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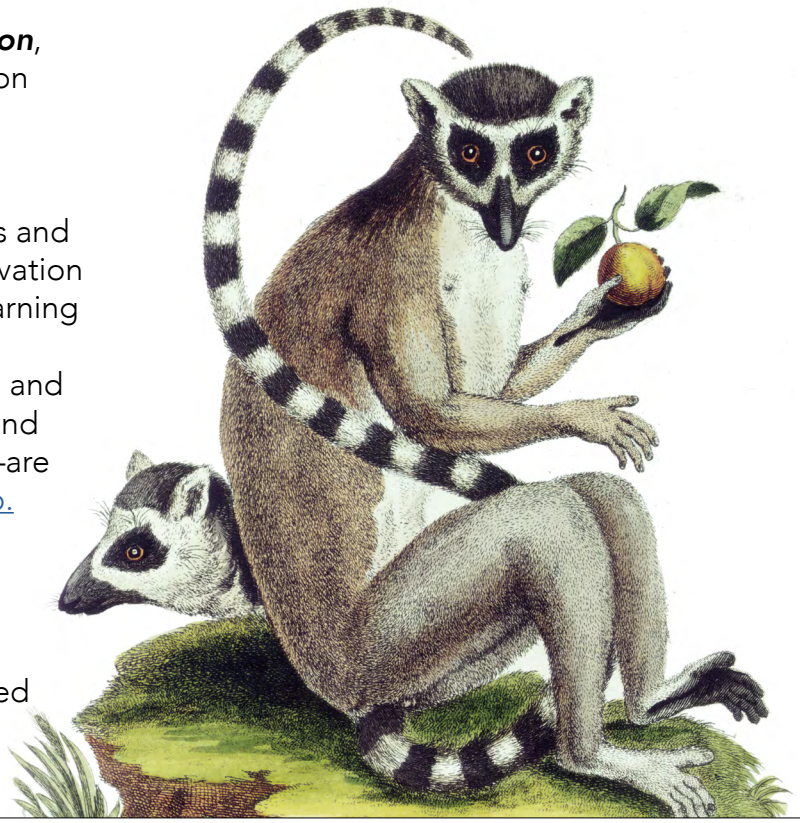

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Parasite Biodiversity: Fish Dissection and Assays for Parasites

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ABSTRACT

This exercise is a wet lab that involves dissecting an easy (and disturbing) source of live parasite material: fresh fish from your local seafood market. Students will search for both ectoparasites (on the outside of the host) and endoparasites (inside the tissues of the host). They will create a lab notebook entry, and they will also discuss observations of parasites within their fish and patterns among other fish dissected in the class.

INTRODUCTION

It's tough to learn fish ecology without ever seeing a fish in its natural habitat. By the same token, it's tough to learn parasite ecology without ever seeing a parasite in its natural habitat. For endoparasites, the innards of hosts are prime habitat; getting to know the parasites often involves getting your hands dirty. To view parasites in their natural habitat, dissection of freshly killed hosts provides the best material for examining living parasites.

In this lab exercise, you will be examining and dissecting freshly collected teleost (i.e., bony) fishes. Fishes serve as intermediate and/or definitive hosts for a diversity of parasites—you may be surprised to find that a fish you might purchase from the market has many parasites living in and on it.

In order to find parasites within your host fish, you will need to develop a "search image." A search image is a picture in your mind that you can match to the outside world to recognize an organism. The better your search image is, the easier it will be for you to see parasites in host tissues, but it takes time and care to develop a search image. You will be searching for both endoparasites (i.e., parasites that live within their host), and ectoparasites (i.e., parasites that live on the external surface of their host). Many parasites are small, so make sure to take your time while dissecting your fish. Review the representative drawings of fish anatomy (Figure 1) and fish parasite taxa (Figure 2) before you begin your dissection.

LEARNING OBJECTIVES

By the end of this exercise, students will be able to:

1. Safely perform a parasitological dissection of a fish.
2. Locate parasites within host tissues.
3. Classify parasites found into broad taxonomic groups.

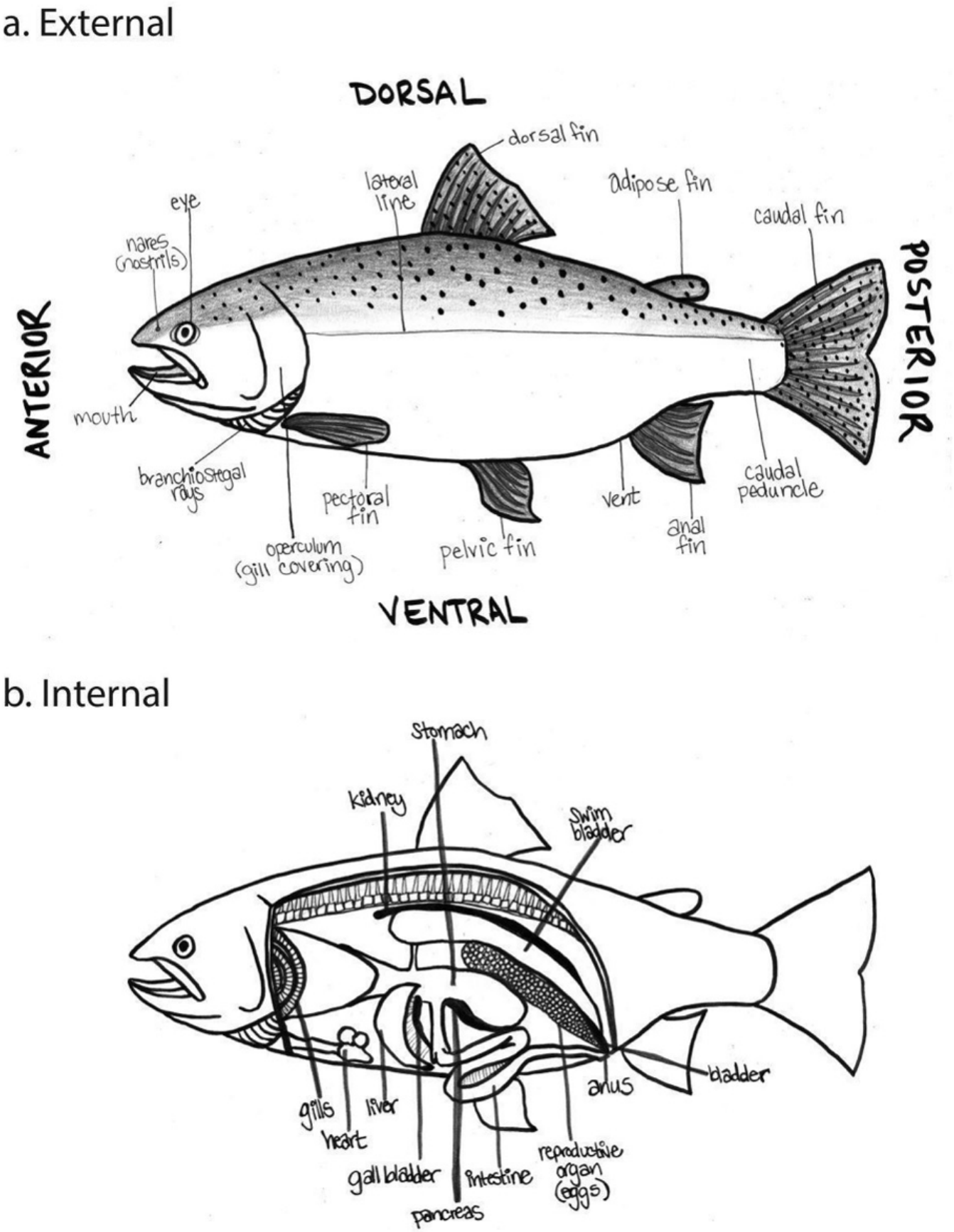


Figure 1. Teleost fish anatomy. a) External anatomy, b) Internal anatomy. Image credit: Danielle Claar.

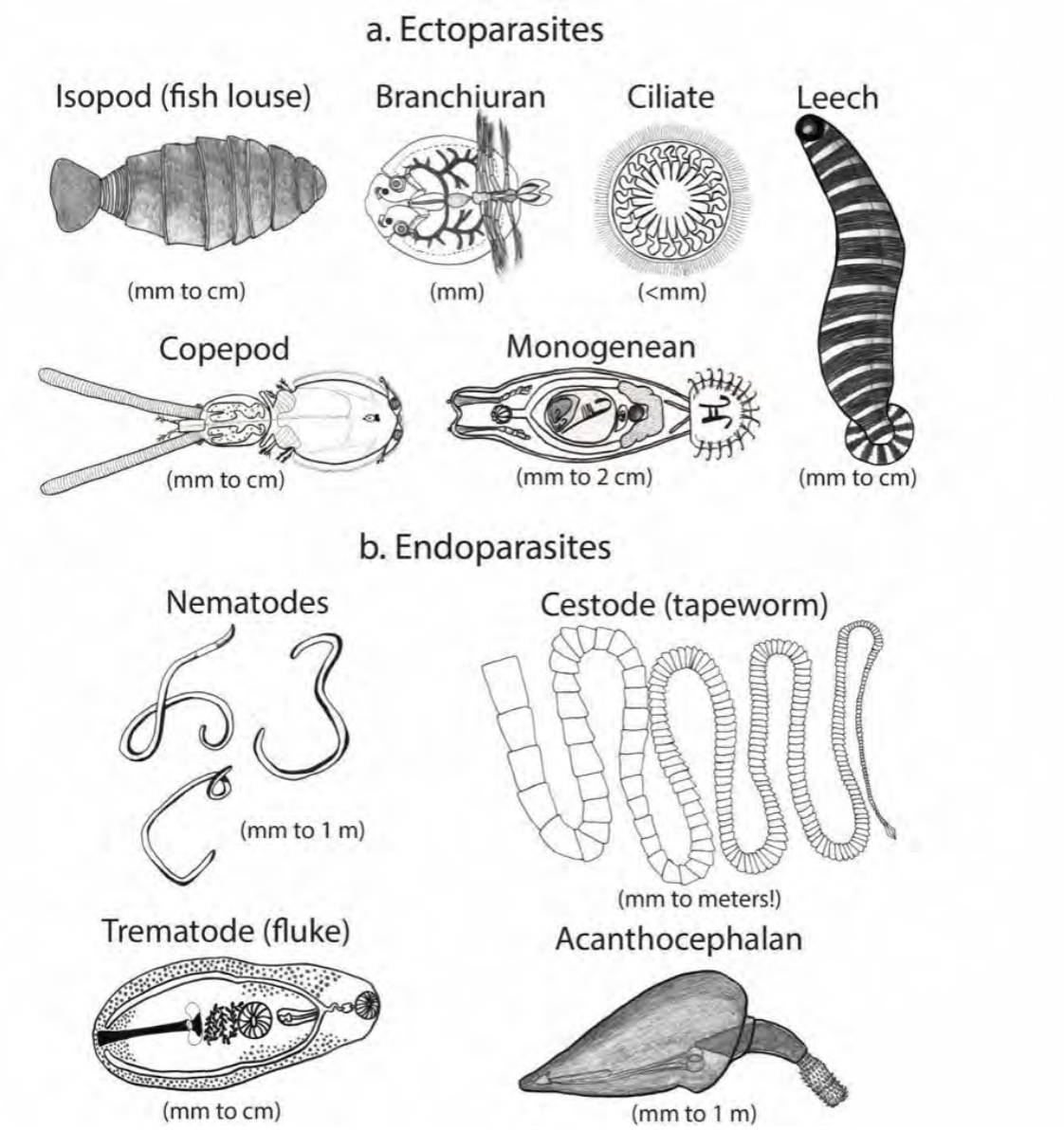


Figure 2. General types of fish parasites. a) Ectoparasites, which are found on the external surface of their host, and b) endoparasites, which are found internally within their host. Note that images are not to scale, but an approximate scale is provided for each parasite type. Image credit: Danielle Claar.

MATERIALS

- Fish
- Dissection tray
- Ruler to measure fish size
- Probes
- Small, fine-tipped forceps
- Dissecting scissors
- Scalpel
- Glass microscope slides and cover slips
- Glass dishes/watch glasses/bowls
- Squirt bottle of salt water
- Dissecting microscope/stereomicroscope and compound microscope

FISH DISSECTION PROCEDURE

Before Dissection

1. Read Box 1 to review all safety recommendations.
2. Confirm you have the needed materials (listed above) and obtain your fish and place it on a dissection tray.
3. Review the "Expected Results" section of the Student Resources handout, to get an idea of what you are likely to find.
4. In a lab notebook, record the species of the fish that you will dissect and the collection data supplied (this information will be provided by your instructor).
5. Record the length measurements of the fish: total length, standard length, and fork length (see Student Resources Figure 4 for how these measurements are made). Be sure to always use metric units (e.g., cm, mm)

Box 1. Safety recommendations

1. Wash your hands thoroughly with soap and water after handling fish.
2. Wear protective gloves. This is mostly to protect your nose—fish are smelly and handling them without gloves will leave your hands smelling fishy for days.
3. Beware of fish's sharp parts. Many fish species have sharp dorsal spines, so be especially carefully when holding the fish from the dorsal side.
4. When using scissors, scalpels, or knives, always make sure that you are cutting away from yourself and that your lab partner is standing behind you or at a safe distance. Handle sharp objects with care.
5. You might think it's a good idea to eat a filet from your fish after you dissect it. Do not do this. To be safe for consumption, fish filets need to be maintained at a cool temperature (40°F or below) and in a clean place (i.e., not a dissection pan). As fish warms to room temperature, dangerous bacteria can grow on it. For more information on safe handling of fish intended as food, check out the US Food and Drug Administration website.

Documenting Your Observations

This is a three point overview that you should read through prior to starting the parasite assays. The actual steps of the assays are in the sections below.

1. As you dissect your fish, you will remove each parasite you find and place it in its own bowl or watch glass (you can put parasites that are the same type and found in the same location together in one bowl). For each parasite, record the site of infection, how many parasites were present, the approximate size of the parasite, and any other distinguishing features in your lab notebook.
2. For some parasites, you may want to view them more in depth using a compound microscope. To do this, place the parasite on a slide with a drop of salt water and cover with a cover slip. Start with the lowest power on the microscope and increase magnification as necessary (but do not use oil immersion).
3. In your lab notebook, you will sketch the parasites that you see in the host (see Student Resources for an example). Don't forget to record the size of the parasite and/or the magnification used, if viewed with a microscope. You can also take pictures of parasites by holding a camera or phone to the eyepiece of the microscope. Even if you take pictures of the parasites with a camera/phone,

it is worth drawing each parasite, as drawing can help you notice distinguishing features. You are required to draw at least one parasite in your lab notebook for this exercise.

Ectoparasite Assay

Be sure to read through each step completely before starting the procedure and review the Student Resources handout.

1. Develop a search image for the parasites that you might encounter (Figure 2). Pay particular attention to the general shape as well as the size range of each parasite taxon, and where you found them on the host. Which parasites might you be able to see with your naked eye and which will need magnification to identify?
2. Search for ectoparasites: copepods, branchiurans, isopods, leeches, and monogenes. Examine the skin, fin rays, and lateral line with your naked eye, and then remove the fins using either the scalpel or scissors and use a dissecting scope to search for smaller parasites that may be hiding in the fin tissue.
3. Check for ciliates: carefully scrape mucus from the fish's lateral line using either a razor blade or a glass slide and use a compound microscope to search for ciliates.
4. Probe the mouth and nasal capsules of the fish to check for parasites.
5. Check for monogenes on the gills: examine the gills while they are still in the fish, and then carefully remove the gills by snipping the ends of them with scissors and examine them under a dissecting microscope.

Endoparasite Assay

Be sure to read through each step completely before starting the procedure and review the Student Resources handout.

1. With a sharp scalpel or dissection scissors, make a cut from the vent to the operculum on the abdomen/ventral side of your fish, but slightly off the midline so you avoid cutting through the digestive tract. The incision depth needed varies by fish and takes some practice. Begin gently and shallowly, and then cut deeper. If the fish has large/tough scales (that make it difficult to cut through) you may need to remove them by scraping before making the incision.
   - If using a scalpel, make multiple shallow cuts to avoid damaging internal organs.
   - If using scissors, carefully lift the skin away from the internal organs so that you are only cutting the skin and muscle, and not anything inside of the fish.
2. To open the fish for endoparasite examination, use the scalpel or scissors to cut two more perpendicular incisions—one behind the operculum perpendicular to the original cut towards the spine and another in front of the vent perpendicular to the original cut towards the spine.
3. To view the internal organs (see Student Resources Figure 5), either open this flap of skin/muscle or remove it, being careful not to cut the vent or other internal organs in the process.
4. If possible, determine and record the sex of your fish. If the fish is female, also record its reproductive status (see Student Resources Figure 6).
5. Remove the entire digestive tract (esophagus to vent) by snipping with scissors at the top near the esophagus and at the bottom near the anus. Place it in a dish, being careful not to puncture any part of it in the process. If you do accidentally puncture it, pay close attention to any material that comes out—noting which part of the digestive tract it came out of, and placing it in a separate bowl if possible. This could be mucousy material, or identifiable organisms like a smaller fish or crab.

6. Check the body cavity and mesenteries (surrounding connective tissue) for the presence of nematodes and cestodes.
7. Find and remove the gallbladder; place it in a small bowl with salt water.
8. Open the stomach. Record any contents found—you may be able to identify the fish's last meal. Carefully examine the stomach contents for parasitic nematodes, and record what you find.
9. Examine the intestine—this is often a hotspot for parasites. Search for adult nematodes, cestodes, and acanthocephalans with your naked eye, and then use the dissecting scope to search for trematodes. You can use a squirt bottle with salt water to "clean off" and more clearly view parasites that you find, just make sure you check that what you are rinsing away doesn't contain other, smaller parasites.
10. Search for encysted nematodes and cestodes within the fish's muscles.

Gallbladder: Myxozoa Assay

Be sure to read through each step completely before starting the procedure and review the Student Resources handout.

Myxozoa are parasitic cnidarians (i.e., they are related to jellyfish). They are very tiny, so the only way you can see them is to view them on a compound microscope.

1. The gallbladder should be in a separate bowl or watch glass. Using a scalpel and forceps, macerate (thoroughly cut up/open) the gallbladder.
2. Examine the gallbladder for myxozoan parasites (Figure 3) by pipetting a small amount of liquid (about a drop) onto a glass slide. View the slide under a compound microscope to search for myxozoans.

Recording and Collating Data

In your lab notebook, document every parasite you see. This includes writing down the identity of the parasite (see Figure 2), the approximate number you observed, the site of infection within the host, and the approximate size of the parasite. See the example lab notebook entries (Figures 7 and 8). When you draw a fish or parasite in your lab notebook, it is important to add a scale for the size of that organism (e.g., the scale bar with 1cm at the bottom of Figure 7).

As you finish your dissection, please write on the board the number and identity of each parasite your group found within each host tissue. If you don't find many or any parasites, try to examine some of the parasites your classmates have found. Make sure you write down the overall results in your own lab notebook before you leave.

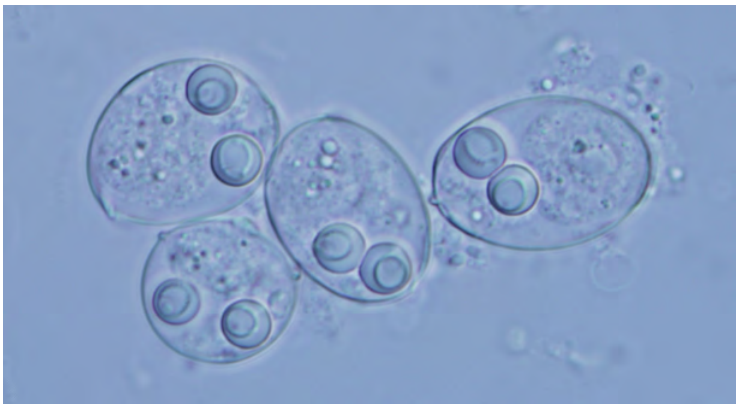


Figure 3. Myxozoa (*Sinuolinea* sp.) from a monkfish (*Lophius piscatorius*). Image credit: Ivan Fiala/ToL (CC BY-NC 3.0).

STUDENT RESOURCES

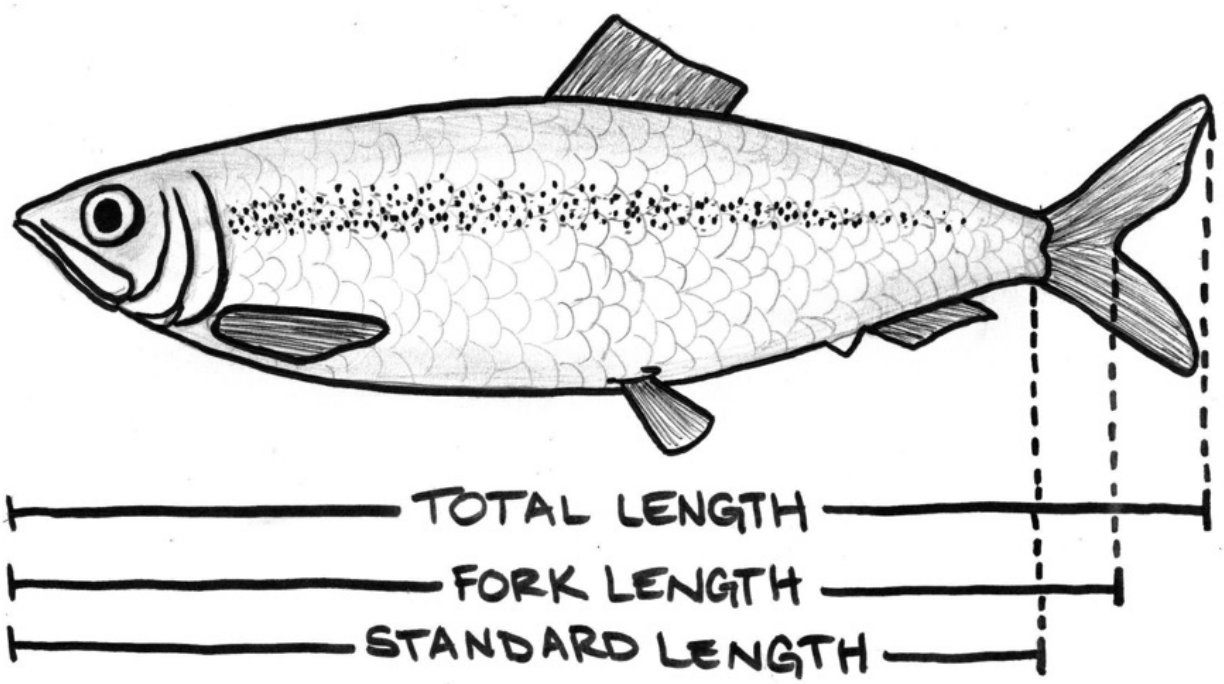


Figure 4. Measuring total length, standard length, fork length. Note: not all fish have forks, so for those fish you only need to measure the standard and total length. Image credit: Danielle Claar.

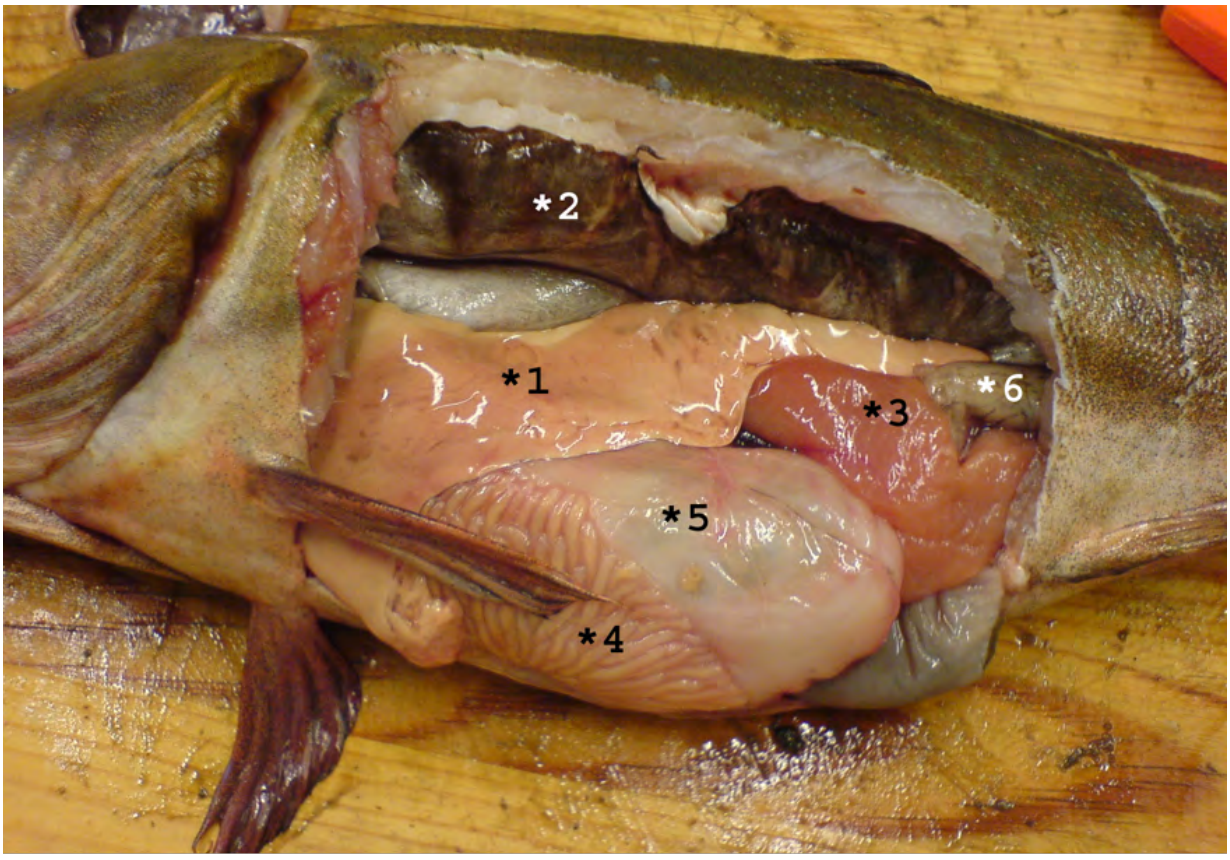


Figure 5. Internal fish anatomy image. 1) liver, 2) swim bladder, 3) ovaries/roe, 4) duodenum, 5) stomach, 6) intestine. Image credit: H. Dahlmo/Wikimedia Commons (CC BY-SA 3.0).

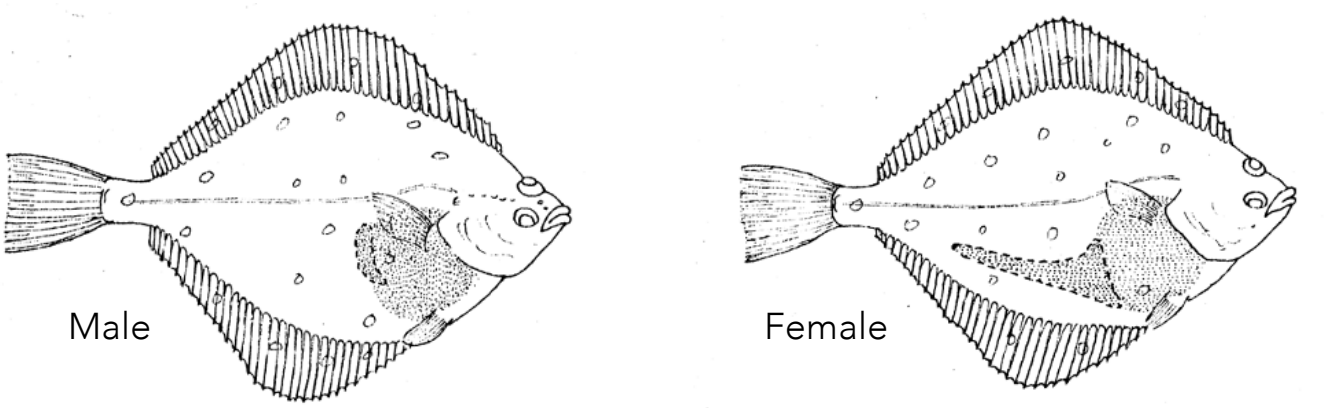


Figure 6. How to determine the sex and reproductive status of your fish. In flatfish (flounder, sole, etc.) the gonads can be found posterior to the body cavity: one on each side of a bony plate. Both testes and ovaries will appear triangular in shape, although ovaries will be more elongate towards posterior (as seen in shaded areas with dotted line borders in the images above). Image credit: Food and Agriculture Organization of the United Nations.

- For most fish (other than flatfish, see Figure 6), the gonads will be on the outer edge of the body cavity and can be found by tracing two structures attached bilaterally to the vent. Male testes will generally be elongated, sometimes stringy in appearance. Testes appear smooth in coloration and texture. Female ovaries will be plump and grainy in appearance when examined—the more defined the granulation, the more developed are the eggs.
- Females with ovaries that are smoother in color are usually designated F1 (least reproductively developed). Females with ovaries of moderate granularity are designated F2 (intermediate reproductive development). And females with clearly visible, defined eggs in their ovaries are designated F3 (highly reproductively developed, or ripe). See https://www.necropsymanual.net/en/teleosts-anatomy/reproductive-system/ for more photos of fish gonads.

Expected Results

Common infection sites in teleost (i.e., bony) fishes include:

- Exterior surface (including fins) – copepods, branchiurans, ciliates, isopods, leeches, monogenes
- Lateral line – copepods
- Gills – copepods, isopods, monogenes
- Stomach – nematodes
- Intestines – cestodes, nematodes, trematodes, acanthocephalans
- Muscles and mesenteries – larval nematodes, larval cestodes, larval trematodes
- Gall bladder – myxozoans

Fish Dissection 3

Host ID: Olive Rockfish (Sebastes serranoides)

Collection Info: Baja, CA
3 Jan 2010

Host Parameters: HL = 15.cm
SL = 34 cm
TL = 46 cm
sex = F (gonad full of eggs)
age = unknown (otoliths & scales not examined)

Ectoparasite Assay:
- dumped contents of bag used to store fish into watch glass
- rinsed bag & dumped rinse into watch glass
- examined with naked eye & under dissecting scope

* Nematodes: 8 total
- ranged between 1.5cm – 5cm
- found in material rinsed from bag

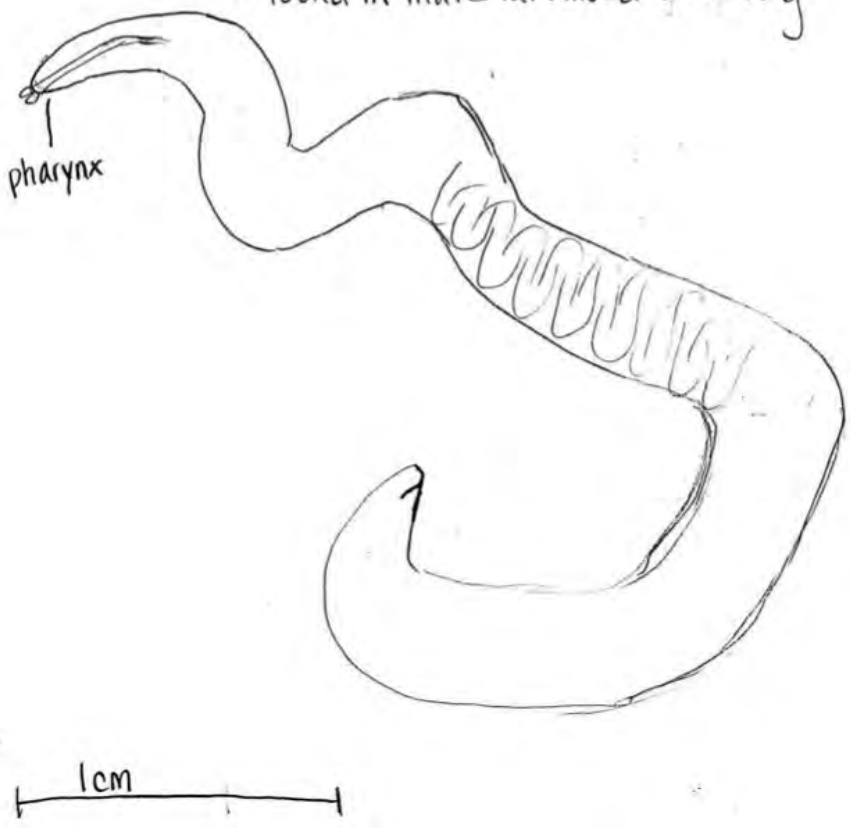
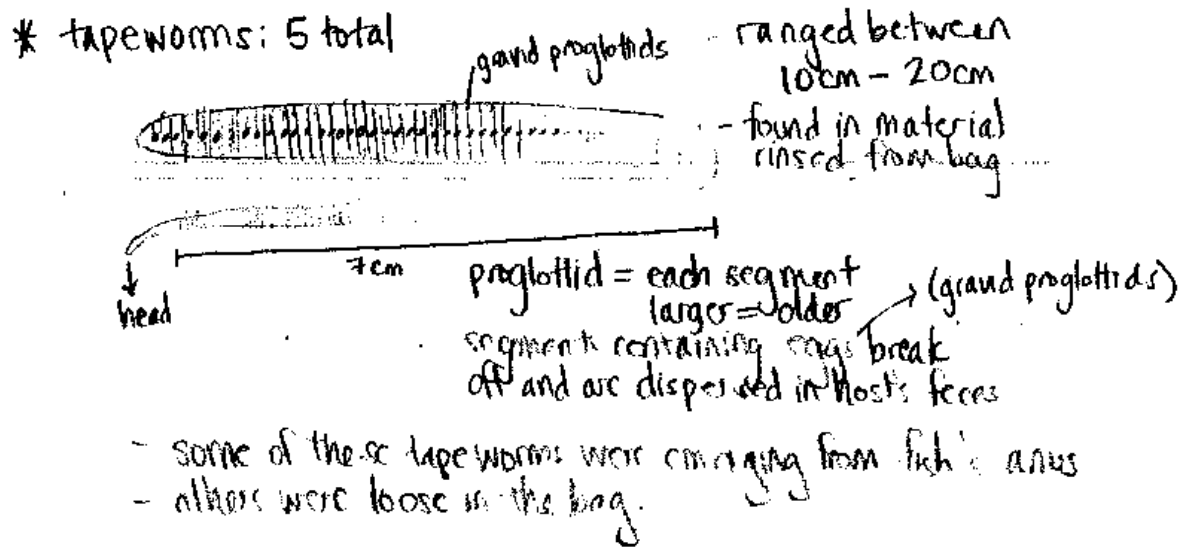


Figure 7. Example lab notebook page showing general information and ectoparasite assay. Image credit: Chelsea Wood.



- carefully examined external surfaces of fish → no parasites
- scraped mucus from lateral line & examined under dissecting scope
  → no parasites
- removed fins & examined under dissecting scope
  - L pectoral – no parasites
  - R pectoral – no parasites
  - D1 – no parasites
  - D2 – no parasites
  - Caudal – no parasites
  - L pelvic – no parasites
  - R pelvic – no parasites
  - Anal – no parasites

- removed operculum & examined gills in situ → no parasites
- removed gill arches and examined under dissecting scope → no parasites
- examined mouth & probed nasal capsules → no parasites

Figure 8. Example lab notebook page showing notes, description of a tapeworm parasite, and ectoparasite assay (i.e., examining fins under a dissecting scope). Image credit: Chelsea Wood.