Botryllid) that often forms stalked buttons, mushroom or club-shaped colonies, or large mounds. In contrast to *Distaplia*, Botryllid tunicates are generally more sheet-like and thinner, though they may also grow in larger gelatinous blobs or even strings depending on substrate availability.

The photo on the right is a close up of a *Botryllus* colony*;* note the flower-like arrangement of the zooids.

The photo below shows a piece of a *Botrylloides* colony. Note the meandering, less flower-shaped arrangement of zooids.



Collecting Botryllids

We are interested in collecting both types of Botryllids: *Botryllus*, with its flower-shaped zooid arrangements, and *Botrylloides*, with its meandering zooids. We intend to use these samples for molecular and morphological identification. One of our major goals in this project is to gather specimens representing different morphologies, so please keep your eyes open for differences in color, zooid arrangements or shape and include these in your collection. Below are some examples of the different color morphs of Botryllid tunicates.


ARKive
© D. W. Greenslade / ARDEA LONDON


ARKive


ARKive

For genetics and collection questions please contact Sarah Cohen, Sarahcoh@sfsu.edu 415-338-3750 or Karen Alroy, Karen.alroy@gmail.com 415-338-3787, and give your name and contact information.
If you have any questions regarding shipping please contact Chela Zabin zabinc@si.edu 415-435-7128.

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Citizen Marine Science Network:

Understanding Change in Coastal Marine Environments

Protocols for the SETL Adopt-a-Plate Program

Version 2.0 (April 2007)

Gregory M. Ruiz, Chela J. Zabin, Kristen Larson
Smithsonian Environmental Research Center
&
Sarah Cohen
San Francisco State University

SETL: Adopt-a-plate program

Program Goal: The SETL program is being established to (a) detect biological invasions by non-native marine species and (b) measure changes in the distribution and abundance of marine invertebrates more broadly.

Approach: The SETL program relies upon a distributed network of volunteer participants, who take and share standardized measures across many geographic locations. Each participant examines key organisms at a single site and collects descriptive physical measures. Although the individual commitment and time required per participant is small, the combined effort can produce a significant amount of critical information. Together, we seek to address fundamental gaps in our understanding of coastal ecosystems and improve stewardship of coastal resources.

A. Getting Started: Deployment Protocol for Settlement Plates

1. Program Structure. Each network participant or group will assess marine invertebrates and collect physical data (including temperature and salinity) at only one site.

If measures are made by a group, a lead must be identified. Thus, whether measures are made alone or by a group, each site has designated "**site leader**". The site leader assumes primary responsibility for (a) proper implementation of protocols, (b) coordination with group participants, (c) consistency of site and methods over time, and (d) interaction across network participants.

For each site, 10 PVC settling plates will be deployed, retrieved, and analyzed following standardized protocols. The settling plates serve as passive collectors for colonization of marine organisms, providing an easy and standardized method to assess the presence of key species. We are focusing initial attention on non-native tunicates (sea squirts), which are spreading northward along western North America.

The restriction of one site per participant/group is intended to provide good quality control and a manageable time commitment. We estimate the time required for one site (10 plates) @ 1 day every 3 months.

2. Timing. As a minimum, the plates will be deployed initially on or about 15 June and retrieved 3 months later, on or about 15 September. This standard time period allows comparison across many sites, including North America and overseas.

Ideally, the network participant/group will also be able to sample with replacement, setting out new plates upon retrieval of the initial summer collectors. For those able to implement such quarterly measures, we wish to synchronize these to occur on or about September 15, Dec 15, March 15, and June 15. Thus, at each time, new plates would be deployed as the previous plates (after being in the water for 3 months) are retrieved.

Site leaders should indicate whether they are able to implement collections at a frequency of 1x (deploy in June and collect in September) or 4x (quarterly, as above).

3. Supplies and Equipment. We will send each site leader an initial set of 20 plates (enough for 1 site), cable ties, and data sheets. We will also supply line (1/4" nylon) and bricks, as possible. We will send temperature loggers with instructions separately. We are working on locating inexpensive salinity measurement tools; if you already have a way to measure salinity, please let us know. A few common tools, clipboard, and pencils will be needed in addition.

A checklist for deployment is provided at the end of this document.

4. Site Selection and Placement of Plates. A site should be selected to provide ready access to water that is at least 1.5 - 2 meters (6 feet) deep at the lowest low tides. An ideal site is a floating dock, such as a marina or private dock. Because these float up at down with the tide, floating docks require a minimum of line for deployment (see below), whereas fixed docks and piers require sufficient line to include the distance to the water, the tidal range, and a depth of 1 meter below low tide. Depth can be estimated with a weighted line, rope, or tape measure. In addition, salinity should be no lower than 25, as tunicates are unlikely to live in such locations.

Our goal is to get representative measures of a specific area in which you are collecting. Small-scale differences in the site, such as degree of shading, current flow, and proximity to adult colonies already present, can make a difference in what settles on a plate. We try to capture all this variation by distributing plates as follows:

- 5 plates placed together at a core location;
- 5 plates distributed individually (one each) among 5 outlying locations.

Obviously, it is important to confirm permission from property owner, business, city, or other entity with oversight or jurisdiction. Participants will usually know members of the community, making this a formality, but an important step nonetheless.

One a site is selected, deployment occurs as follows.

Core Location: For the core location, an ideal site is a floating dock that is 5-10 meters (15-30 feet) long, where settling plates can be hung into the water from either side. If a longer dock is available, select a section that is 10 meters. Five plates would be deployed at roughly even intervals (1-2 meters apart) as described below.

Outlying Locations: Five additional locations are selected in the general area (< 1 mile from Core Location), and a single plate is deployed at each. Exact spacing will vary among sites, based upon available structures. The main goal here is to disperse plates as much as possible, to represent the general area --- but not at great distance. For example, the Core Location may be at one dock of a large marina, and Outlying Locations may be selected throughout the marina to include endpoints and center locations.

If you have access to a single dock only, you might cluster 5 plates closer together (but not less than 1 m apart) and deploy the remainder farther apart.

Key data are recorded, as described below, about the exact location of each plate at the time of deployment.

5. Permits. Most states require a collection permit to sample (collect) any marine life. This requires a brief explanation of the purpose and methods to be used. Permits are

usually renewable on an annual or biennial basis, upon receipt of a brief report of past activity.

Depending upon the location, additional permits may be required. This is often the case for state, regional, or local parks. While this is rarely an obstacle, it is also an important step in avoiding potential problems or interruption of your activity.

6. Assembling the Settlement Plate Units.

Step A: Lace a smaller (yellow in figures) cable tie from top of one hole in the plate, under (sanded) bottom of plate and up through second hole on the SAME side. Close cable tie so there is a loose loop. See Figure A.

Step B: Repeat on other side of plate.

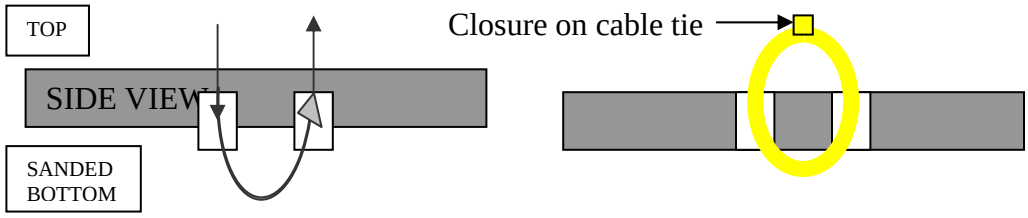

Figure A. Side views of PVC Plate Construction

Step C: Place brick in middle of plate on top (smooth) side so the cable tie loops are on the sides of brick.

Step D: Lace a large cable tie (red in figures) through one loop, through middle hole of brick, under loop on other side and back through same hole to the other side to close cable tie. See Figure B.

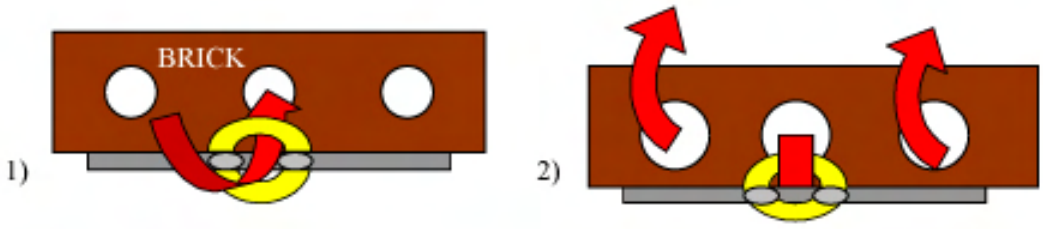

Figure B. Side views of brick attachment to PVC plate

Step E: Tighten all cable ties.

Step F: Loop a large cable tie through one hole on each end of the brick (1 tie per side). Do not close these cable ties tightly; line gets attached to these loops. See Figure C.

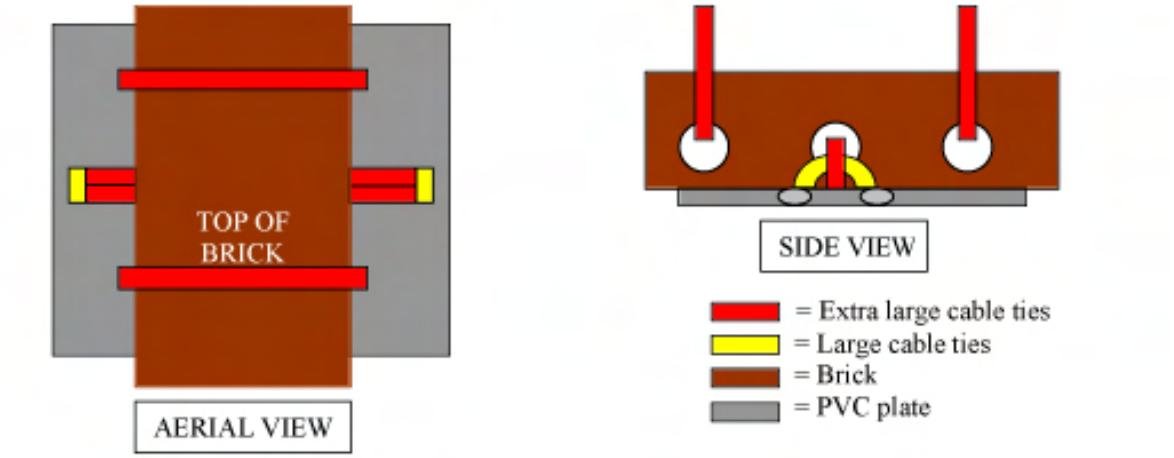

Figure C. Final views (before line attachment) of brick/plate units.

The units are now ready for attachment to a line (rope) and deployment into the water. Lines may be attached at this point or upon arrival to the field location, following instructions below.

7. Line Length. Communities of marine organisms tend to vary with depth, with some species found only in very shallow sites and others only found deeper.

To be able to make comparisons across multiple sites and dates, we have selected a standardized depth below the water surface for deployment. We also require a minimum distance from the bottom, so the settling plates are not scraped or buried by contact with rocks, sand, or mud.

Plates should be deployed such that they are 1 meter (3.3 feet) below the surface of the water at the lowest low tide. It is easiest and preferable to achieve this by deploying the plates from floating docks, which move up and down with the tide. In this case, there should be 1 meter of line between the brick and the surface of the water. *Remember: you must make the line longer than 1 meter to be able to tie it to the dock, such that the plate lies 1 meter below the surface upon deployment*.

If you do not have access to a floating dock, you can deploy from a stationary dock or pier, but you will have to figure out how long to make your line. The goal is to deploy the plate 1m below the lowest tide that will occur at that site. To do this, you will need two pieces of information, both of which can be gathered from local tide tables. You need to know the lowest tide your site will experience (these generally fall around the summer

and winter solstices). You also need to know the tide at the time you plan to set up the plates.

For example, if you put out plates at a +2.0 foot (+ 0.6 meter) tide and the tide at your site can drop to -2.0 feet (-0.6 meter), the plate must be suspended 1.2 + 1 (=2.2) meters below the surface of the water when you set it out at that time. This assures that your plate will always be 1 m below the water's surface. Thus, to deploy your plate, measure out the length of line that will go under the surface of the water, in the example given, this would be 2.2 m. Place colored tape around the line at this length, or mark the line with a thick permanent marker (Sharpie brand is good), attach the brick and plate, and lower your line until the colored tape is just above the surface of the water. If your plate touches the bottom, then you cannot deploy from this site.

Tide charts in the US generally show tide predictions in feet, so you will have to convert to the metric system to calculate length of line in meters (1 foot = 0.3 m). Tide charts can be found at fishing supply stores and surf shops and on the internet at sites such as http://tbone.biol.sc.edu/tide/ or http://tidesandcurrents.noaa.gov/tide_pred.html
.

8. Line Attachment and Deployment. Lace the rope through the two loose cable ties (see Figure C), attaching one end of the line to the settling plate unit. A bowline knot works best for this purpose (see Figure D).

Attach line to dock. Methods for doing this will depend on the dock you are working on. The most secure method is to hammer a fence staple (u-shaped nail) into the side of the dock; for this approach, the line is inside the u-shaped nail and a knot is placed in the line above the nail, to prevent slipping. Other methods include tying off to existing structures or looping line around planks on the dock. As for attachment to the settling plate unit, the bowline (shown below) is the most secure knot to use.

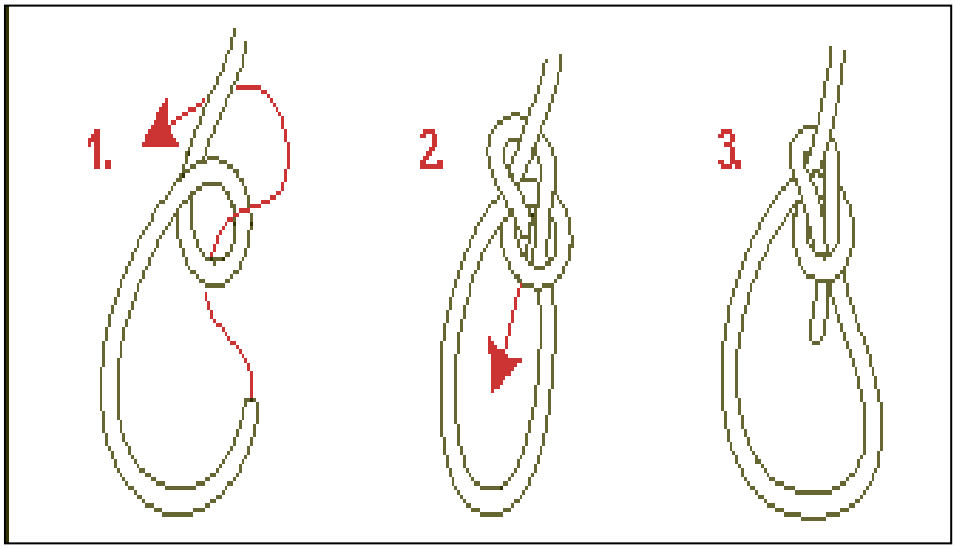

Figure D

9. Filling Out Deployment Data Sheet. When you first deploy, key information should be recorded about the deployment site. As a minimum, the following information is needed:

- Name of the site (marina or harbor).
- Location of each plate. Each plate location is given a unique identification code (state, site, number). The location is then described, using map, verbal description (e.g., Dock A, berth 22, south side), and GPS location if available.
- Date of deployment.

You should include a description that gives us a sense of both the water use and nearby land use (i.e., yacht club in urban location, private dock near forest reserve, etc.).

Some physical measures can also be recorded. As equipment is available, we wish to record surface temperature and salinity. We may also send a temperature logger to attach to one of the plates at the Core Location, providing a continuous recording of temperature.

A photograph of the specific plate locations is useful. Also please include notes on any problems encountered with setting out plates.

10. Questions & Coordination. If you have further questions or concerns about deployment, please contact one of us:

Chela Zabin (zabinc@si.edu; phone: 415-435-7128)
Greg Ruiz (ruizg@si.edu; phone: 443-482-2227)
Kristen Larson (larsonk@si.edu; phone: 443-482-2309)

Deployment Supply Checklist

Plates mounted onto bricks
Line for brick deployment
Extra cable ties
Extra line
Tools and hardware as needed for tying off bricks and cutting line (possibly hammer, nails, and knife)
Measuring tape
Local tide tables (Possibly for first deployment, if from non-floating dock)
Deployment data sheet
Clipboard
Pencil

Thermometer
Camera (Optional for deployment)
YSI or other device for measuring water temp, salinity (optional)
GPS unit (optional)

Citizen Marine Science Network:

Understanding Change in Coastal Marine Environments

Retrieval and Redeployment Protocols for the SETL Adopt-a-Plate Program, August 2007

Chela Zabin and Gregory Ruiz, Smithsonian Environmental Research Center
and Sarah Cohen, San Francisco State University

SETL: Adopt-a-plate program

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Supply list for retrieval/redeployment

We will supply the following items

Photo labels
Size bar
Retrieval/Redeployment Data Sheet
Plate Data Sheets
Vials for specimen collection
Set of 10 clean plates for redeployment
1 ibutton temperature logger
Cable ties
Extra line
Field ID Cards (We suggest that you print these and laminate or place in plastic protectors)

You will need to supply

Scissors or knife for cutting cable ties
Large tub(s) for holding plates
Small clear plastic tub for photographing plates
Bucket on a rope or other means for getting seawater for holding plates
Camera
Umbrella (for providing shade for photos)
Clipboard, pencils
Single-edged razor, tweezers, small paint scraper for removing specimens from plates
Tools and hardware as needed for tying off bricks and cutting line
GPS unit (optional)
YSI or other device for measuring water temp, salinity (optional)
Secchi disk or other device for measuring turbidity (optional)
Other field guides for your area (optional)

Part 1. Timing

Retrieval and deployment of plates is intended to occur quarterly. Plates in the water should be pulled up and new plates deployed as local conditions permit, on March 15, June 15, September 15 and December 15 each year. Please let us know if you are not able to follow this schedule.

Part 2. Field protocol

Step 1. Pull plates out of the water slowly and with caution. Place them on the dock, preferably in a bucket of seawater, with the plate side up. Use a scissors or knife to cut the plates free of the brick. Deploy clean plates and bricks (see Plate Deployment Protocol). You can re-use the line you already have if it is not too heavily fouled or worn.

Retrieve the temperature logger (ibutton or HOBO) and deploy the new one we have sent you.)

Step 2. Fill out the data sheets. Once each retrieval date, fill out a Retrieval/Redeployment Data Sheet with information about the site: specifically, the date, your name and the names of assistants, and if possible, water temperature, salinity and murkiness of water and any additional notes or observations you think are important. In addition, we ask you to fill out a Plate Data Sheet for each plate. On these sheets you should note the presence of Botryllid tunicates (see Introduction to Tunicates and Field ID Cards) and what percent of the surface area of the plate they cover. To supplement the photos, write a description of each morphospecies (each apparently unique type, based on differences in appearance), including color, texture and shape. Example: "flat yellow and orange colonial tunicate, firm to touch." You may write a description for organisms on the plate that are not Botryllid tunicates (i.e., solitary tunicates, mussels, barnacles) but this is not necessary.

Step 3. Photograph the plates. We recommend a camera with at least a 5 megapixel resolution and a macro setting. For each plate, fill out a Plate Photo Card with the relevant information. Place the plate into small clear plastic tub along with its Photo Card and the size bar. Sink the Photo Card and size bar and place along one edge of the plate. Stand over the plate, zooming in or bringing the camera close to the plate so that the plate takes up nearly the entire frame of the view finder. Reduce the glare on the water by setting up in some shade, or creating your own shade with an umbrella or your body. Glare and reflections on the water's surface make it hard to see the organisms and can confuse a camera's automatic focus. For this reason, we recommend turning off your flash. You may need to adjust brightness levels on your camera and to shoot several photos to ensure a good shot.

After you have shot the entire plate, take a close-up photo of the Botryllid tunicates on the plate (if you have several that look alike, you only need to shot one close up). Use the small photo labels (numbers 1-40) for these, one number per tunicate. Sink these and the scale bar next to the specimen or otherwise ensure that they are visible in your close-up shots. Then record this number on the plate data sheet. This allows us to keep track of which close-up shots go with each plate and to be able to calculate the size of the organism. A super macro setting may work best for these shots. If you have this feature on your camera, play back the photo and zoom in to check for sharp edges on the organism you have photographed. Below is a photo of a fouling plate, showing a photo label.

In the photos below, some examples of close ups of individual species on the plate.

Step 4. Preserve specimens. Collect a sample of each type of Botryllid tunicate for preservation. If you have several that look alike, you only need to collect one, preferably one of which you have taken a good close-up photo. The specimens should be preserved in 95 percent ethanol or Everclear and placed into the plastic vials which we will supply to you. Place the small photo labels into the containers with the specimens. Use the parafilm provided to make a tight seal over the lid. Even the best containers often leak a little, so make sure to transport and store these upright. Contact us and we will advise you on how to get the sample to us.

NOTE: If you are unable to complete steps 2-4 in the field, you can transport the plates in buckets/tubs of seawater, but make sure the plates do not jostle one another, which can knock off or damage the attached organisms, and label plates so you can remember their position on the dock. Make sure you are able to finish processing plates within 3 hours. Heat will stress and kill organisms on the plates, so don't leave these in bright sunlight or in a locked car for long.

Part 3 Managing/Sending Data

Download the photos and burn them to two CDs. Make photocopies of the data sheets and send the originals, along with one of the CDs, to us. Keep your CD and photocopies of the data sheets for future reference and to ensure that data are backed up. In the near future, you will also be able to enter the data and download the photos to the SETL website. Send us the temperature logger you retrieved in the postage-paid box.

Part 4. Finishing up.

Clean the plates and bricks. Remove remaining organisms with a paint scraper, rinse them down with freshwater and scrub with wire brush and/or sandpaper. Soak these in a 30 percent bleach solution. Now the plates are ready for reuse.

Retrieval/deployment data sheet

Collector's name
Name(s) of assistant(s)
Date of retrieval/redeployment
Bay
Harbor/marina name
Dock number/name
Finger number/location/GPS coordinates
Notes on nearby land use
Notes on nearby water use and activities
Water temperature
Salinity
Turbidity

Other observations and/or problems with plate retrieval or redeployment

Plate data sheet (use one for each plate)

Surface = Percent of surface area covered
P = Small photo label number.
V = Specimen taken (check off if specimen is collected)

Description: Color, form, size, etc.	***Surface***	***P***	***V***

Plate #	
Date	
Location	
Total # of macro shots taken this plate	
Total # of specimens taken this plate	

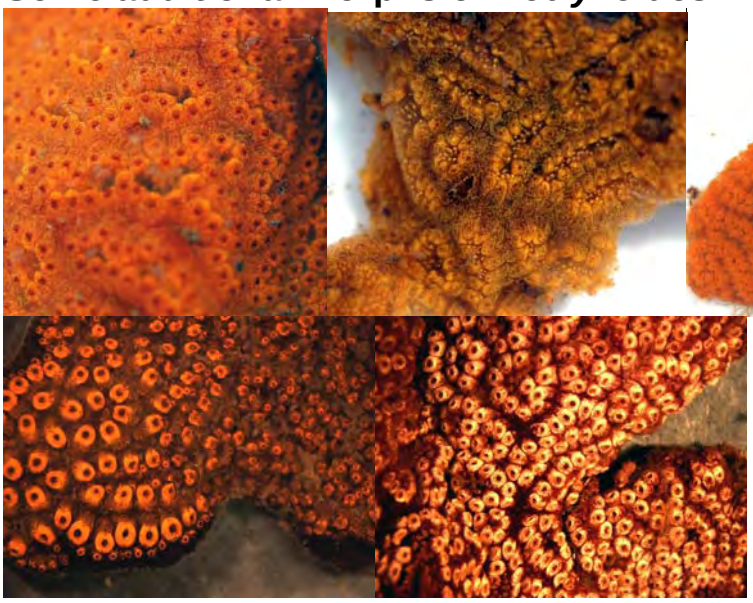
TUNICATE PHOTO IDENTIFICATION CARD

Some examples of *Botryllioides* species

Botrylloides violaceous (orange morph)

Botrylloides violaceous (pink morph)

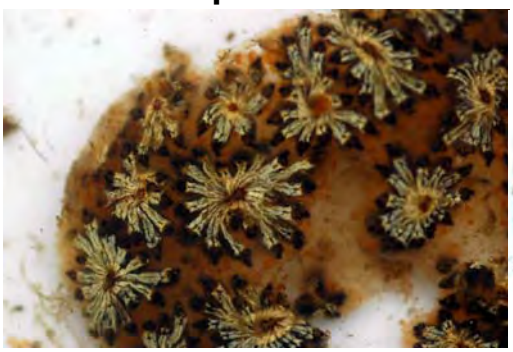
Some additional morphs of *Botrylloides*

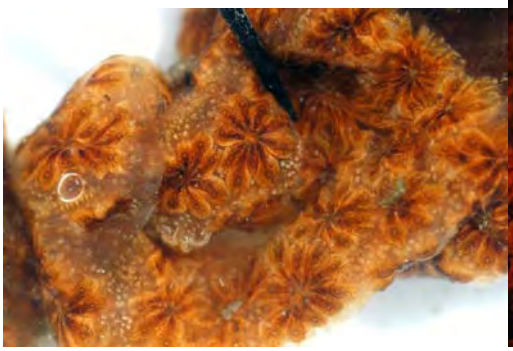


Some examples of *Botryllus* species. Note flower-like arrangement of zooids.

Botryllus schlosseri (white morph) *Botryllus scholesseri* (orange morph)

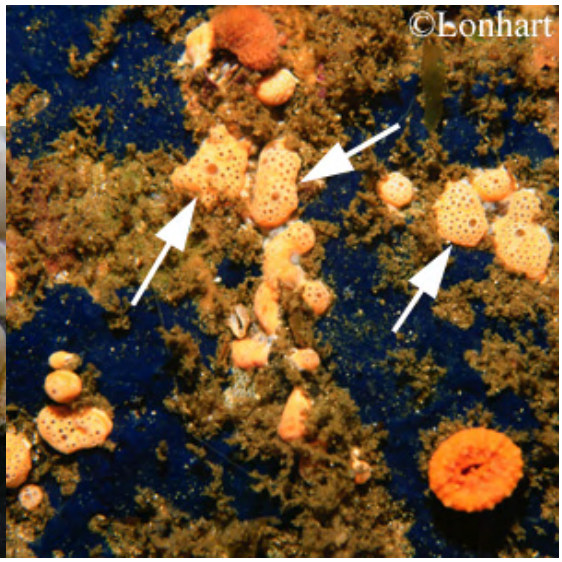
Some examples of different morphs of *Botryllus*





Some examples of other colonial tunicates

Didemnum albidum



Didemnum carnulentum

Distaplia occidentalis (left, side view, orange morph, note peduncle, or stem-like structure; right top down view, white morph top and center).

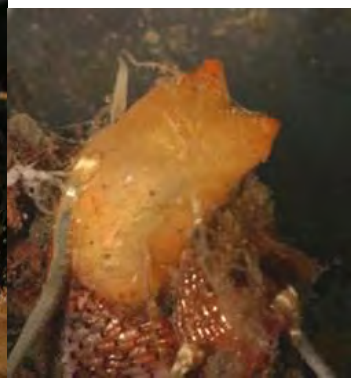
Some examples of solitary tunicates

Ascidia callosa

Corella inflata

Halocynthia aurantium

Molgula species



Styela truncata

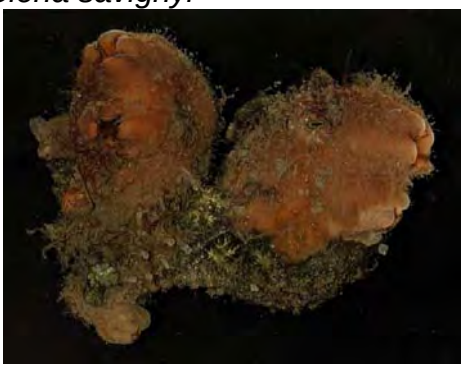
Perophora annectens, an example of a “social” tunicate: tiny grape-green tunicates connected by a runner-like “stolon.”

TO WATCH OUT FOR:

Some invasive tunicates not yet reported from Alaskan waters

Ciona intestinalis

Ciona savignyi



Styela plicata