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March 2015, updated May 2020

LIONFISH COLLECTION AND DISSECTION MATERIALS

Collection gear:
- hand-nets and/or spear
- Ziplocs with sliding seal: ranging in size from 1-5-gal. (the sliding seal is for easier handling underwater – if you spear the fish, immediately and carefully place it in its own bag to capture any ectoparasites that may come off)
- clear dry-bag for holding live fish underwater
- goodie bag for holding equipment and samples
- cooler(s) for live and/or dead samples

General materials for dissection:
- puncture-resistant gloves
- large sharp kitchen knife
- basic dissecting kit, with at least the following items:
    - small set of sharp scissors
    - scalpel and blades
    - pipette (glass or plastic)
    - tweezers and pointed devices for manipulating contents of Petri dishes
- paper towels to blot lionfish dry
- volumetric flasks (50 mL) for interstitial fat analysis

Parasite examination materials, not already listed above:
- saline solution (about 1 quart per fish) = 1 part seawater (filtered) to 3 parts tap water
- a few pint-sized jars with lids (plastic or glass, so long as the lid seals)
- a few clear Petri dishes
- squirt bottle of freshwater (for the external parasite exam)
- container big enough to submerge body of fish in water (for external parasite exam)
- funnel (for external parasite exam)
- plankton mesh (55-micrometer filter size, for external parasite exam)
- dissecting microscope, ideally with both top and bottom light sources

Sample storage:
- Sharpie permanent markers
- 2-mL vials for storing parasites and genetic samples
- >5-mL vials for preserving larger tissue samples
- 5% buffered formalin for preserving soft-bodied parasites (trematodes, cestodes, acanthocephalans, monogeneans)
- >70% ethanol for preserving hard-bodied parasites (crustaceans, nematodes)
- >70% ethanol for preserving genetic samples

SAFETY: If you get spined by a lionfish, immerse the affected area in non-scalding hot water immediately! Most incidents occur when dissecting/filleting dead lionfish, even lionfish that were previously frozen (only heat denatures the venom). If the effects are serious, DAN insurance covers care for lionfish stings (http://www.alertdiver.com/Revenge_of_the_Lionfish).

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EXTERNAL ANATOMY

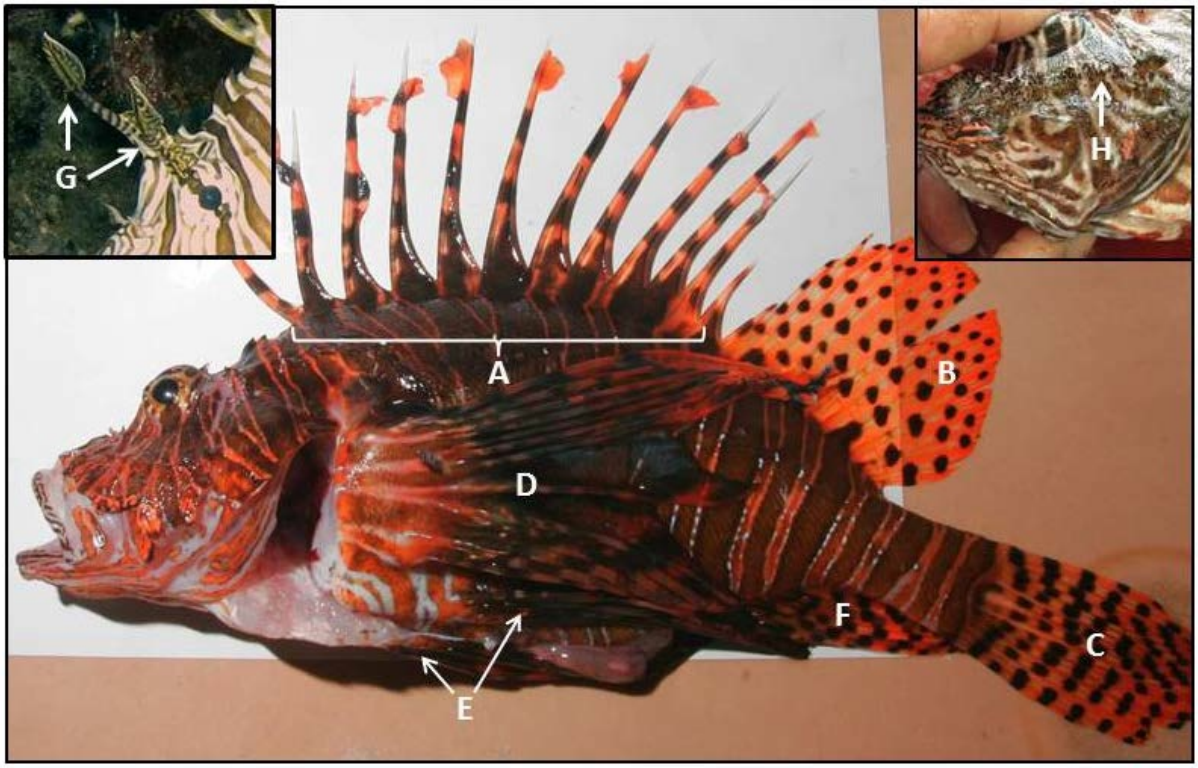

Fig 1. Lionfish external anatomy. Photo credit C. Calloway (main photo).

Code	Description	Venomous	Notes
A	Dorsal spines	Yes	13 spines, all are venomous
B	Soft dorsal	No	Secondary dorsal fin; non-venomous
C	Caudal fin	No	Non-venomous
D	Pectoral fin	No	Non-venomous
E	Pelvic fin	Yes	First spine on each pelvic fin is venomous
F	Anal fin	Yes	The first three spines of the anal fin are venomous
G	Super-ocular tentacles	No	Missing in this specimen; *left inset*
H	Opercular spines	No	Larger individuals may have prominent bony protrusions on the operculum and head; *right inset,* also referred to as "cheek mail"

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INTERNAL ANATOMY

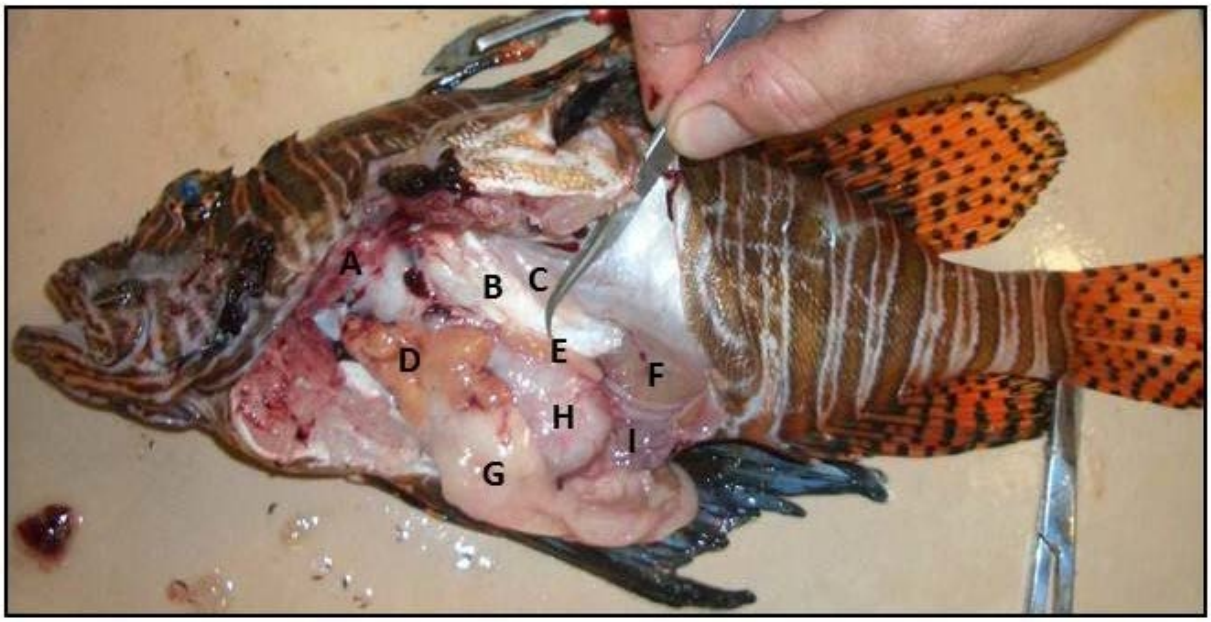

Fig 2. Lionfish internal anatomy.

Code	Description	Notes
A	Gill rakers and filaments	
B	Swim bladder	Often over inflated in specimens brought up from depth; may be deflated in speared fish
C	Swim bladder muscles	Used to control position of swim bladder, often confused with gonads in smaller specimens
D	Liver	Color can be an important indicator of health
E	Gonad (this specimen: testis)	Often lay along inflated swim bladder Two gonads; second in same position on the other side of the body; Males: testes are thin and elongated with edges Females: ovaries are rounded. Size depends on development (see additional photographs figures below)
F	Urinary bladder	Clear membrane, usually only visible when filled with urine; Easily ruptured during dissection
G	Interstitial fatty deposits	White tissue, often surrounding stomach and intestines
H	Stomach	Can expand up to ~30 times when full
I	Intestine	Often contains dark-colored digested matter

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LIONFISH DISSECTION METHOD

(0) *Killing the lionfish*

If the fish is not already dead when you begin the exam, a quick way to kill the fish is to use a sharp kitchen knife to cut through the top of the head down through the spinal cord. Be careful not to pass through the area of the head where the otoliths are (see step 12 below). Keep in mind that this may affect the weight and length measurements of the fish. If you plan to do a gill and/or external parasite exam, you should not euthanize the fish with any sort of chemicals because chemicals also affect any attached parasites.

External measurements and sampling

(1) *Fish information*

Begin by writing down important details for each fish, and take pictures if you think that will help document what you see. Briefly examine the external surface and in the mouth of the lionfish for any obvious parasites or other interesting characteristics.

- specimen ID number(s)
- date (make a note if date of exam is different from date of capture)
- location where fish was collected (and any known details about the habitat/depth at which it was found)
- whether the specimen was previously frozen or preserved
- other notes: e.g., unusual color, emaciated, clouded/beady eyes

(2) *Weight*

Before weighing the lionfish, place your weight boat or specimen tray on the scale and tare it. Blot the lionfish dry using paper towel, as excess water can be a large source of error. If the specimen has been euthanized in ice slurry, check the mouth cavity for any remaining ice. When balancing the lionfish on the scale, ensure that no portion of the fish is touching the table (Fig. 3A). Read and record the unit weight measurement in grams. **Note**: When possible, do not take weight measurements on a moving surface (i.e., boat) as the movement of the vessel will make accurate weight measures difficult.

(3) *Length*

Use a metric fish board or metric measuring tape secured to a flat surface for all length measurements, and be sure to place the lionfish ON TOP of the measuring tape. The tape should run parallel to the fish's midline from snout to tail (Fig. 3B). Placing the measuring tape on top of the fish introduces measurement error due to curvature of the tape. To measure total length (TL), obtain fish length from the tip of the snout to the longest point on the tail (caudal fin), recording to the nearest 1 mm (Fig. 3B). Ensure that the specimen's mouth is closed during measurement. Measure standard length (SL) as the length from the tip of the snout to the last vertebrae to the nearest 1 mm. The last vertebra is at the base of the end of the fleshy portion of the caudal peduncle (Fig. 3B) and can be located with manual probing or by flexing the caudal peduncle to find the last vertebrae. **Note**: Accuracy of weight and length measurements may decrease once fish body cavities have been opened or altered (through filleting or other handling procedures). These conditions should be noted in any altered specimens.

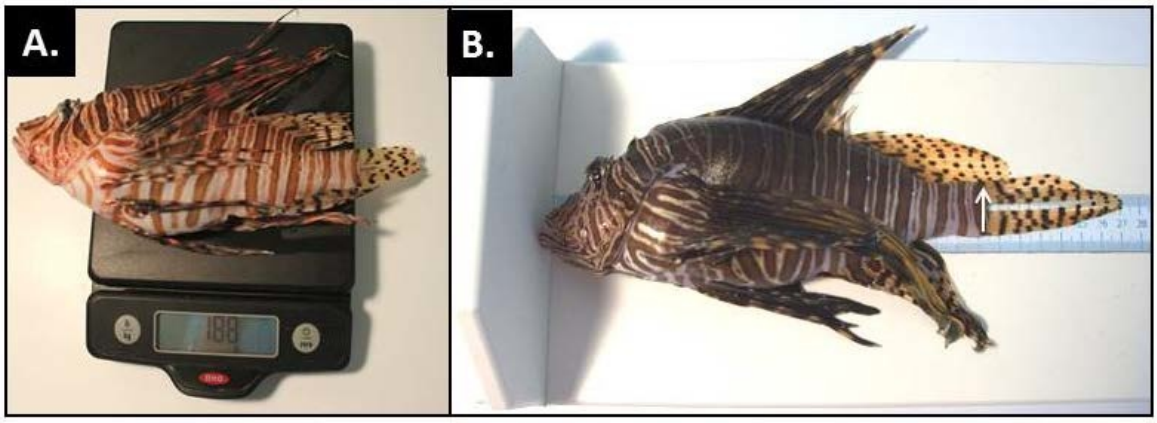

Fig. 3. A) Lionfish weight measurement. Ensure that the specimen is thoroughly blotted dry and that the entire specimen is balanced on the scale. B) Measuring lionfish total and standard length to the nearest 1 mm. The arrow indicates the location of the base of the caudal fin.

(4) *Meristics*

Count the dorsal spines and fin rays, the anal spines and fin rays, and the pectoral rays of all lionfishes collected (see Fig. 1, External Anatomy).

(5) *Tissue sampling*

When collecting tissues for genetic purposes, it is important not to contaminate samples. Ensure that all dissection equipment (i.e., scissors, scalpels, forceps) are cleaned with ethanol prior to taking each sample. All the tissues listed below will provide ample DNA for genetics studies and can be placed in fixative (ethanol or DMSO buffered solution) or frozen.

- *Gill tissue:* Using dissection scissors, remove a 1-cm^2 section of gill tissue from an inside arch including both arch and filament (Fig. 5A).
- *Fin clip:* Remove a 1-cm^2 section of pectoral fin, including membrane using dissection scissors or a scalpel (Fig. 5B).
- *Muscle tissue*: Using dissection scissors or scalpel, dissect a 1-cm^3 section of white muscle tissue (including skin) from the shoulder of the fish (behind the head and above the lateral line; Fig. 5C).

Fig. 5. Sample collection of three tissue types for genetic and stable isotope research: A) gill, B) pectoral fin, and C) muscle tissue. Tissue size for gill/fin sample is 1-cm^2 and for muscle is 1 cm^3.

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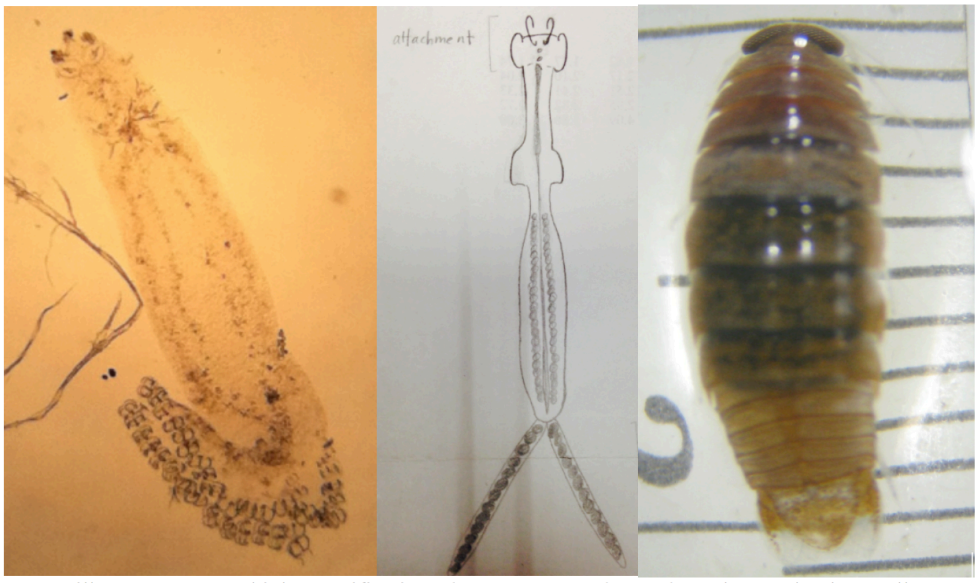
(6) *Gill parasites*

This method is designed to dislodge parasites from gill tissue. It relies on the fact that parasitic worms and crustaceans are more dense than saline and will sink over time. Once you begin the parasite exam, please record all information on the 'Lionfish Parasite Exam' data sheet.

1. Prepare a jar, filled up to about 80% capacity with saline solution (keep lid off).
2. With a clean set of scissors, snip the arch bones of the top gill at top and bottom, and place the gill arch in your jar of saline. Be sure to cut the top end of the arch first (if you cut the bottom end first you risk flooding the gills with blood, which makes microscope examination more difficult).
3. Also cut out the second gill arch on that side, and the front 2 gill arches of the other side and place them in the same jar of saline.
4. Screw the lid on the jar and shake **vigorously** for 30 seconds.
5. Put the jar on the counter and immediately unscrew the lid; this will save you from disturbing the sediment after all has settled.
6. Wait 5 minutes for the contents to settle.
7. Using your pipette, remove any sediment from the bottom of the jar a Petri dish. Carefully remove the gill arches from the jar with your tweezers, and place them in the same Petri dish.
8. Place the Petri dish on the dissecting scope, and examine both sides of the gill arches at the lowest magnification, looking for large gill parasites. At the highest magnification (often ~60x), look for small parasites (often partially transparent, round "monogeneans") attached to the arch bone or gill filaments.
9. Note the number of each type of parasite found. If there are many parasites, I usually remove them to a different Petri dish where they are easier to see, keep track of, and count (so you're not counting the same parasite twice). If you can, take a picture of the parasite by putting a camera to the eyepiece. ***Drawing simple pictures is extremely helpful, and encouraged!***
10. Preserve any parasites found in a centrifuge tube (or more than one if necessary) with formalin (for soft-bodied parasites) or ethanol (for hard-bodied parasites). **Label the tube with the date, location, lionfish ID, solution (e.g., "5% formalin," "70% EtOH"), and contents (e.g., "gill worms")**. It is not necessary to segregate all parasites by type (with the exception of soft-bodied vs. hard-bodied), unless you find that helpful.



Left: Gill monogenean at high magnification, *these can vary a lot in shape*, but tend to be small; **Middle**: Drawing of gill copepod with egg cases (bottom) easily seen at high magnification, even with naked eye; **Right**: Mouth cavity isopod, can be very large (up to several cm).

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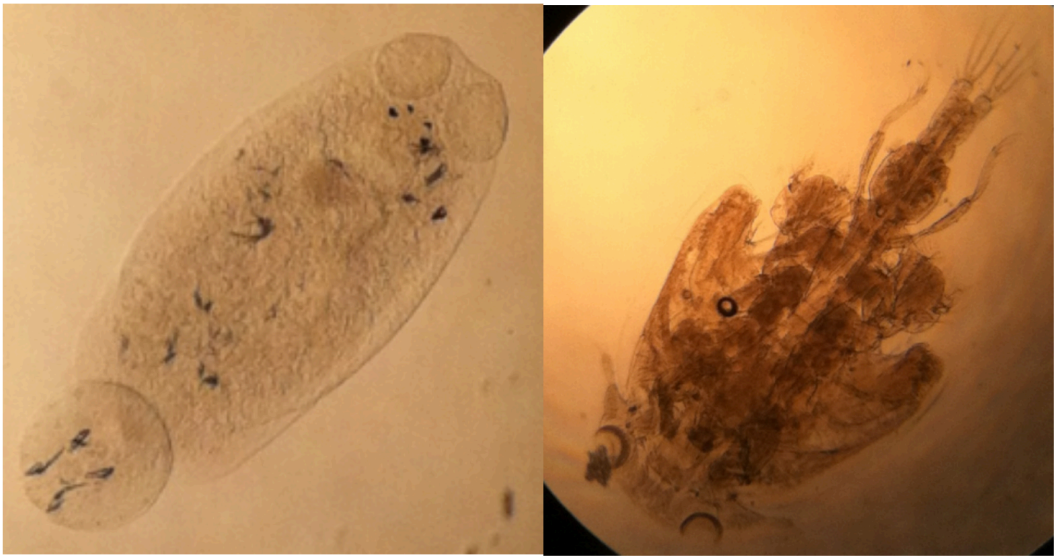
(7) *Skin parasites*

This method is designed to dislodge parasites from fishes' skin. It relies on osmotic pressure created by placing the fish in freshwater to cause the parasites to "pop" off. Once you begin the parasite exam, please record all information on the additional data sheet.

***If the lionfish has speared and placed in an individual Ziploc bag underwater, please pass the bag contents through the filter (see step 5) in addition to the freshwater bath described below.*

1. After removing gill arches, submerge fish in a container of freshwater.
2. With your scalpel, scrape a 3 x 3 cm area of the skin/scales from both sides of the fish. A good place to scrape is usually the area just below the posterior dorsal fin rays.
3. Wait 5-10 minutes.
4. Lift the fish out of the freshwater and lightly squirt its body with a bottle of freshwater, so that the water goes into the fish's container.
5. Cut a piece of the plankton mesh to fit into the funnel so that when you pour the freshwater into the funnel, no water is left un-filtered.
6. Pass the freshwater that the fish was soaking in through your funnel-filter.
7. Remove the plankton mesh from the filter and carefully transfer anything on it into a Petri dish (I often turn the mesh upside down over a Petri dish and gently squirt the mesh with my squirt bottle).
8. Place the Petri dish on the dissecting scope, and examine the contents at low magnification to progressively higher magnifications. External parasites are often relatively large, round copepods, or small, round "monogeneans."
9. Note the number of each type of parasite found. If there are many parasites, I usually remove them to a different Petri dish where they are easier to see, keep track of, and count (so you're not counting the same parasite twice). If you can, take a picture of the parasite by putting a camera to the eyepiece. ***Drawing simple pictures is extremely helpful, and encouraged!***
10. Preserve any parasites found in a centrifuge tube (or more than one if necessary) with formalin (for soft-bodied parasites) or ethanol (for hard-bodied parasites). **Label the tube with the date, location, lionfish ID, solution (e.g., "5% formalin," "70% EtOH"), and contents (e.g., "skin worms").** It is not necessary to segregate all parasites by type (with the exception of soft-bodied vs. hard-bodied), unless you find that helpful.



Left: Skin monogenean at high magnification (usually transparent and relatively small); **Right**: Skin copepod at low magnification, can sometimes be seen with the naked eye.

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Internal measurements and sampling

(8) *Opening the gut cavity*

Using dissection scissors, make a shallow incision from urogenital opening towards the base of the pelvic fins (pelvic girdle) along the ventral surface of the fish (Fig. 6A). Take care to cut shallowly; lifting up with scissors will extend the tissue away from organs helping to avoid damage to the internal organs. As you approach the pelvic fins, a deeper cut will be required to sever the pelvic girdle. A pair of utility scissors may be needed to sever the pelvic girdle in larger specimens. Continue cutting anteriorly beyond the pelvic girdle to the isthmus of the gill openings and snip through the thin flesh of the isthmus (Fig. 6B). Next, place the fish on its side and cut along the rear edge of the gill arch toward the dorsal fin (Fig. 6C). Finally, lift the lateral musculature to expose the gut cavity and internal organs, cutting through any minor connecting tissue as needed (Fig. 6D).

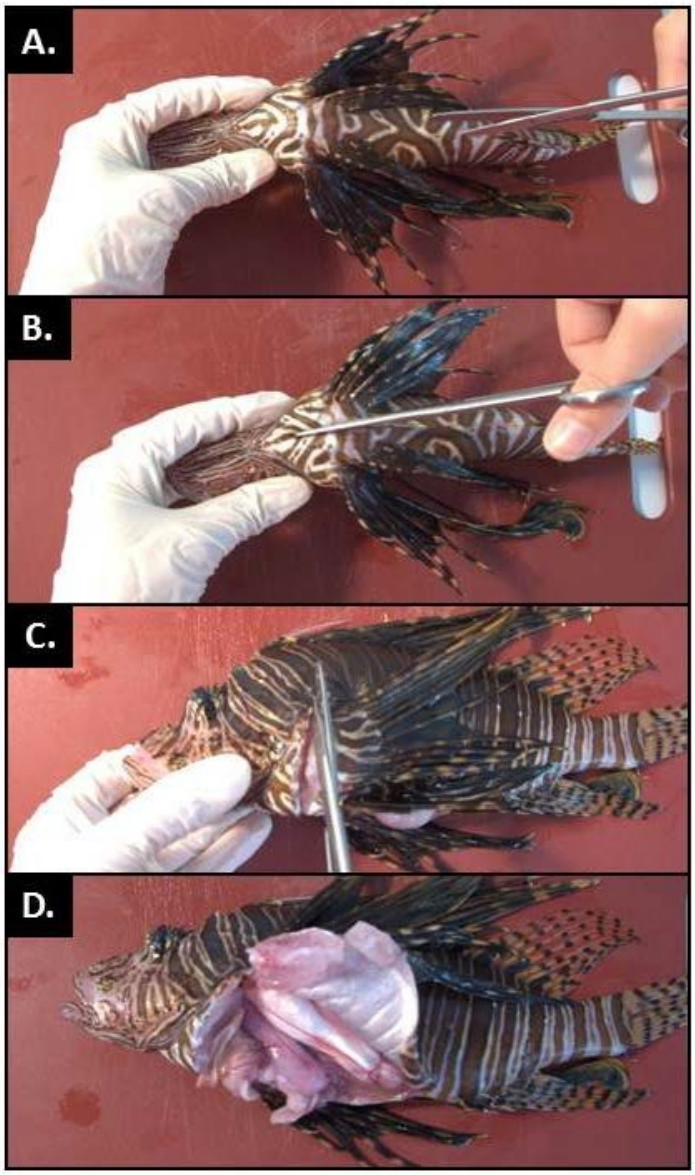

Fig. 6. Opening the lionfish's gut cavity. A) A shallow incision from urogenital opening towards the base of the pelvic fins (pelvic girdle). B) As you approach the pelvic fins, a deeper cut will be required to sever the pelvic girdle. C) Cutting along the rear edge of the gill arch toward the dorsal fin. D) Lifting the flank to expose the gut cavity and internal organs, cutting through any minor connecting tissue as needed.

(9) *Gender Identification and Reproductive Staging*

Using a dull dissecting probe or finger, move away the stomach, liver and fat to expose the swim bladder, which lies along the dorsal wall of the body cavity (Fig. 2B). Identify gonads lying along the ventral side of the swim bladder (Fig. 2C). Avoid confusion with swim bladder muscle tissue, which lies along the dorsal edge of the swim bladder (Fig. 2D). Gonads of smaller fish may be identified by looking for the presence of a thin blood vessel leading into and out of the gonad. Use a magnifying glass or dissecting microscope to locate lionfish gonads in small individuals as needed.

Using information on the size of the lionfish specimen, and the size and appearance of its gonads greatly aids in gender and reproductive stage identification. Immature gonads from both sexes are threadlike. As a result, testes are largely indistinguishable from immature ovaries and may be misidentified without histology in specimens smaller than 180 mm TL (Table 1). For specimens greater than 180 mm TL, immature testes, appearance and size of the gonads can be used to determine reproductive status (Table 1).

Gonads may be removed, weighed and stored in 10% neutral buffered formalin solution for histological examination. For long-term storage, gonads that have been fixed in formalin may be transferred to 95% ethanol. Gonads that have been previously frozen may not provide accurate histological information because of cellular damage caused during freezing.

See Table 1 (next page) for more details.

(10) *Interstitial fat deposits*

Interstitial fat deposits appear as white, waxy bodies and masses around the stomach and intestines (see Appendix 2 for orientation). Using dissecting scissors and forceps, carefully cut fat away from the intestines and remove any other large fat deposits from the gut cavity (Fig. 7A). Partially fill a 50-ml graduated cylinder with freshwater and note the volume at the meniscus. Completely submerge the extracted fat deposits in the graduated cylinder and record the final volume of fat and water. The difference between final and initial volume is the volume of the specimen's interstitial fat; record this value to the nearest 1ml (Fig. 7B). **Note**: In fish that have warmed to room temperature, fat may begin to liquefy and become difficult to accurately handle or remove. It is recommended that fish be refrigerated or stored on ice prior to dissection.

Fig. 7. A) Removal of interstitial fat deposits from intestines and B) measurement of interstitial fat volume.

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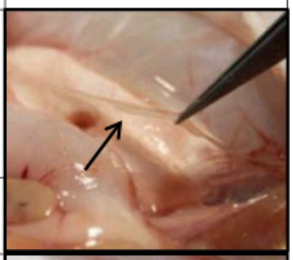
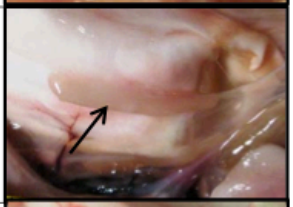
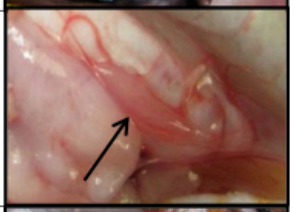
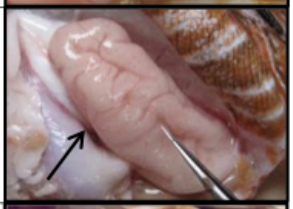
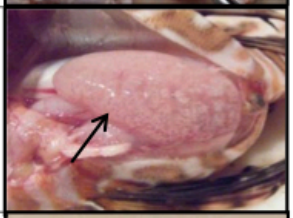
Table 1. Sex determination and gonad staging key for lionfish.

Fish length (TL)	Appearance	Gender	Stage description	Stage	Image
<180 mm	Gonads are oval masses, cream-pink in colour, with ratio of length:width less than 2.	Female	Additional information required to determine reproductive stage.	Immature (virgin) or Early Developing	See Immature or Early Developing
	Gonads elongated with ratio of length: width greater than 2.	Histology required to distinguish between male and female.	Clear, threadlike structures 1-3 mm in diameter and 5-10 mm in length. Immature ovaries are largely indistinguishable from immature testes.	Immature (virgin)	
>180 mm	Gonads elongated with ratio of length:width greater than 2.	Male	Testes appear threadlike; 1-3 mm in diameter and 5-10 mm in length.	Immature (virgin)	
			Testes appear as cream-colored with well-defined edges. Testes are typically not larger than 10 mm in diameter and 20 mm in length in the largest of specimens.	Spawning capable	
	Gonads are oval masses, cream-pink in colour, with ratio of length:width less than 2.	Female	Ovary is cream colored and round with no edges. Width may vary from 5 - 15 mm. Eggs not visible macroscopically. Ovary is more firm than during the Developing stage.	Early developing	
			Ovary cream colored with some pinkish portions. Eggs visible as small white spheres. Size may vary from a width of 15 mm to 30+ mm. No gelatinous mucus visible around periphery.	Developing	
			Ovary large with clear gelatinous mucus containing visible eggs peripheral to central stroma. Size may vary from a width of 15 mm to 30+ mm.	Spawning capable	
			Large number of clear eggs encompassed in gelatinous mucus visible along periphery of the ovary. Note: ovary wall is removed in picture.	Actively spawning	

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(11) *Gut parasites*

This method is designed to dislodge parasites from gut mucosa and associated mucus and food debris. It relies on the fact that parasitic worms are more dense than saline and will sink over time. Gut washing should always be done if accurate counts of parasites are needed. Once you begin the parasite exam, please record all information on the additional data sheet.

1. Prepare a jar, filled up to about 80% capacity with saline solution (keep lid off).
2. If you haven't already, open the fish body cavity from just behind the gill cover back to the anus. It's often easier to work after cutting away excess skin and muscle tissue in this region.
3. Remove the gut (stomach and intestines) from the fish you are examining. The stomach starts just behind the mouth and the intestines end at the anus. Interstitial fat makes gut parasite exams more difficult because it creates a white film at the top of your Petri dishes, so remove as much fat as possible before proceeding.
4. Using a small set of clean scissors or a scalpel, open the stomach and the intestines lengthwise – if intestines are too difficult to cut lengthwise, roughly cut into small sections (about 2 cm long). The idea is to open the guts so that parasites on the inside can be more easily dislodged.
5. Put the cut stomach and intestines in the jar with saline. If the gut is too large, fatty, etc., it is acceptable to split the contents into two or more jars. Screw on the lid, and shake **vigorously** for 30 seconds.
6. Put the jar on the counter and immediately unscrew the lid; this will save you from disturbing the sediment after all has settled.
7. Wait 5 minutes for the contents to settle.
8. Carefully decant the supernatant and pour the sediment into a clean Petri dish (*you will likely need to do this repeatedly to look at all the sediment*). **Alternatively**, you can use a pipette to remove the sediment from the bottom of the jar to a Petri dish. Be sure to examine the external and internal surface of the stomach and intestines for any parasites still embedded in the tissue (e.g., acanthocephalans – "thorny-headed worms" – are particularly good at remaining embedded in gut tissue, even after shaking).
9. If you gently swirl the dish in an orbital fashion, the parasites will accumulate in the center of the dish, which can make looking for them easier.
10. Sort through the sediment, sucking out parasites with a pipette. If there are many parasites, I usually remove them to a different Petri dish where they are easier to see, keep track of, and count (so you're not counting the same parasite twice).
11. Note the number of each type of parasite found. If you can, take a picture of the parasite by putting a camera to the eyepiece. ***Drawing simple pictures is extremely helpful, and encouraged!***
12. Preserve any parasites found in a centrifuge tube (or more than one if necessary) with formalin (for soft-bodied parasites) or ethanol (for hard-bodied parasites). Label the tube with the date, location, lionfish ID, solution (e.g., "5% formalin," "70% EtOH"), and contents (e.g., "gut worms"). It is not necessary to segregate all parasites by type (with the exception of soft-bodied vs. hard-bodied), unless you find that helpful.

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Upper Left: Nematode at low magnification ("round worm"; can usually be seen with naked eye); **Upper middle**: Acanthocephalan at low magnification ("thorny headed worm", sometimes brightly colored but sometimes not, sometimes seen by the naked eye); **Upper Right**: (Stained) larval cestodes at very high magnification ("tape worm"; these guys are very small but are NOT colored like they are in the pic – they're often transparent or opaque); **Lower Left and Right**: Trematodes at high magnification ("fluke worm"; these are not usually visible to the naked eye but can vary in shape, size).

(12) *Otolith removal*

Otoliths, or ear stones, are small bone-like structures in the otic bulba of the cranial cavity of fish. As a fish grows, deposits are made on otoliths in the form of rings. These rings can be used for determining fish age and growth rates (Fig. 9A through C). Otoliths are composed of calcium carbonate and are brittle. Care must be taken during removal to avoid damaging them. There are three otoliths on each side of the otic bulba in the cranial cavity (grouped into pairs). Sagittal otoliths are the largest and their dissection is described below and in Fig. 10.

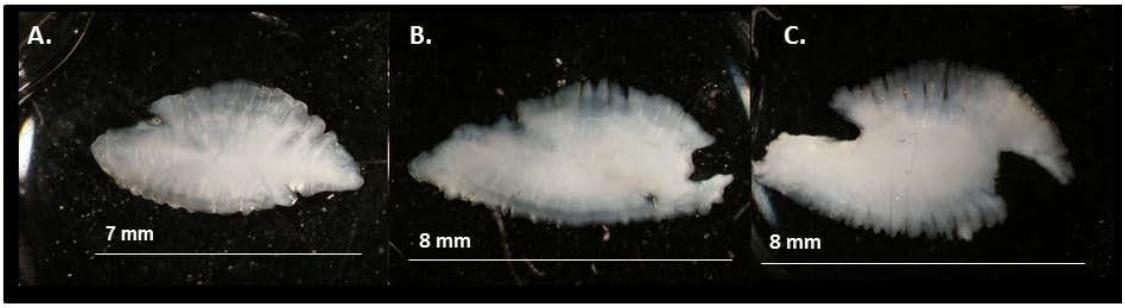

Fig. 9. Ontogenetic change in sagittal otolith shape, taken from lionfish of A) 250 mm, B) 325 mm and C) 357 mm total length. Photo credit: Jennifer Potts.

There are several ways to perform otolith removal. Here we provide instructions for otolith removal using a technique that involves removal of the head for easy access to the sagittae. In some cases where trauma to the head of the carcass is an issue, other methods may be more appropriate (e.g., trophy fish caught during tournaments). Detailed instructions for alternate techniques of sagittal otolith removal can be found in Secor et al (1991).

Remove the specimen's head by cutting vertically from between the head spines and the first dorsal spine to the isthmus of the gill opening using a large, sharp knife. Turning the head nose down following severance of the spinal cord (Fig. 10A) reduces difficulty in cutting through cartilage. Holding the head dorsal-side down, grasp gill filaments and arches and sever their attachment to the roof of the oral cavity (Fig. 10B). Fold the gill arches forward to reveal the cranial cavity, which can be easily located by running two fingers along both sides of the spinal cord anteriorly until a slight bulge is felt (the cranial cavity; Fig. 10C). Place two fingers on the cranial cavity and make a cut on the posterior side of the cavity down through the spine (Fig. 10D). This incision allows access to the cranial cavity which contains the sagittal otoliths. Proper position of this incision is critical. Cutting too far forward into the cranial cavity will dislodge the otoliths and make them difficult to recover and cutting too shallow will prevent access to the cavity (Fig. 10E). Using a pair of fine-tipped forceps, reach into the cranial cavity at an oblique angle (~30o) to the dorsal plane, holding the forceps perpendicular to the dorsal plane. Remove the otoliths by reaching into the cranial cavity. Otoliths are free-floating inside the cranial cavity and resistance felt while searching inside the cranial cavity indicates that the structure held is not an otolith (Fig. 10F). Once removed, clean and dry the otoliths and place in a storage container.

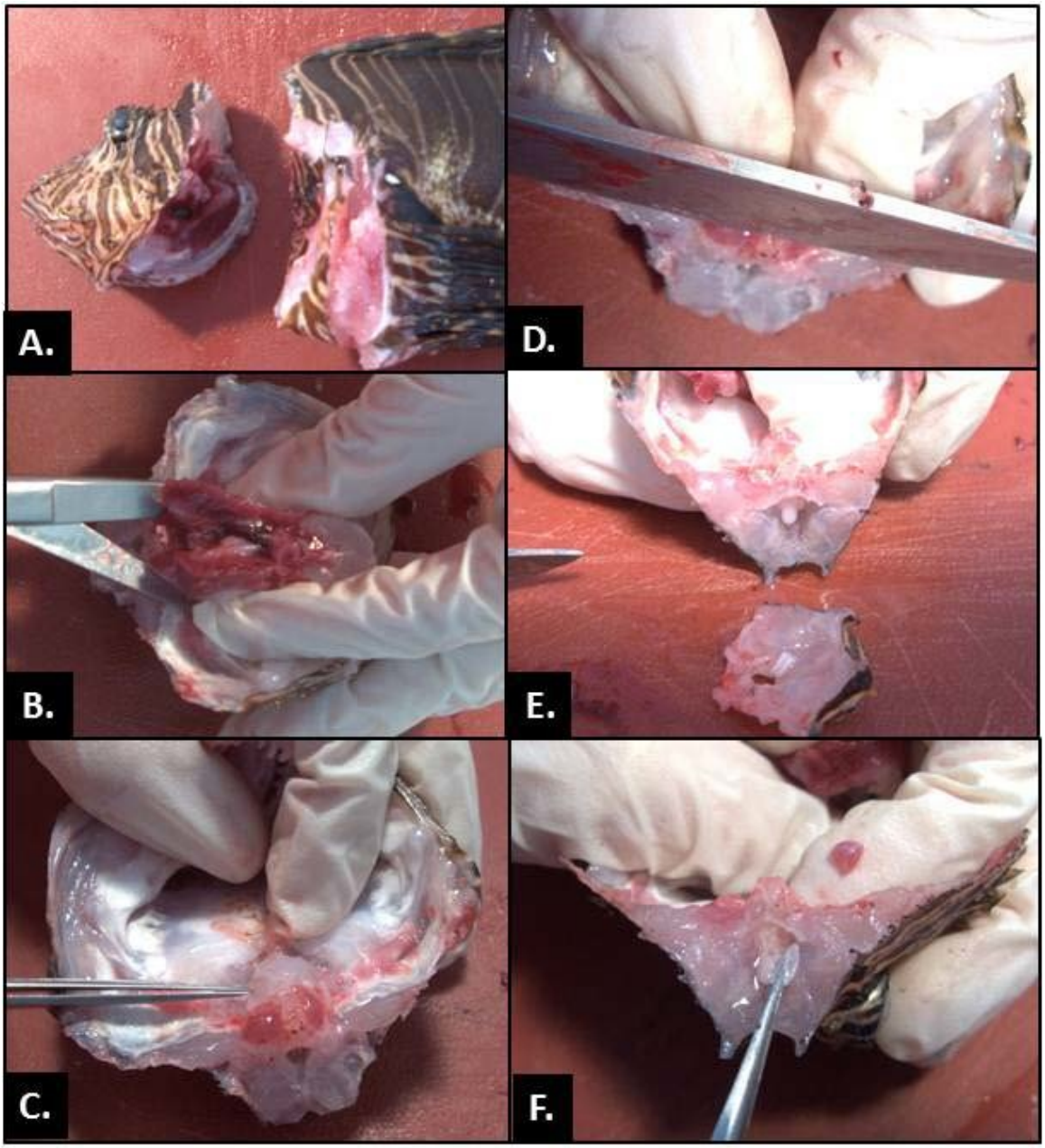

Fig. 10. Sagittal otolith removal. A) Severe the spinal cord between the head and the first dorsal fin ray. B) Grasp the gill filaments and arches and severe their attachment to the roof of the oral cavity. C) Locate the cranial cavity by running two fingers along both sides of the spinal cord anteriorly until a slight bulge is felt. D) Make a cut on the posterior side of the cavity down through the spine. This incision allows access to the otic bulba, which contains the sagittal otoliths. E) Brain matter (white, soft tissue) will often be exposed by opening the cranial cavity. F) Reach gently into the cranial cavity to remove the free-floating otoliths.