

Langerhans Lab Protocols

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DRILL PROCESSING:

While each project's needs vary, this protocol assumes that lateral and dorsal photographs will be taken for each fish. The procedure will generally follow this protocol, with the addition/substitution of image/video types.

See Langerhans Lab DRILL Overview Protocol for options and format of entries into the DRILL spreadsheet.

Processing Basics:

1. Prepare specimens:
 - a. Write collection # (C + 5 digits) in red on jar lid. Do not open more than one collection jar at a time, to prevent confusion.
 - b. Take some fish from ethanol/formalin collection jar and put them on the dissecting tray. Gloves, lab coat, and eye protection are recommended. Put the lid back on the jar to prevent evaporation.
 - c. Squirt fish pile gently with water. Cover with inverted petri dish to prevent drying.
 - d. Sort fish into piles of males and of females. Squirt these piles of fish with water and keep each pile covered with an inverted petri dish. The goal for most collections is 30 fish of each sex. Choose fish with a size range representative of the population (i.e., don't choose only the largest fish). Choose fish that are mature and have straight bodies as much as possible. If you're not sure about an individual's sex, don't use it, unless you've looked at all the other fish in the collection and you need more to make the 30-fish goal. Avoid fish under 14 mm if possible.
 - e. Sort each pile into size order (largest to smallest, or vice versa). This will make photographing and x-raying easier.
 - f. Put fish into tubes (see next step), head down. If a specimen is too large or wide for a tube, put it into a vial.
2. Prepare tubes for individually preserving specimens and tissues:
 - a. Set 2.0 mL plastic tubes into 2 tube boxes: one tube for each specimen in one box, and one tube for each corresponding tissue sample in another box. Label each box with your name, collection #, genus & species, and "tissue" or "specimens".
 - b. Place slip of Rite-in-the-Rain paper with pre-printed ID # into each tube, one for tissue and one for specimen.
 - i. To print slips (usually the lab manager will provide these): The specimen ID template is in DRILL>protocols and templates>DRILL labels. Modify the template as necessary, print it out on Rite-in-the-Rain paper, cut the labels as needed. Keep your labels in a sheet protector with your name on it so you can cut a few at a time.
 - c. Label each tube on lid (VWR pens are best) with 5 digit specimen ID #.
 - d. Fill specimen tubes with 70% ethanol, enough to cover the fish. Fill tissue tubes about half full with 95% ethanol (you'll add the tissue after photographing).
3. Process a specimen:
 - a. Prepare a spreadsheet for your collection. Start with the DRILL Photo Template.xlsx, and fill in as much info as you can about your collection (from jar lid &/or slip of paper in jar). Enter Specimen ID #s, two rows per specimen (one for dorsal and one for lateral photos).
 - b. Using forceps, gently dry off excess water from the next individual fish to be processed on a clean paper towel. Leave head/dorsal region slightly wet to obtain best images.
 - c. Weigh the fish & record mass and sex/age class (capital M, F, or J) into the DRILL spreadsheet (on 2 rows for each specimen). See below for automated weight recording method, or type into spreadsheet by hand.
 - d. Take dorsal and lateral photographs of the fish; may need other image perspectives such as lateral x-rays or lateral gonopodium distal tip (see X-ray Processing and Gonopodium Imaging Protocols for methods).
 - i. *see examples and read all steps below before taking any photos!*

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- ii. To send images to the correct DRILL folder:
 1. Turn on the camera and wait for the EOS Utility box to appear on the computer.
 2. Choose Camera Settings/Remote Shooting. This opens the camera controls on the computer.
 3. Click on the folder icon (below the shutter button) and choose destination folder: Folder name = 5 digit collection ID number inside of species folder. Check Download Images.
 4. To change file name: on camera control box, click Preferences, then file name. File name should be date + 3 digit number (e.g., 2012_03_09_001). Change the start # at the beginning of the day.
 - iii. Photo platform is either a) black wax dissecting tray, with ruler (metric) next to specimen (clear ruler with black marks and white paper on underside). If tray slides around on the tabletop too much, put a small piece of modeling clay under one edge; or b) white plastic board with ruler printed on it.
 - iv. Fish should face left in all images (facing right on tray with dorsal side toward photographer), with minimal shadows and even lighting on body.
 - v. Dorsal photos: use dissecting pins (on wax tray) or wooden picks (on plastic tray) to support fish so that photo captures direct top view. Straighten fish body as much as possible.
 - vi. Lateral photos: In was, use dissecting pins to separate (lift up) dorsal and anal fins from body so morphometric points are visible (see diagram below). Make sure pin shadows don't fall on fish. On plastic, separate fins before setting fish onto plastic board. Press on left pectoral fin (but not obscuring morph points 2 & 10) and body with a pin to roll fish, if necessary, so that view is directly of side of fish.
 1. Make sure fish's right gill cover (opercular flap near "throat") or top of head (dorsal side of fish, mainly between eyes and mouth) do not show in photo—if either shows, the fish is rolled too far. Re-take photo while holding fish in better position with pin.
 - vii. Take photo:
 1. Position fish correctly.
 2. With lights off, manually focus camera and check fish and ruler position (look through camera's viewfinder). Fish should be in center of field and should fill most of the view field. The camera can slide up and down on vertical arm to optimize fish size in field. Ruler should be parallel to fish and to bottom of view field, with 1 & 5 mm marks (but not numbers) visible.
 3. Flip on lights using power strip switch, use computer to take photo, then immediately switch lights off. Lights should be on for a second or less. Don't look at the specimen while lights are on unless you enjoy being blinded.
 4. Adjust the 4 fiber lights for even, shadow-free lighting. Black sleeve on each light can be slid up or down for broad or narrow focus of light. Once they are positioned correctly, they shouldn't need much adjusting.
 5. View enlarged photo to check for morphometric points, lateral position, lighting, shadows, and ruler position. Re-take photo as many times as necessary to get a good image, keeping fish moist with water (but don't have puddles in photo). Delete poor photos on ZoomBrowser EX and keep only one best.
 - a. Photos deleted on ZoomBrowser EX will also be deleted in DRILL folder; photos deleted in DRILL folder will not be deleted in ZoomBrowser EX.
 6. Copy and paste photo name from ZoomBrowser to DRILL Excel spreadsheet in column AO ("Image Filename"). Record "Dorsal" or "Lateral" (capitalized) in column AM ("Image Perspective"). Record photo date in col. AP ("Image or Video Date") in format "5-Mar-2012".
4. Collect tissue sample (omit this step if the specimen has been stored in formalin):

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- a. Rinse snippers/scissors and forceps in water to remove any previous tissue. Wipe with paper towel.
 - b. Clip the right pectoral fin and small amount of muscle from the base of the pectoral fin / opercle region with snippers/scissors. Use forceps to transfer tissue to labeled tube of 95% EtOH.
 - c. Rinse snippers/scissors and forceps in water. Wipe with paper towel.
 - d. Place the specimen in labeled tube of 70% ethanol.
5. Frequently check that the specimen ID # you are working on matches the # on both tubes and the line on the Excel spreadsheet.
 6. Store specimens in 4°C refrigerator and tissues in -20°C freezer.
 7. Save remaining organisms (plants, shells, shrimp, etc.) in a 20 ml vial w/ collection ID # in 4°C refrigerator.
 8. Sort photos: within the Collection # folder, make 2 new folders: dorsal and lateral (and others as needed). Sort your photos into these by choosing Views -> Large Icons, then hold Ctrl key while clicking on all dorsal views, then drag them into the dorsal folder. Select all remaining photos (laterals) and drag to lateral folder.

ADAM-DU Scale automatic data recording:

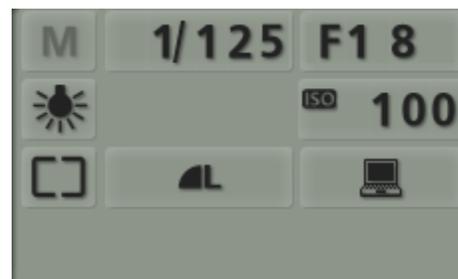
1. Double click on the ADAM-DU icon on the desktop to open the program. Click on “Start New Data Collection Session”.
 - a. If you try to start the program and it doesn’t open, look for the icon in the bottom right corner of the screen. It might already be running, and you just need to maximize it.
2. Choose “HCB” from the Select Scale/Balance drop-down menu. Choose COM8 for the Port, 4800 for the Baud/Speed, and n.8.1 for the Mode. Click on Confirm and Start Collecting Data.
3. To export files to an excel sheet: Data>Continual Data Logging to File>Enable; Name the file with the range of specimen ID numbers contained. Save in C:\DRILL\Mass Backup\ADAM data sessions as a .csv file.
4. Put dry-ish fish on scale, click Get Reading on computer ADAMDU Session 1... screen (make sure reading is taken).
5. When finished: Session>Stop Data Collection, and then exit the session.
6. Go to C:\DRILL\Mass Backup\ADAM data sessions and open your data file. Paste your data into correct specimen rows (remember to enter each mass on 2 lines).

Camera & EOS Utility Settings For Copy-Stand Photography Methods:

Be sure the USB cable is connected from the camera to the computer. Turn on the camera. Dialog box opens; click on Camera settings/remote shooting. The EOS Utility Settings Screen should look like this (for small preserved fish):

Recommended manual settings (with 2 pairs of fiber lights on specimen):

- small preserved fish (< 3 cm):
Shutter speed: 1/125, Aperture: F18, Flash: off, White Balance: Tungsten, ISO: 100, Center-weighted average metering; use 2 pairs of fiber lights
- medium-large fish (approx. 3 – 7 cm):
Shutter speed: 1/125, Aperture: F14 or 16, Flash: off, White Balance: Tungsten, ISO: 100, Center-weighted average metering; use 3 pairs of fiber lights. To aim fiber lights, turn on one pair at a time and look at where light falls on the specimen. One pair is for the upper half, one for the lower half, and one for the center, to lighten the shadowed area between the other pairs.
- large preserved fish specimens (big sleepers):
Shutter speed: 1/125, Aperture: F14, Flash: on, White Balance: auto Tungsten, ISO: 100, Center-weighted average metering; no teleconverter lens attached



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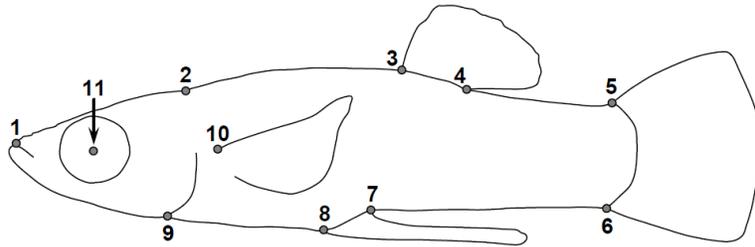
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- live fish specimens:
Shutter speed: 1/100, Aperture: F7.1, Flash: off, White Balance: Tungsten, ISO: 100, Center-weighted average metering

Photography Notes:

- Be sure that none of the key landmarks on the body used for morphometrics are obscured (see figure below).
- Make sure that posterior insertions of the dorsal and anal fins (#s 4 & 7 in figure below) are visible in photographs.
- Avoid leaving fish under fiber lights for long periods to prevent drying out.
- Camera battery is rechargeable.

Morphometric points that must be visible in every photo:



example dorsal view:



example lateral view:



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white background example dorsal view:



white background example lateral view:

