

Fishing for Parasites at your Local Seafood Market

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In this workshop, we will search for helminth worm parasites in whole, un-gutted, freshly-caught fish obtained from a local seafood market. As a hands-on wet lab activity, students will use an inquiry-based approach to examine fish for the presence of helminth parasites and to assess parasite robustness against various treatments. Such treatments may include varying the temperature by freezing or heating, or altering the pH by adding vinegar or a “citrus marinade”. The fish are then dissected, and the organs are soaked in a “sea water” saline solution. When viewed under a dissecting microscope we have often observed parasitic worms swim out of the organs. The students can attempt to key out the worms, quantify, and assess selected treatment efficacy. In addition to learning a bit about the prevalence of parasites and their life cycles, they learn the importance of cooking food, or flash-freezing (as is done with sushi in NYC) in protecting oneself against ingesting parasites.

Keywords: inquiry-based learning, parasites, helminth worms, fish dissection

Link to Supplementary Materials: <http://www.ableweb.org/volumes/vol-39/Callahan/supplement.htm>

Introduction

Ingestion of fish parasites poses a major health threat to humans. Such parasites are diverse and may include a variety of helminth worms including nematodes, tape worms, or fluke worms (Lima dos Santos, 2011). For example, *Anisakis simplex* is a common nematode that persists in many fish. As an intermediate host, colonization of the fish by *Anisakis* may occur upon consumption of crustaceans harboring *Anisakis* larvae. The larvae may then encyst in the fish tissues. Infections of humans can occur when infective larvae are ingested from raw or undercooked fish (Nieuwenhuizen, 2016). Humans may serve as a “dead end host” for this nematode which can burrow into the intestinal lumen (Sakanari, 1990). In severe human infections *Anisakis* infection is treated by removal of the larvae using endoscopy or surgery (Baird, 2014). Proper cooking of fish fillets to an internal temperature of 63° C can kill most of these helminth parasites (Vidacek, 2011).

This laboratory investigates the prevalence of such fish parasites with students and determines conditions necessary to eliminate them. The idea for this lab came to us while viewing the television show “Monsters Inside Me” when a patient who consumed undercooked fish was diagnosed with the disease Anisakiasis. In the episode, parasitologist, Dr. Judy Sakanari, describes the high prevalence of *Anisakis* nematodes in freshly-caught fish

and methods which may be used to eliminate them (Hambleton, 2010). This investigative procedure does not require IACUC (Institutional Animal Care and Use Committee) approval as the vertebrates are non-living. This experiment is ideal for a parasitology, vertebrate, invertebrate biology, or a student research laboratory course. It is highly adaptable and varied based on fish species examined and treatments rendered. This laboratory exercise can be introduced in an investigative manner following a classroom discussion of helminth parasitic infections. This exercise synthesizes many areas of biology including dissection/anatomy, microscopy, infectious disease, and inquiry. Dead, raw fish and shellfish purchased at your local seafood market will often harbor live, moving, parasites that your students might enjoy seeing. In this exercise, you may purchase freshly caught whole un-gutted fish (or have students bring in their own) from a local seafood market to dissect and examine for parasites.

Students love to dissect various organisms, and they seem to enjoy dissecting fresh tissue (of course, the caveat of this lab is its odor after a prolonged stint in the lab). Students can dissect the fish and remove organs such as the gills, liver, intestines, spleen and then place them in a 3% saline solution, which can draw out visible nematode worms. Flukes and tapeworms might also be discovered. Identification of worms can be attempted by using a parasite atlas (Sullivan, 2009). Students can work in teams

and design inquiry-based experiments based on their findings. One of the most interesting and relevant inquiries that students designed was a temperature gradient to see which temperature actually killed the parasites. Other experiments students can conduct might mimic what one might do to try to kill parasites from places such as a fish filleting factory to the home including various concentrations of acetic acid, citrus marinades, or temperature treatments. The key here would be a real-world application that can extend to their kitchen- to kill the parasites, but not ruin the flesh or kill the taste of the fish.

This exercise might lead into a wider discussion of fish parasite ecology, and students might want to look at fish that harbor only one type of parasite (specialists) versus those with multiple types of parasites (generalists). They could conduct a survey of their various fishes, count

the parasites they find in each one, and track which parasites keep showing up and which ones are rare. This would be modeled after Sassal et al. (1999) who actually examined 48 different species of ocean fish! Another easy exercise for students to do is simply to measure the length and body weight of the animal as a ratio. These indicate the body condition of the animal. It is thought that fish with a larger value for body condition would contain less parasites, but, paradoxically, this is not always the case (Lagrue and Poulin, 2015). A comprehensive review article could spark discussions about climate change and parasites (Lõhmus and Björklund, 2015 and Laaksonen, 2010). Macnab and Barber (2012) discussed worm temperature preference, and how this can be coupled with host microclimate preferences.

Student Outline

Objectives

- Examine the gills, liver, intestines/stomach, swim bladder, and muscles of a freshly-caught fish for the present of helminth worms
- Identify and quantify the type of helminth recovered (e.g. nematode, tapeworm, or fluke worm)
- Determine which treatments are best at inhibiting survival of parasitic helminths

Introduction

Fish are the largest group of vertebrates, comprising nearly half of all vertebrates. Fish are highly adapted to life in an aquatic environment. However, it is estimated that the majority of fish serve as a host for a variety of parasites, including helminth worms (Lima dos Santos, 2011). Helminth worms include a variety of pathogenic phyla including Nematoda and Platyhelminthes. In this laboratory you will observe the internal and external structures of a typical untreated control un-gutted bony fish (whiting, mackerel, or similar) and examine its organs and flesh for helminth worms. In addition you will treat another experimental fish using heat or freezing, or altering the pH with a vinegar or citrus marinade. This is a highly experimental investigation and we do not know what your investigation will uncover. Many of the parasitic worms we may encounter, such as the nematode *Anisakis simplex*, trematode *Heterophyes heterophyes*, and cestode *Diphyllobothrium latrum* are linked with illness, use caution!

Methods and Data Collection

Treatment Stations (feel free to suggest/ add your own):

Heating: The heating method will “cook” your fish to a minimum desired internal temperature (65 ° C) the recommended internal temperature for fish.

Previously frozen: As in sushi, this treatment will involve previously frozen fish.

Marinating: in lemon juice, and/or vinegar of varied concentrations.

Control Untreated: Stored at 4°C and not previously cooked.

Slides of known helminth parasites you will examine include:

Nematodes: *Trichinella spiralis* Female, w.m.

Cestodes: *Diphyllobothrium latum* Eggs, w.m., Tapeworm Proglottids

Trematodes: *Fasciola hepatica* (sheep liver fluke), w.m. *Heterophyes heterophyes* (Human intestinal fluke) w.m.

Caution: Wear gloves when performing dissection and be careful not to touch spines in dorsal fin. Follow guidelines carefully in provided dissection guide.

1. Within your group decide which treatment you will use to prepare your fish from the options listed below. Work in teams to perform the desired treatment for your fish and visit the appropriate “station.” You will be comparing a “treated” fish with an untreated fish to compare worm type, worm burden, and survival. Use the provided atlas (Sullivan, 2009) and known slides for reference in identification. While your experimental fish treatment is being carefully monitored by one team member, the rest of your team can dissect and examine the control fish.

Stations and instructions:

Citrus marinade:

Soak fish in Pyrex dish containing lemon juice mixture for 30 minutes.

Vinegar marinade:

Soak fish in Pyrex dish containing vinegar solution mixture for 30 minutes.

Frying:

Using a lightly-oiled Pyrex dish sear your fish on the hot plate set to medium heat for approximately 4 minutes on each side. Carefully remove the fish with tongs, measure the internal temperature and place on ice to cool prior to dissection. **DO NOT PLACE HOT PYREX DISH ON THE BENCH! IT WILL SHATTER!**

Poaching:

Place your fish in the Pyrex dish containing the simmering water for 10 minutes on medium heat. Carefully remove the fish with tongs, measure the internal temperature and place on ice to cool prior to dissection. *DO NOT PLACE HOT PYREX DISH ON THE BENCH! IT WILL SHATTER!*

Freezing: Fish frozen for a minimum of 3 hours prior to lab session (these fish are kept on ice while they thaw).

Control: Kept at 4° C in the refrigerator.

2. **GILLS:** Using the Carolina fish dissection guide provided, work in teams to examine the gill filaments. This can be easily done by inserting the probe into the mouth and pushing through the operculum. Place the fish under the dissecting microscope to search for the presence of helminths. Note these may be quite small, as fluke worms are common in this location. Carefully remove the gill arches by cutting them out. Place gills in Petri dish of “sea water”. How many do you see? What type(s) do you observe? Are they alive or dead? Record your results for the treated sample in Table 1 and the control in Table 2.
3. **LIVER:** Next, using the dissection guide and the dissecting microscope, cut the fish ventrally by inserting scissors into the anal opening. While lifting upward on the scissors, cut from posterior to anterior toward the gills of the fish. This will expose the inner organs of the fish. Locate the liver, it should be light brown and attached to the gallbladder. Examine the liver under the dissecting scope to search for worms. The liver is often a “hotspot” for nematode worms. Do you see any? How many do you see? What type(s) do you observe? Are they alive or dead? Remove liver and place in “sea water” dish. Record your results for the treated sample in Table 1 and the control in Table 2.
4. **STOMACH AND INTESTINE:** Again using the dissection guide and dissecting microscope identify the stomach and intestine to examine for the presence of worms. Do you see any? How many do you see? What type(s) do you observe? Are they live or dead? Remove organs and place in “sea water” dish. If you are able to extract the worm with forceps do so carefully and place in tube of “sea water” and label tube. Record your results for the treated sample in Table 1 and the control in Table 2.
5. **SWIM BLADDER:** Again using the dissection guide and dissecting microscope identify the swim bladder. While viewing under the microscope, gently cut open to examine for the presence of worms. Do you see any? How many do you see? What type(s) do you observe? Are they live or dead? Remove organs and place in “sea water” dish. If you are able to extract the worm with forceps do so carefully and place in tube of “sea water” and label tube. Record your results for the treated sample in Table 1 and the control in Table 2.
6. **FILLET MEAT/ MUSCLE:** Lastly, use the dissection guide to carefully “fillet” your fish and remove its muscles. This is the part of the fish that is generally consumed. Examine the muscle carefully as worms may not be readily moving but rather “encysted” within the muscle. Do you see any? How many do you see? What type(s) do you observe? Are they alive or dead? Carefully slide the fillet and place sections in the “sea water”. Record your results for the treated sample in Table 1 and the control in Table 2.
7. Share your group’s findings on the class master data sheet for analysis.
8. If you find any interesting worms, notify your instructor and under their supervision carefully use forceps to place them in conical tubes containing “sea water.” Place tube(s) on ice.
9. Clean up: Place your fish, fish organs, and gloves in the disposal bag provided by your instructor. Carefully wash and dry your dissecting tray and dissecting tools using soap and water.

Table 1. Treated Fish Results.

Organ	Gills	Liver	Stomach/ Intestine	Swim Bladder	Muscle/Fillet
Number of parasites: Type 1 _____ Type 2 _____					
Description Type 1: Type 2:					

1. Treatment Assigned:

2. Did the treatment rendered kill the worms?

3. Which organ(s) contained the most worms?

4. Which types of worms did you observe? (e.g. nematodes, tapeworms, and/or fluke worms)

Table 2. Control Fish Results.

Organ	Gills	Liver	Stomach/ Intestine	Swim Bladder	Muscle/Fillet
Number of parasites: Type 1 _____ Type 2 _____					
Description Type 1: Type 2:					

1. Did you find worms present?

2. Which organ contains the most worms?

3. Which types of worms did you observe? (e.g. nematodes, tapeworms, and/or fluke worms)

Cited References

Lima dos Santos CAM and Howgate P. 2011. Fishborne zoonotic parasites and aquaculture: A review. *Aquaculture* 318(3):253-61.

Sullivan, JT. 2009. *A Color Atlas of Parasitology*, 8th ed. University of San Francisco 158 pp.

Materials

(This will be set up for 6 groups of 4)

- 12 Fish from your local seafood market (whiting, mackerel, or similar) ---ask for “un-gutted” (2 per group)
- Dissection pans (1 per group)
- Dissection instruments---forceps, scissors, probe, (1 set per group)
- Dissecting Microscopes (1 per group)
- Microscope Camera/ Projector (optional)
- Ice bucket (2 per lab)
- Petri dishes and/or finger bowls for excised organs (2 per group)
- “Sea water” prepare 3% w/v NaCl in distilled water (2 liters per class)
- Vinegar and lemon juice solutions (2 liters/class)
- pH meter (for preparation)
- Cooking oil (500 ml)
- Cooking stations: Hot plates (4/class)
Pyrex baking dishes or metal pans.
- Perch Dissection Mat: Carolina Item #229960
Suggested Microscope Slides to supplement this procedure (1 each /group):
- *Trichinella spiralis* Female, w.m. Microscope Slide Carolina # 307020
Diphyllobothrium latum Eggs, w.m. Microscope Slide Carolina # 306622
Fasciola hepatica (sheep liver fluke), w.m. Microscope Slide Carolina # 306394
Heterophyes heterophyes (Human intestinal fluke) w.m. Microscope Slide VWR # 470182-010
Tapeworm Proglottids Comparison, w.m. Carolina # 306610
- 15 ml conical test tubes/and or microfuge tubes for freezing parasites for future analysis
- Color Atlas of Parasitology (Sullivan, 2009)

Notes for the Instructor

This lab was designed for a class of twenty-four, consisting of six groups of four. Control fish are stored in the refrigerator at 4 ° C. Previously frozen fish are “treated” in the freezer (-20 ° C for minimum 3 hours). This lab is relatively low-cost (especially if the students bring in their own fish). It doesn’t require any IACUC proposals because the vertebrate fish are already dead, and the invertebrate parasites do not require a protocol. It is highly adaptable for species of fish examined and treatment rendered. You may also perform this lab without the treatment step, if you are just interested in examining the

parasite load. Other organs may be investigated or examined. If the students perform a cooking step, be sure to reinforce necessary safety procedures. This step may alternatively be performed in a metal pan. Hot glass Pyrex dishes may shatter when placed on a laboratory bench. If students wish to make a gradient of vinegar or citrus marinades they can use various dilutions and compare worm viability.

Disposal: We keep the fish on ice in the dissection pans to help prevent odor issues (Fig. 1), and after dissection freeze the fish carcasses until they can be properly disposed. Another alternative is to have the students take their own fish home and dispose of them. Cooking material, dissecting trays, and dissecting sets are washed with soap and water. Benches can be wiped down with ethanol or a 10% bleach solution.

During the workshop participants noted challenges from lack of worms seen in their samples. This obstacle may occur with older fish samples which no longer harbor viable worms. Whenever possible, use fish that are freshly caught (within twenty-four hours). When we performed this experiment in the New York City Metropolitan area, we used un-gutted whiting purchased at Sea Breeze Fish Market at 541 9th Avenue, New York, NY just after they opened at 8 am. The fish mongers assured us they were “freshly caught.” The fish were then stored in the refrigerator at 4° C used in the lab the same day at 1 pm. Using these whiting we have had much luck locating nematodes, particularly in the liver. At the workshop we used a regional species, the walleye which also harbored tapeworms and flukes within its organs. When performing this laboratory, it is important to include slides of expected helminth worms for reference, and for students to view.

If this procedure is performed with freshwater fish, the excised organs can be soaked in a freshwater solution. Also other organs and scales can be investigated for the presence of helminths. Our experiences have shown nematode worms are more likely to swim out of organs, while cestodes (tapeworms) and trematodes (flukes) are more likely to be located within the organs and require “surgery” to remove. Dissecting microscopes projected on a screen can facilitate location of this small organisms. Follow up studies, including DNA extraction and PCR can identify the types of helminths observed.

Supplemental video links for discussion:

- <https://www.youtube.com/watch?v=1nVLiDlzlac>
- <https://www.youtube.com/watch?v=MEDbNcFaJz8>
- <https://www.youtube.com/watch?v=XzU5LzOJ1dE&list=EL4RAHzbmvVQDjCt-He0iq5A&index=1> (Monsters Inside Me episode. Must be purchased for \$1.99)

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Appendix

Selected Results from Workshop and Classroom Exercise (Visual and Tabular)

During the major workshop, participants dissected sardines and a larger fish, walleye (**Fig. 1**) was analyzed as a classroom demonstration model. It is important to keep the fish on ice to maintain freshness and limit the odor in the laboratory. If the students are able to extract worms follow up studies DNA extractions and barcoding can identify the types of helminths observed.



Figure 1. Control (Untreated) walleye fish dissection from major workshop.

During the major workshop multiple helminth worms were found including Phylum Platyhelminthes (Classes *Cestoda* and *Trematoda*) and Phylum *Nematoda*.

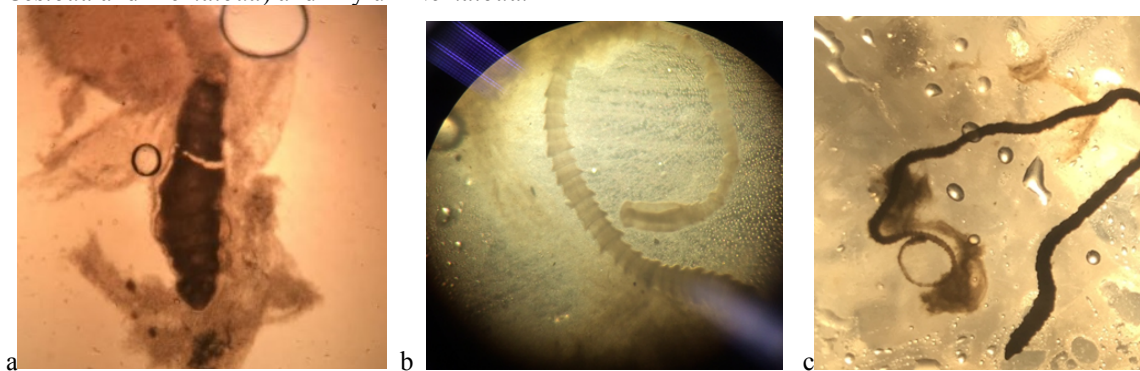


Figure 2. Dissection microscope images obtained during major workshop a) Trematode (flake) recovered from liver. b) and c) Cestode (tapeworm) recovered from the swim bladder

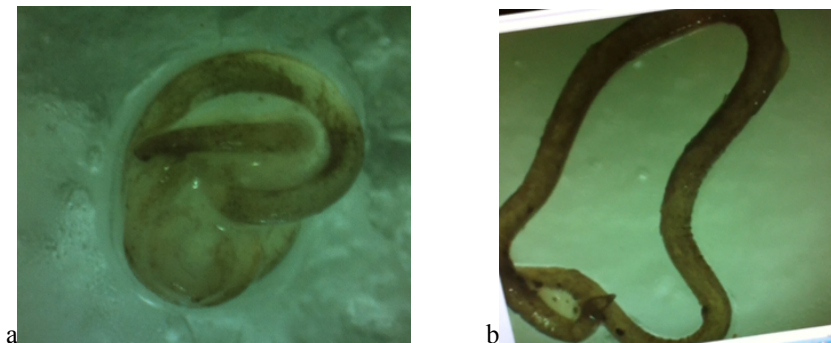


Figure 3. Dissection microscope images obtained during classroom exercise. a) Encysted nematode obtained from the liver of whiting b) Nematode identified in liver of whiting April 2016, Saint Francis College.

Table 3. Selected Tabular Results from Classroom exercise April 2016, Saint Peter's University

Organ	Gills	Liver	Stomach/ Intestine	Swim Bladder	Muscle/Fillet
Number of parasites: Type 1 _____ Type 2 _____	<i>0</i> <i>0</i>	<i>2</i> <i>0</i>	<i>1</i> <i>0</i>	<i>0</i> <i>1</i>	<i>1</i> <i>0</i>
Description Type 1: <i>yellow nematode</i> Type 2: <i>none</i>	<i>none</i>	<i>yellow nematode</i>	<i>yellow nematode</i>	<i>None</i>	<i>yellow nematode</i>

1. Did you find worms present? *Yes*

2. Which organ contains the most worms? *Liver*

3. Which types of worms did you observe? (e.g. nematodes, tapeworms, and/or fluke worms)

Yellow nematodes living

Tapeworms living

Table 4. Selected Tabular Results from Classroom exercise April 2016, Saint Peter's University

Organ	Gills	Liver	Stomach/Intestine	Swim Bladder	Muscle/Fillet
Number of parasites: Type 1 _____ Type 2 _____	<i>0</i> <i>0</i>	<i>3</i> <i>0</i>	<i>1</i> <i>0</i>	<i>0</i> <i>1</i>	<i>1</i> <i>0</i>
Description: Type 1 <i>yellow nematode</i> Type 2 Not observed	<i>none</i>	<i>yellow nematode</i>	<i>yellow nematode</i>	<i>none tapeworm</i>	<i>yellow nematode</i>

1. Did you find worms present? *Yes*

2. Which organ contains the most worms? *Liver*

3. Which types of worms did you observe? (e.g. nematodes, tapeworms, and/or fluke worms)

Yellow nematodes living

Tapeworms living

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