Invasive tunicate sampling protocol (special targeted collections, non-plate) Sarah Cohen, Greg Ruiz, Chela Zabin August 2006

Colonial ascidians are highly variable in color, size, shape, and attachment substrate Botryllids may be any color including orange, yellow, black, cream-colored, red, blue, and others. They may have striking patterns with multiple colors.

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



Photos in this document show 2 general kinds of colonial botryllids: *Botryllus* spp. and *Botrylloides* spp. We would like to have samples of both.

In left photo above, a mussel with 2 *Botryllus* sp. colonies: a large orange sheet, and a small yellow/orange colony in upper left. Also present (bottom middle of upper left photo), small *Distaplia* colony (a different type of colonial tunicate that often forms stalked buttons, mushroom or club-shaped colonies, or large mounds). In contrast, 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).

In photo below, a piece of a *Botrylloides* colony. Note the meandering, less flower-shaped arrangement of modules (zooids). In right photo above, note flower-shape in close up of *Botryllus* modules (zooids).



We are interested in having samples of both of these types, *Botryllus*, with its flower-shaped zooid arrangements, and *Botrylloides*, with its more meandering zooids. We would also like samples of different colors and shapes of these two groups of tunicates as well.

We are interested in obtaining samples of any botryllid-like colonies for molecular and morphological identification. We can send tubes, pre-filled with high-grade ethanol for preserving tissues for DNA analysis. Samples of at least 2 cm square per colony are preferred, but we can use much less for DNA (as little as a few zooids, in theory). The extra material will aid in morphological work and provide backup tissue. Tubes should not be overfilled at the material will not preserve well. When the sample is placed in the tube, it should be able to slosh up and down the tube if it is tilted back and forth. So, the ethanol should be able to flow easily around the sample. Samples may be broken in half within a tube to facilitate ethanol mixing around the sample. Tubes will have small amounts of ethanol that may be legally mailed by FedEx (packages provided) back to us at our lab. They should be very well wrapped in parafilm (we'll also provide) and triple bagged in plastic bags to catch any leakage.

Sample information: Please number any tubes by writing in pencil on a piece of paper and placing that inside the tube. Also, please provide a separate piece of paper, written in pencil, stating your name, where the samples were collected, on what date, and on what substrate, whether they are abundant at your location, hard to find, or extremely hard to find. Also, if you know, please tell us other locations where they may be found. And, if you know either how long they have been in the area, and at that specific location, that would be very helpful. For the location where you collected, please tell us when you first became aware of them and how you came to notice them. Finally, if they are of either immediate or long-term concern currently to you or others in your area, please let us know about that.

For questions, please contact, Sarah Cohen, <u>sarahcoh@sfsu.edu</u>, 415-338-3750 and give your name and contact information.

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