

Sea Urchin Dissection Protocol

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Before you begin:

1. Make sure you are working in a clean area/bench top
2. Always wear latex or nitrile gloves during dissections
3. Remember to wear eye protection when working with liquid nitrogen
4. If collecting tissues and then immediately shipping them, make sure the dry shipper is 'charged' and the FedEx paperwork is ready to go*

Sea Urchin Species (adults only)

- *Heliocidaris erythrogramma*
- *Holopneustes purpurascens*
- *Centrostephanus rodgersii*
- *Tripneustes gratilla* (if you get it great, if not, no big deal)
- (2-3 individuals for larger urchins; 5-6 individuals for smaller urchins)

Sea Urchin Dissections

1. Wear new latex gloves at all times
 - a. The human hands contain RNA degrading enzymes that can digest delicate RNA molecules
2. Pre-label the cryovials with
 - a. Date of dissection
 - b. Urchin genus and species name
 - c. Collection location
 - d. Tissue type (example: digestive, intestine, stomach, etc)
 - e. ID number (example: Individual 1)
3. Dissect each urchin one at a time. Use a clean plastic dissecting tray or pan.
4. Make a cut through the circumference of the urchin with sharp scissors or bone shears (Figure 1). Be careful not to cut too deep into the body cavity to avoid cutting internal organs.

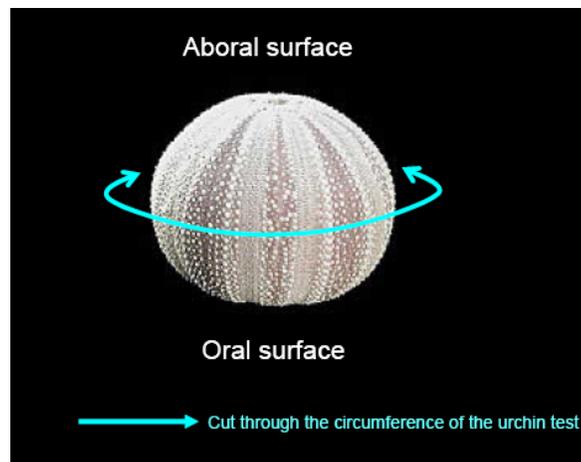


Figure. 1

5. Gently separate the two halves
6. Using clean forceps remove the digestive tissue, which includes: (see Figs. 2 and 3)
 - a. Esophagus
 - b. Cecum or stomach
 - c. Large and small intestine
 - d. Rectum

Comment on the digestive system of a sea urchin:

The large calcareous mass in the center of the floor of the body cavity is called the **Aristotle's lantern** and operates as the mouth. The lantern is enclosed in its own peripharyngeal coelom surrounded by a thin transparent **peripharyngeal peritoneum**. The mouth opens into a spacious **pharynx** entirely within the lantern. The pharynx rises vertically through the core of the lantern and emerges from it as the esophagus (Figure 2, Section B). The **axial gland** and **stone canal** also emerge from the center of the lantern but is smaller in diameter and extends to the **madreporite** at the aboral surface.

Shortly after exiting the lantern, the esophagus turns toward the periphery and, upon reaching the body wall, widens dramatically to become the stomach. The **stomach** (or **cecum**) makes a complete counterclockwise circle around the inside of the test on the floor of the perivisceral coelom.

The **siphon** is a thin tube on the inside curve of the stomach (Figure 2, Section A). The siphon connects with the stomach at the beginning and the end and is used as a water channel to reroute water around the digestive tissues. This is a convenient marker for recognizing the beginning and end of the stomach. It may not be obvious.

At the end of the stomach the gut reverses direction and, now is known as the **intestine**, makes a second circle, this one clockwise and aboral to the stomach (Figure 3). So the intestine lies on top of the stomach. The intestine is usually wider and flatter than the stomach but paler in color. The intestine is attached by **mesenteries** high on the walls of the test and can often be damaged during removal of the aboral hemisphere of the test. Both the stomach and intestine are very delicate and easily torn (try to keep them intact when dissecting).

Upon completing its clockwise loop the intestine becomes the **rectum** which extends vertically (aborally) to the anus that is attached to the outer surface of the test.

7. Place organs (stomach, intestine, rectum, esophagus) into pre-labeled cryovials and screw the cap on tightly. If all the tissue does not fit into one cryovial, separate the tissue and use two vials, but mark the vials (1 of 2) and (2 of 2). Do not pool tissues from different individuals. Use a separate cryovial for each urchin.
8. If the digestive organs rupture, (Don't panic), work fast and place the tissues in the cryovial and then immediately freeze according to step 9.
 - a. Ruptured organs can release digestive enzymes that can break down RNA molecules if the samples are not frozen right away.

9. Submerge the sealed cryovial containing the tissue immediately in liquid nitrogen for 1 min.
10. Use forceps to remove the cryovial and place cryovials in a -80°C freezer (ultra-low freezer) until you are ready to ship me the samples in the dry shipper. Tissues must stay at -80°C until shipment.
11. Clean off all metal dissecting tools with ethanol and wipe dry with a Kimwip or paper towel for the next urchin dissection. Rinse dissecting pan with water between dissections.

Charging the Dry Shipper with Liquid Nitrogen*

Vapor shippers (or dry shippers) are ideally suited for the transportation of cryobiological materials. The liquid nitrogen is retained in an absorbent material and the cold nitrogen vapors maintain the Cryo Storage Area at -190°C. The absorbent retains the liquid nitrogen and prevents accidental spills (See Figure 6).

Always use extreme caution when handling liquid nitrogen, or transfer vessels or any objects which have come into contact with liquid nitrogen. Always wear proper safety attire, glasses, cryogenic gloves, etc.

I am going to try and purchase a dry shipper through KIScientific (an Australian supplier of scientific ware). This product will come with an instruction manual, but I will briefly go over how to fill a dry shipper for transport of frozen samples.

1. Carefully inspect the dry shipper for cracks or damage upon arrival.
2. Start charging the dry shipper 4-6 days prior to shipment. Make sure you have sufficient liquid nitrogen for charging (~ 10 liters should be plenty)
3. Take out the metal cylindrical sample holder before filling with liquid nitrogen.
4. Add 2 liters of liquid nitrogen to a warm vessel.
5. Allow this amount of liquid nitrogen to sit in the covered vessel for a minimum of 2 hours. This liquid will be absorbed into the sides of the vessel. This will limit the stress caused by sudden temperature change associated with adding liquid nitrogen to a warm vessel.
6. Add additional liquid nitrogen to the vessel, 2 liters at a time. I usually add 2 liters, then check the dry shipper 2-6 hours later to see if all the liquid nitrogen has been absorbed. If most of it has been absorbed, I add an additional 2 liters of liquid nitrogen and keep repeating this process until **you are able to maintain the inner chamber half full with liquid nitrogen**. You can check the level of liquid nitrogen in the dry shipper by using a flash light. If you see that half of the inner chamber is full of liquid nitrogen and this level is maintained for more than two hours, the dry shipper is “fully charged”.
7. It is OK to leave the inner chamber filled with liquid nitrogen for an extended period of time prior to shipment (3 days); it won't hurt the shipper, but this liquid will be dumped out prior to filling the chamber with samples.
8. Before I put my cryovials in the dry shipper, I drain any remaining liquid not absorbed in the dry shipper by gently tipping it over. Once the bulk of the “nonabsorbed” liquid is out, I then place the cylindrical sample holder back in the dry shipper, place in my

cryovials, and place the cap back on the dry shipper and secure the cap with zip ties (or cable ties).

9. Tape one of Peter Steinberg's business cards to the outside of the hard plastic shipping container. Write UNSW or put their logo on the outside of the container. **VERY IMPORTANT:** Dry shippers must have the following written on the outer packaging to indicate the shipment is not restricted or non-hazardous (DRY SHIPPER – NON-HAZARDOUS, NOT RESTRICTED (IATA-FX-08)). (See Figure 8)

10. FedEx papers should be filled out in advance to avoid shipment delay and the samples will be shipped to the following location:

c/o Kristen Whalen and Gretchen Hofmann
University of California, Santa Barbara
Marine Science Institute
Building 520, Room 4013
Santa Barbara, California, 93106-6150, USA

Phone: 1-805-893-6176

Fax: 1-805-893-4724

Ship the dry shipper using FedEx International Priority Freight having the package arrive on or before September 9, 2008*.

If you have any questions or concerns please email me at:

kwhalen@whoi.edu

Figure 2.

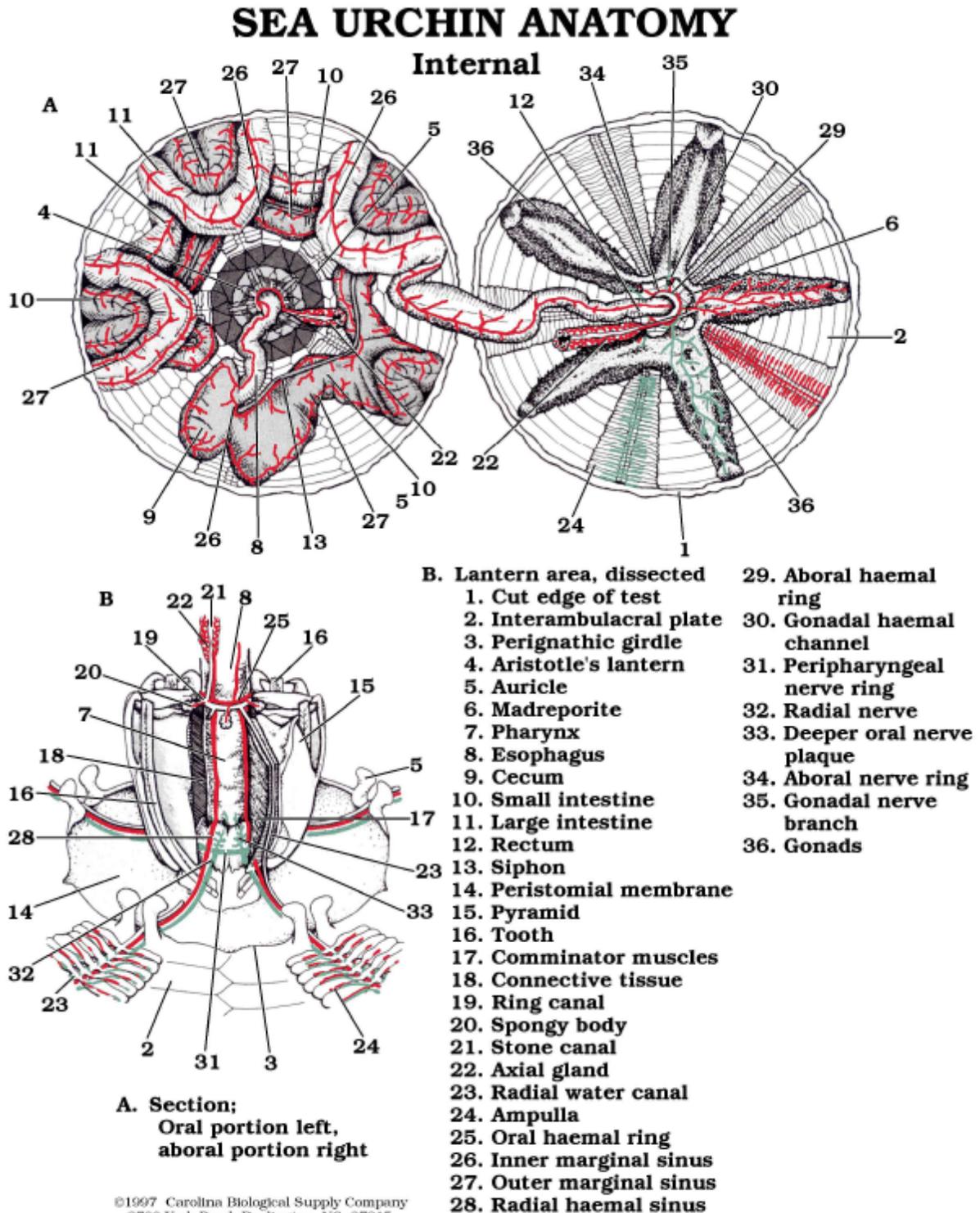


Figure 3.

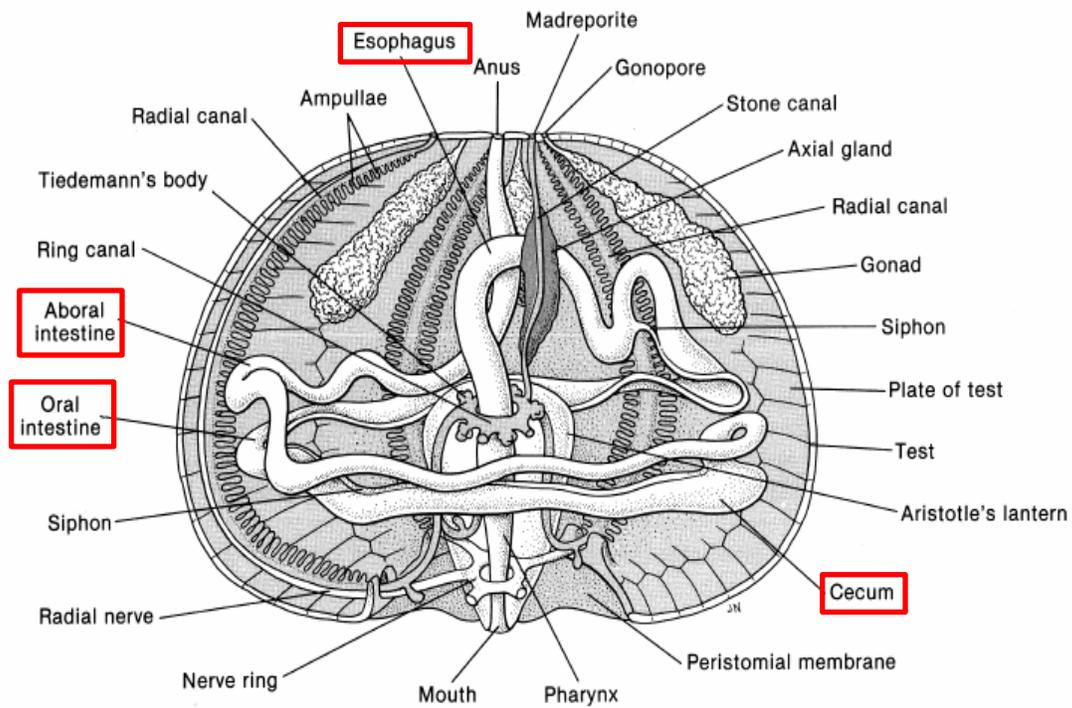


FIGURE 18-35 Internal structure of the regular urchin, *Arbacia* (side view). (Modified after Petrunkevitch from Reid.)

Figure. 4 (An example of the internal anatomy from a black sea urchin)

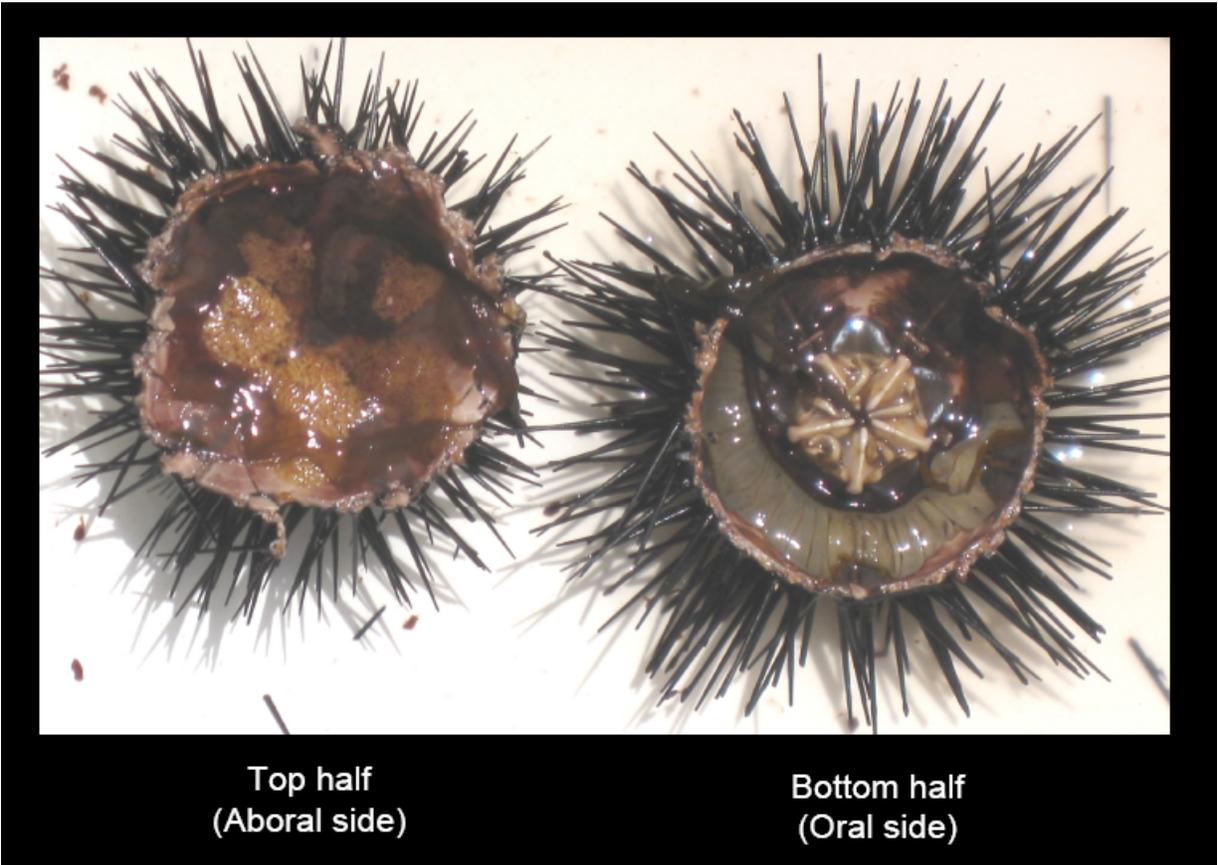


Figure 5

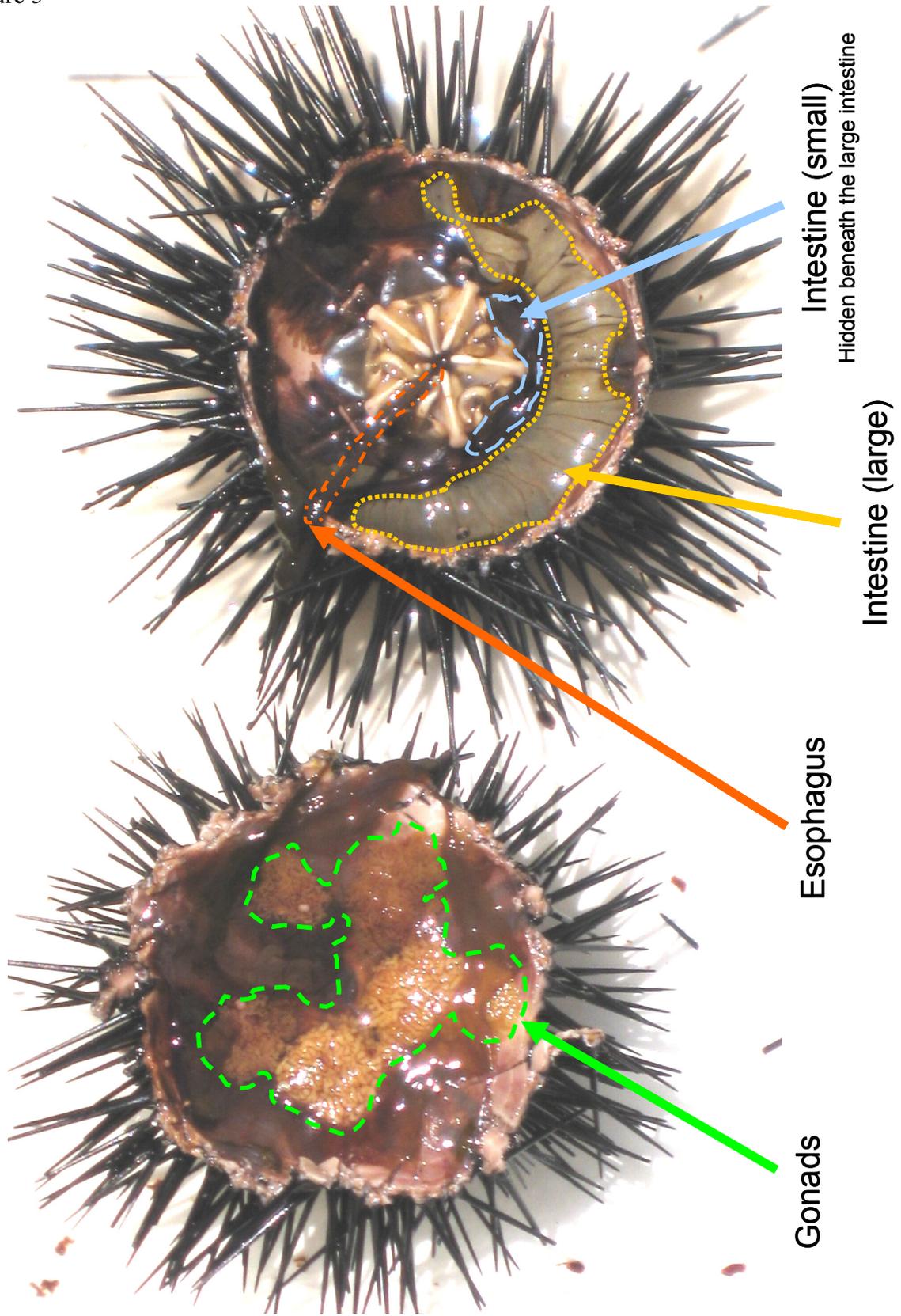


Figure 6.

Parts of a dry shipper



1 = Hard plastic case

2 = Dry shipper

3 = Metal cylindrical sample holder

4 = Dry shipper cap

Figure 7. Quick guide

Step 1: Charge dry shipper

Step 2: Dump out excess LN₂ and insert the cylindrical sample holder

Step 3 Load the dry shipper with cryovials you have stored at -80°C

Step 4: Place cap back on the dry shipper and secure with a cable tie (zip tie) for transport.



Figure 8.

REMEMBER TO:

Label the outside of the hard plastic case with the following information:

UNSW logo

P. Steinberg business card



Write the following:
“DRY SHIPPER – Non-Hazardous,
NOT restricted (IATA FX-08)”