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Animal Care Protocol: Streblospio benedicti

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Abstract

This protocol describes how to collect and care for *Streblospio benedicti.* This protocol outlines best practices for sample collection, adult worm care, crossing worms, and raising worm larvae to adults. Additionally, we have information on how to ship worms and a list of recommended supplies.

Troubleshooting

Complete Animal Care Protocol: *Streblospio benedicti*

Kayleigh McHugh | Zakas Lab | NC State University | August 2024

Table of Contents

Lab Maintenance

Autoclaving Mud

Image 1: set-up for autoclaving mud and algae.

- Get an autoclavable bin
- Fill the bin about ½ inch with water
- Put frozen mud samples (in nalgene containers) and frozen algae (in 50ml falcon tubes) upright in an autoclavable container. As you do so, open the lids and set the lids on top of the tubes and containers.
- Place the containers and tubes into the bin. Add about 1 inch of water to the bin.
- Autoclave the mud at 100C for 45 minutes on the FLUID setting.
- Use autoclave gloves to remove the containers from the bin; set out on the counter.
- Allow the mud and algae to cool to room temperature.
- Add ~10 ml of autoclaved algae to each mud container.
- Screw caps on mud containers and mark as “ready to use”

Note: When using, add artificial seawater as needed depending on the fluidity of the mud.

Making Artificial Seawater (ASW, 30ppt)

Image 2: Set-up for making artificial seawater

- Fill a 5 gal carboy with DI water
- Add 200 ml (~275 g) of Instant Ocean
- Add a magnetic stirring rod to the container
- Put the carboy on a stirring plate and stir on HIGH for 45 minutes - 12 hours
- Double check the salinity is 30 ppt using a refractometer.
- Filter the seawater using a 0.22 um vacuum filter into 1 L bottles

Making and Maintaining Algae Stocks

Image 3: Set-up for making algae stocks.

- Use a disposable pipette to place 3 ml of Tetraselmis stock into an autoclaved flask, 3 ml of Rhodomonas stock into another.
- Add 150 ml of Alga Grow made in artificial seawater (30 ppt)
- Top flask with a sterile cotton ball and aluminum foil
- Label flask with date and contents
- Leave in a window sill or 12-hr light cycle incubator at room temperature (or 20C) for 3-5 days to allow algae to grow
- Allow stocks to grow for 7-10 days.
- After 10 days, reserve old algae in 50 ml falcon tubes and freeze. These can be autoclaved and then added to lab mud stocks as needed.

Storage Conditions

Image 4: Zakas Lab incubator set-up for storing *Streblospio*

- Worms stay in stainless steel incubators
- Conditions: 20C, 12-hour light cycle
- Keep a bowl or graduated cylinder of DI water at the bottom of the incubator to maintain humidity, if needed.
- Worms are in glass finger bowls or plastic six well plates.
- These bowls and plates are placed on clear acrylic trays to prevent spilling of seawater into the incubator, while also allowing light to be distributed to all plates and bowls. 1 population or experiment per tray.
- It is best to keep stacks of plates to a maximum of three.
- Clean the incubator shelves monthly with 10% bleach solution. Make sure to rinse well and dry.
- Replace air filters in the incubator as required by the manufacturer.

Field Work

Image 5: Supplies needed for collecting worms and/or mud in the field

Sample Collection

- Use a garden spade to scrape the first ~4 cm of mud into a 5-gallon bucket. Walk along the marsh, scraping the first ~4 cm, until the bucket is close to full.
- Test the salinity of the water you are collecting in.
- Coarse sieve (4.75mm): Sieve collected mud into a new bucket. Reserve the flow-through and discard the contents of the sieve
- Fine sieve (600 um): Sieve the coarse-sieved mud. Flow-through can be reserved for mud collection. Reserve the contents of the fine sieve. Move sieve contents to tupperware containers.
- From your tupperware search for Streblospio worms in your sample using forceps under a dissecting microscope. Note: Take about 1 tablespoon from your tupperware and dilute with seawater to look for worms. Repeat until the entire sample has been searched through.
- Place the worms into small glass bowls or 6-well plates. Add enough mud to cover the bottom of the plate or bowl. If the site salinity is below 30 ppt, gradually increase salinity by 5 ppt per day until you reach desired salinity. Changing the salinity too fast will shock the worms and they may die.
- For example, if the collection site was 15 ppt, use 15 ppt when you initially plate them. Increase salinity to 20 ppt the next day, 25 ppt the following, and then finally 30 ppt.
- Mark and set aside brooding females to collect life history data and to raise young, if desired.
- How to label 6-well plates for field collected worms:

Image 6: How to label a 6-well plate of field collected worms

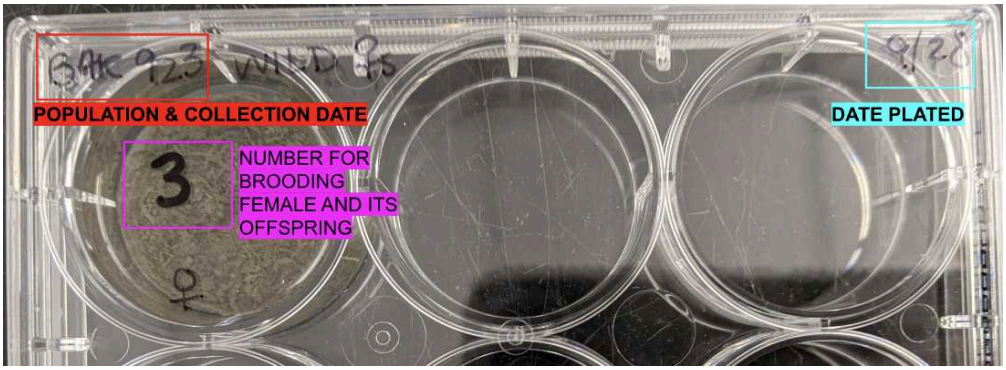

Image 7: How to label a 6-well plate of field collected brooding female worms.

Mud Collection

- Using a garden spade, scrape the first ~4 cm of mud into a 5-gallon bucket. Walk along the marsh, scraping the first ~4 cm, until the bucket is close to full.
- Coarse sieve (4.75mm): Sieve collected mud into a new bucket. Reserve the flow-through and discard the contents of the sieve. Use seawater to get as much mud off the sides of the bucket as possible.
- Rinse out your original mud bucket.
- Fine sieve (600 um): Sieve coarse-sieved mud into the original mud bucket. Use seawater to get as much mud off the sides of the bucket as possible.
- Allow the fine-sieved throughput to settle for about an hour.
- Carefully decant water off the top of the bucket, or using a bit of aquarium tubing, siphon water off of the mud just until the output starts to run brown.

- Back in the lab: Allow your mud collection to sit again for 1-12 hours.
- Using a garden spade and funnel, scoop mud into 125 ml nalgene containers.
- If the bottom of the bucket becomes too thick (notice sand-like particles and lack of fluidity), this mud can be discarded.
- Once all mud is in containers, they can be frozen in a standard freezer.
- Prior to use, unscrew container lids, add approximately 10 ml of autoclaved algae to each mud container, and autoclave for 45 minutes on the FLUID setting.

Crosses

Image 8: Supplies for making crosses. Image Credit: Zach Benfield

Crosses in 6-well plates

- Locate the tray in the incubator for your desired population.
- Find 6-well plates with worms marked as either male or female (preferably from a handful of different families).
- Pick 3 males and 3 females from different families.
- Get a new 6-well plate and label the top left corner with the population and “CROSSES” and the top right corner with today’s date.
- Find your 3 females - check and make sure that they look healthy (no green/blue and not moving slowly) and have plenty of eggs on the side of their body walls. (see Sexing Worms section, if needed)
- Place each female in a well in the top row of your new plate and label each well with that worm’s family ID. Add about 1-2 ml of mud and fill the wells with ASW.
- Find your 3 males - Before looking for the worm check and see if there is evidence of spermatophores on the mud’s surface. Try to only use males that look like they have recently made spermatophores. Check and make sure that they look healthy (no green/blue and not moving slowly).
- Place each male in a well in the bottom row of your plate and label each well with that worm’s family ID. Add about 1-2 ml of mud and fill the wells with ASW.
- Wait 1-2 days and then check your males for spermatophores. Once a male is making spermatophores, move the female in the well above it to the male’s well. Mark on the tray lid with an arrow that the female has been

moved. Clear the female's original well of mud and water.

- After about 5 days from starting the cross, pull out each worm. Check the females for a brood. See image below for a brooding female.

Image 9: Brooding *Streblospio benedicti*

- Using insect forceps, move brooding females to a separate well on a new plate. Zoom into the brood pouch (4-5x) and determine the stage of the offspring.
- If needed, remove an embryo to check the stage of the brood.
- Removing embryo/brood video:

https://www.youtube.com/embed/5xUc4eLt5jk?si=uFRs06VAaoLRJjTh

- If it is a lecithotrophic brood, and the stage of the offspring are swimming or greater, remove the brood; since lecithotrophic larvae burrow into the mud soon after release, they often get missed/lost in the mud.

Image 10: Lecithotrophic larvae in mother's brood pouch. Image Credit: Matt Rockman

- Released planktotrophic larvae will be swimming at the edge of the well

https://www.youtube.com/embed/GoKOJib7WVQ?si=94IVsTG7FPr7Cwna

- If larvae are released or removed, use a glass pipette to move them to a new six well plate with ASW.
- Name the offspring with the name of the cross ("mother's family ID.father's family ID; ex. "15.3"), or the name of the wild female they came from. Include the population, offspring name, birth date, and date plated on the new plates lid.

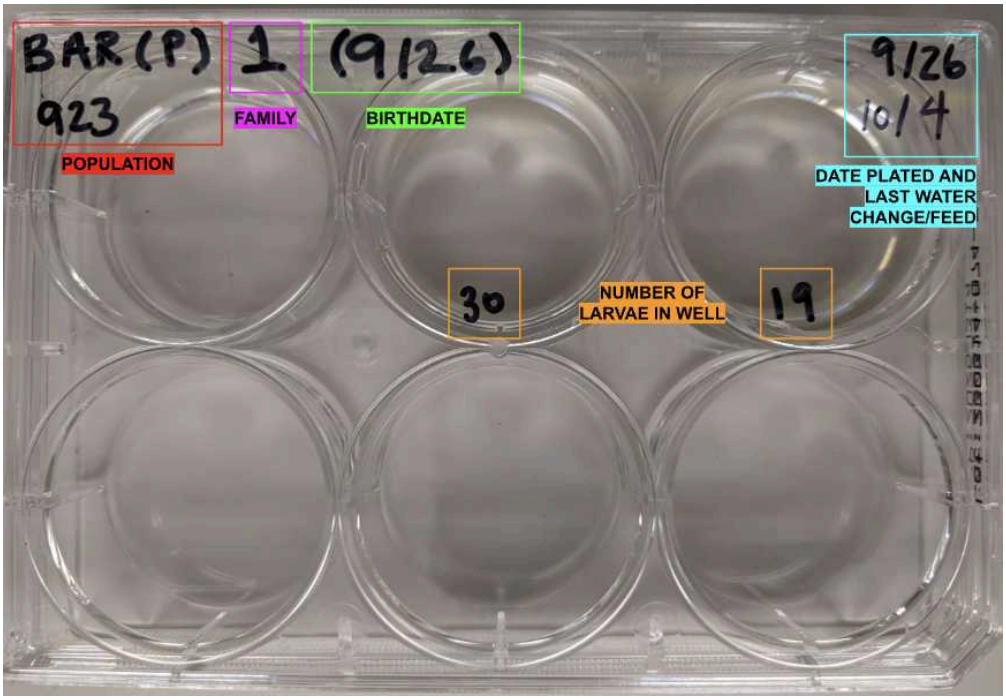

Image 11: How to label a 6-well plate of larvae.

- Once larvae are plated, proceed to the Larvae Care section.

Crosses in bowls

- Find wells marked as male and females or undifferentiated worms that are older than 4 months from your desired population.
- Combine 5-8 females/undifferentiated and 3-4 males in a bowl. Note the family IDs of each worm in your bowl in your lab notebook.
- Designate a name for the bowl.
- Add ~10 ml of mud to your cross bowl. Fill the rest of the bowl ¾ of the way with ASW.
- Label lid with population, the name of the bowl, date the cross was started, and how many worms are in the bowl. Write the name of the population on a piece of lab tape and place it on the side of the bowl.
- After one week, check for brooding females.
- Using insect forceps, move brooding females to a separate well. If you can: zoom into the brood pouch (4-5x) and determine the stage of the offspring.
- If needed, remove an embryo to check the stage of the brood.
- If it is a lecithotrophic brood, and the stage of the offspring are swimming or greater, remove the brood; since lecithotrophic larvae burrow into the mud soon after release, they often get missed/lost in the mud.
- If larvae are released or removed, use a glass pipette to move them to a new six well plate with ASW.
- Name the offspring the name of the cross, or the name of the wild female they came from. Include the population, offspring name, birth date, and date plated on the new plates lid.

Larvae Care

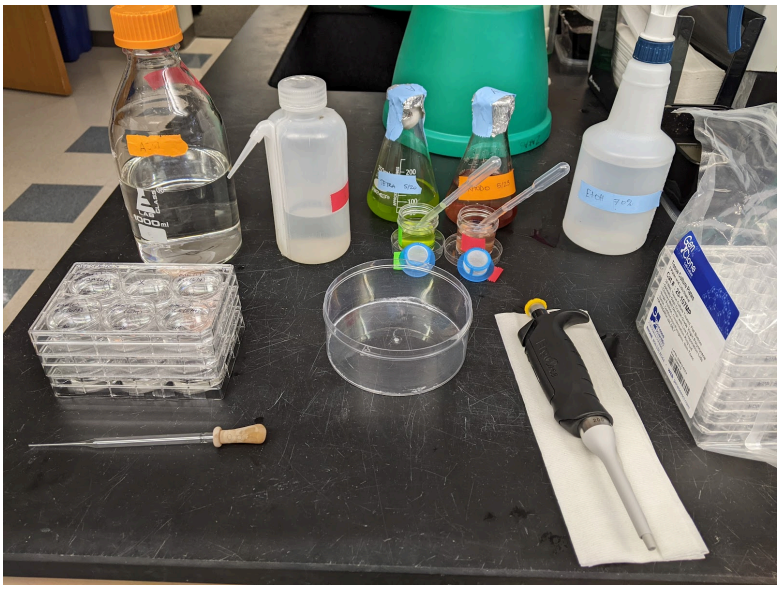

Image 12: Supplies for cleaning lecithotrophic and planktotrophic larvae.

Planktotrophic larvae care

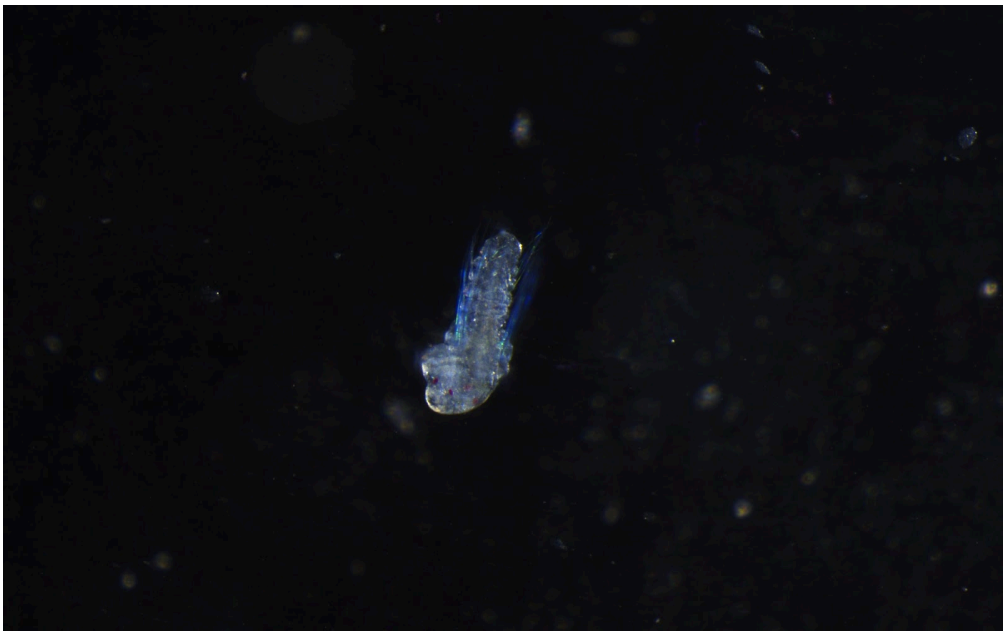

Image 13: Planktotrophic larvae

- Under the dissecting microscope, use a glass pipette to then divide the larvae into wells with only about 30 larvae per well. You may need more than one 6-well plate. Make sure all plates are properly labeled.
- Feed the larvae:
- Strain your desired amount of algae into two small graduated cylinders or cups using a 40 um cell strainer.
- Next, use a disposable pipet to add about 3 ml of Tetraselmis phytoplankton and 1 ml of Rhododomus phytoplankton to each well. Use a different pipette for each species of algae.
- Planktotrophic larvae live in the incubators for 2-3 weeks.
- Twice per week their ASW and algae should be changed.
- Use either a glass pipet or a 10 ml serological pipet & pipetman to carefully remove old ASW and algae from the well.
- Move waste water to a secondary container that can then be dumped down the sink.
- Add new ASW and feed fresh algae as described above.
- After 2-3 weeks, the larvae should be old enough to be switched to juvenile care. Their bodies should be longer, segmented, and there should be the beginning of head gear (palps and branchia). Chaetae may still remain.

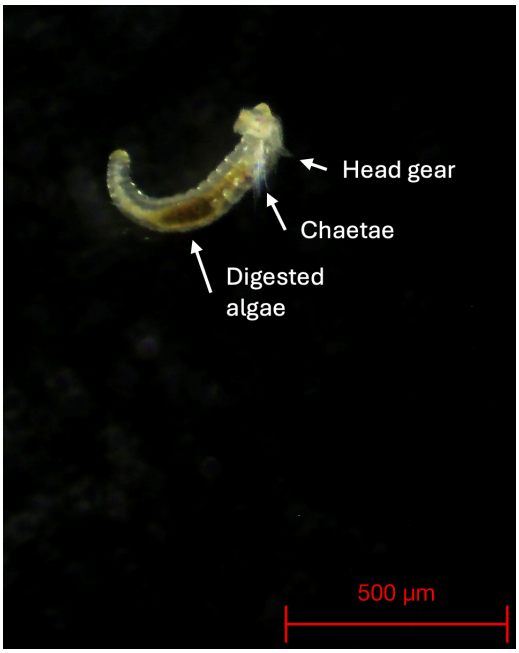

Image 14: Planktotrophic early juvenile that is ready for juvenile care. Notice the presence of chaetae and head gear.

- Proceed to juvenile care.

Note: If the larvae do not look old enough for juvenile care (presence of many long chaetae, short bodies, no headgear) after more than three weeks, they are likely not to survive and should be disposed of in the mud disposal containers.

Lecithotrophic larvae care

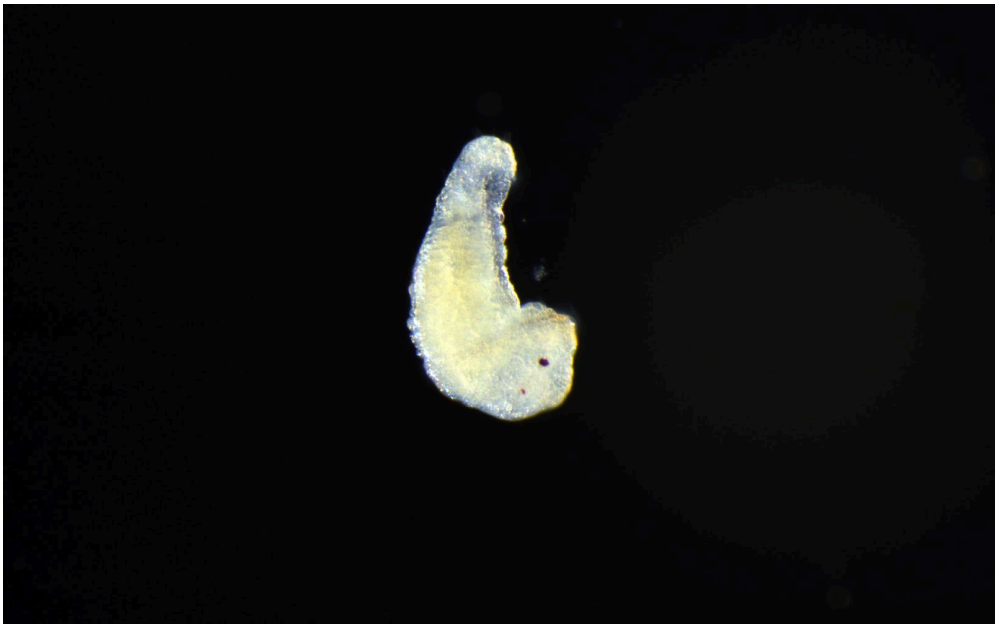

Image 15: Lecithotrophic larvae

- Use the glass pipette to then divide the larvae into wells with only about 30 larvae per well. You may need more than one 6-well plate. Make sure all plates are properly labeled.
- Larvae live in the incubators for 1-2 weeks. Their ASW should be changed once a week. Use either a glass pipet or a 10 ml serological pipet + pipetman to carefully remove old ASW from the well. Move waste water to a secondary container that can then be dumped down the sink. Refill the wells with new ASW.
- After 1-2 weeks, the larvae should be old enough to be switched to juvenile care.

Image 16: Lecithotrophic juveniles

- Proceed to juvenile care.

Note: If the larvae do not look old enough for juvenile care after more than three weeks, they are likely not to survive and should be disposed of in the mud disposal containers.

Juvenile Care

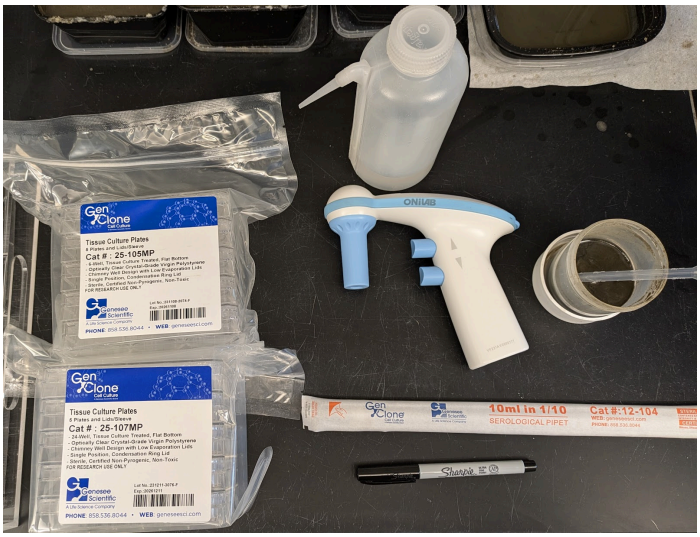

Image 17: Supplies for juvenile care

- As larvae are cleaned, early juveniles can be transitioned into juvenile care (see images 14 and 16 in Larvae Care section).
- Use a 200ul pipette to move early juveniles to 24-well plates. 1 juvenile per well.

Image 18: Labeled 24-well plate of juvenile worms.

- Change pipette tips between families.
- Check the juveniles weekly.
- Juvenile worms are ready to go into 6-well plates as adults when >50% of the mud in their well is gone through. Worms that do not go through mud for 4 weeks since being added to a 24-well plate are not going to survive and are considered dead.

- Example: This tray is 1 week old. The red circled wells are ready to be transferred to 6-well plates. You should come back the following week to check and see if the yellow circled wells are ready to be transferred. The purple circled wells are likely dead based on their inactivity.

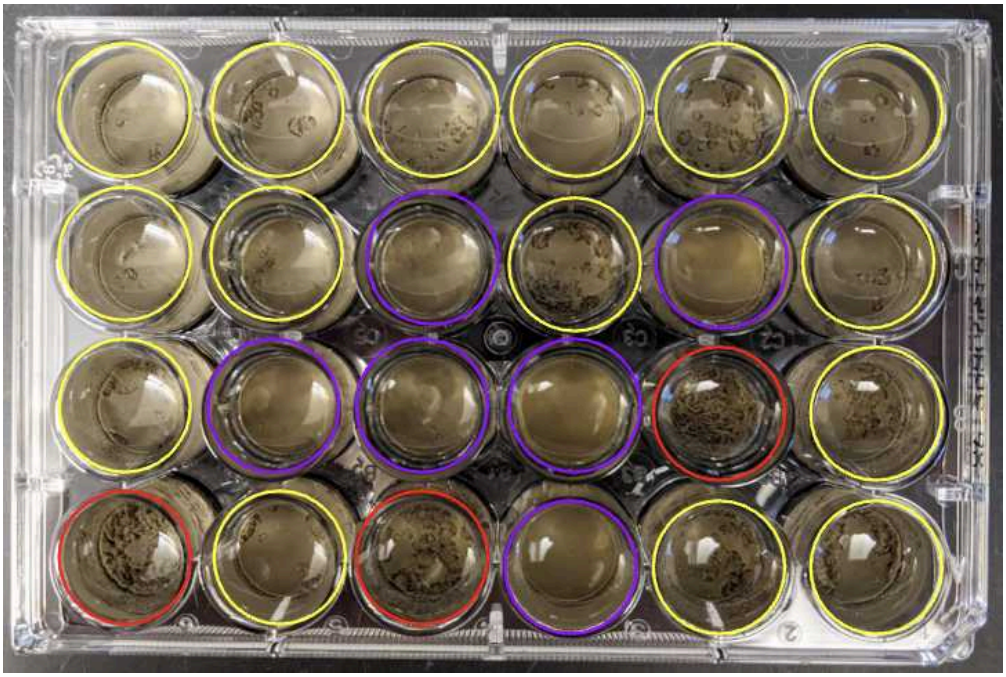

Image 19: Example of juvenile wells to be transferred to adult 6-well plates

- To move juveniles/"young adults" from 24-well plates to 6-well plates: get a 10 ml serological pipette with a pipetman. Pipette the whole well into a new well in a 6-well plate. You may need to add some ASW to the well to ensure all mud is moved over from the 24-well to the 6-well.
- Switch serological pipettes in between populations.
- Once moved, use a disposable pipette to add enough new mud to cover the bottom of the well. Fill the rest of the well with fresh ASW. Make sure to label the 6-well plate with the worm's family information and birthday. Add today's date to the top right corner of the tray. Add the new plate to that worm's population tray.

Adult Care

Cleaning 6-Well Plates

Image 20: Supplies for cleaning worms in 6-well plates. Image Credit: Zach Benfield.

- Get your population's tray from the incubator.
- First, scan the plates for the presence of any red/brownish algae on the surface of the wells. Set these plates aside to be cleaned last, so as not to contaminate the rest of the population. Plates with contaminated wells will all need to be moved to new plates.

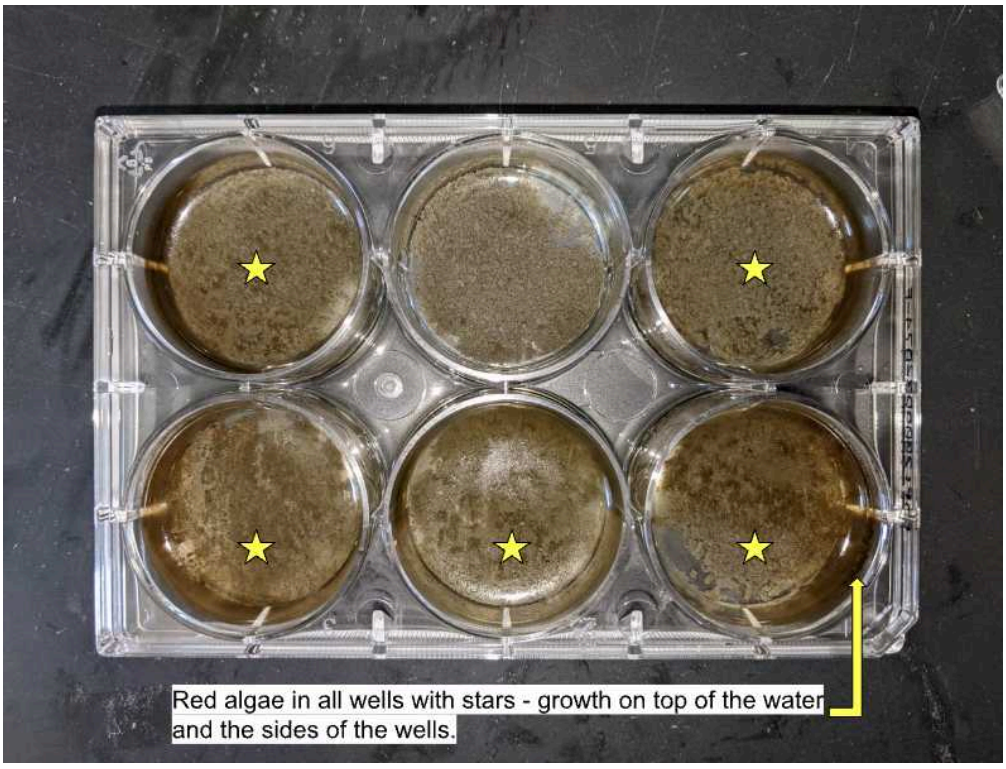

Image 21: Example of a plate of adult worms that has red algae/bacterial overgrowth.

- Start with cleaning the contaminated wells.

- Focus on one well at a time (0.63x will capture the whole well).
- Pause and look for movement. Notice any irregular movement (i.e. other than the water moving) or any head gear that might be poking out of the mud surface.

Image 22: Two Streblospio branchia and palps emerging from the mud. Image Credit: Christina Zakas

- Using soft insect forceps, scoop underneath the area of movement that you suspect is the worm.
- Use the forceps to tap the end of the tube that you think the worm is in to start to scare it out of its tube. Always confirm there is a worm in a tube when you see its branchia and palps. Use this video as an example for how to find a worm:

https://www.youtube.com/embed/U7jvLnzKuWM?si=v_f7UhhQ2ceQyV0S

- Use the forceps to move the worm momentarily to another plate or tray. Add a small amount of ASW to the temporary well. If the worm is not fully out of its tube yet, scare the worm out completely by gently tapping the posterior end of the worm's tube. See this video for an example:

https://www.youtube.com/embed/ElUNYfgtfQs?si=rwTNCYaAkQi4BIlB

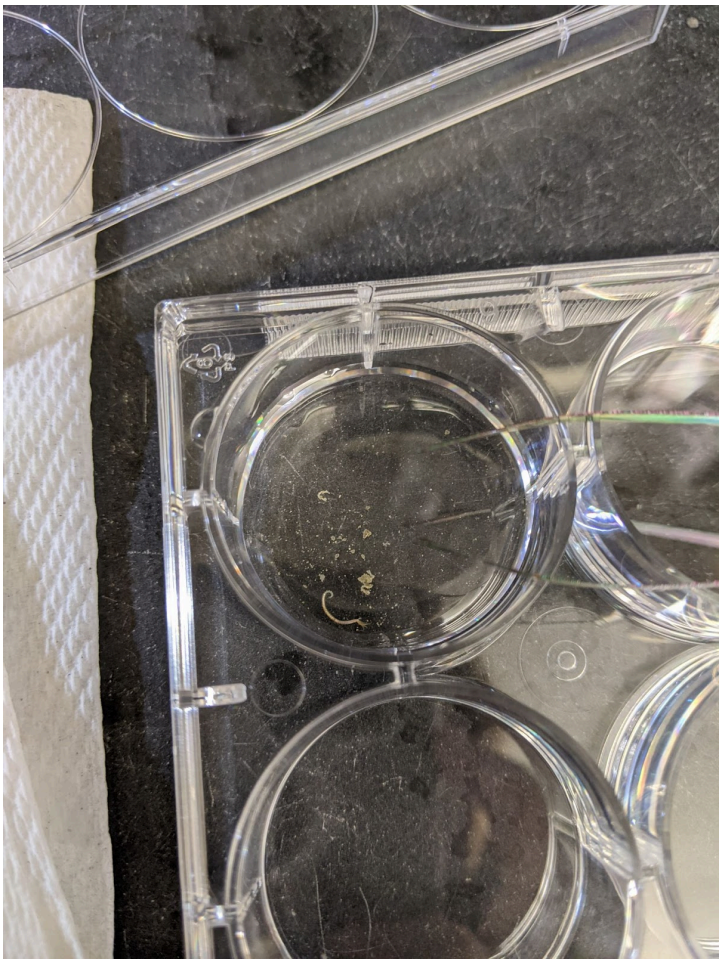

Image 23: Example of a worm moved to a temporary or “scratch” plate during cleaning.

- If the worm has not been marked as male or female, check the worm for eggs. Eggs appear on the lateral sides of the worm. See the section on sexing worms below to determine if your worm is male or female.
- Look at the worm’s plate to check the date of last cleaning in order to determine if the worm is going back to its original well, or if it will go to a new 6-well plate.
- Six-well plates should be changed out every 6 weeks. If the plate is >6 weeks the worm will go into a new 6-well plate. If the plate is <6 weeks old the worm’s original well can be cleared and replaced with new mud and ASW, and the worm can go back into that well.

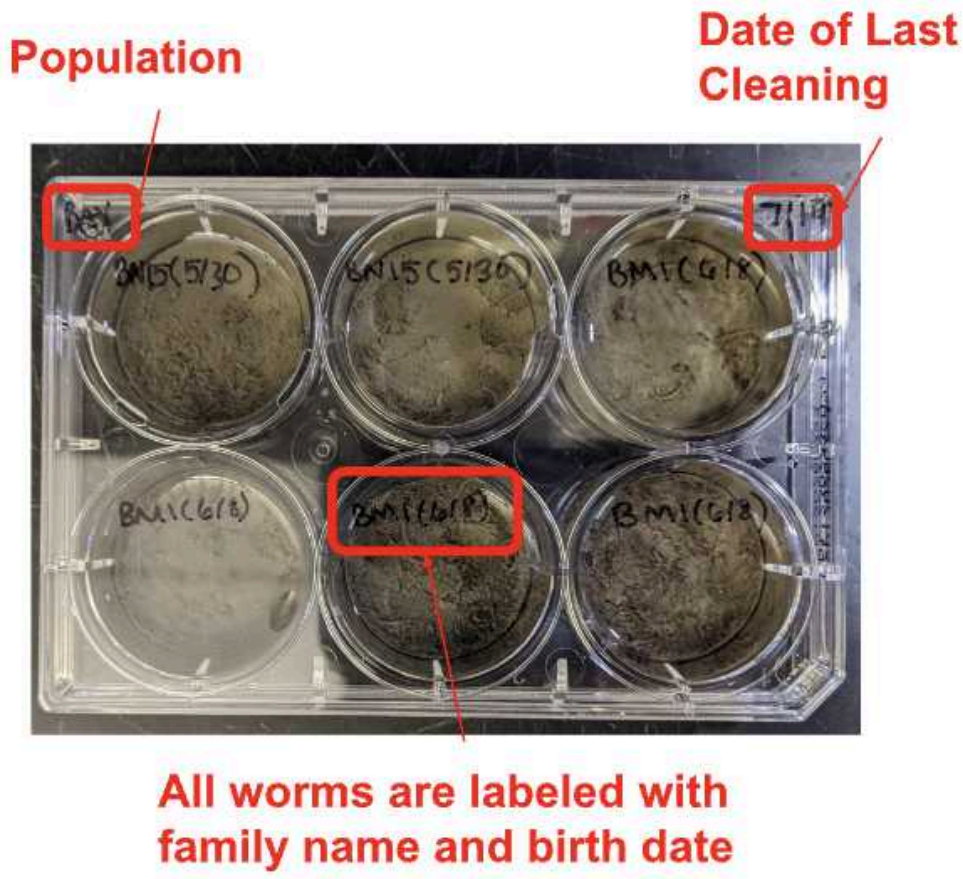

Image 24: An example of a well labeled adult Streblospio plate.

- Use a pipetman and a 25 ml serological pipette to clear the original well of mud and artificial seawater. Use this video as an example for how to clear out a six-well plate:

https://www.youtube.com/embed/4dI29clyeXQ?si=M-T_Mqw8dGWLQgLx

Video Credit: Langston Humes

- Once the well is clear, take your forceps and move the worm either back to its original well or into a well on a new plate. If the worm gets stuck to your forceps, gently spray the worm with ASW into its new well.
- Use a disposable pipette to add approximately 3 ml* of mud to the worm's well - or enough that the mud has completely covered the bottom of the well. Use a squirt bottle to slowly fill the rest of the well with ASW.
- *This volume is very approximate - it largely depends on the fluidity of the mud on how much to add. A good rule of thumb is just enough mud to cover the bottom of the plate and cover the worm entirely. Too much mud will make future cleaning/finding the worm difficult, too little will lead to a hungry, hungry worm.
- Clean forceps with a paper towel and 70% ethanol
- Repeat the steps for cleaning until all of the uninfected plates in your population have been cleaned.
- If the worm cannot be found after 5 minutes, move on to other wells and continue to look after the other wells have been cleaned. If the worm can't be found then after 5 minutes, just change the worm's water:
    - Pipet out the ASW.

- Refill ASW and add a few drops of mud.
- Put a question mark on the well to indicate that this worm could not be found.
- If the worm in question already has a question mark on the well lid, assume the worm is dead and clean out the well.

- For the infected plates:
  - Start with the least infected wells.
  - Search for worms as you did before.
  - Always move the worm to a scratch plate to try to get off as much algae as possible.
  - Move the worm to a new plate.
  - Very important to clean forceps with ethanol regularly when dealing with infected plates.
- Update the plate's lid in the top right corner with the date you have cleaned the plate.

Image 25: How to update a plate with the day you have cleaned.

- 1-3 days after cleaning, scan the surface of the mud of undifferentiated worms for spermatophores. If present, mark the worm as male on the plate's lid. A spermatophore will look like this:

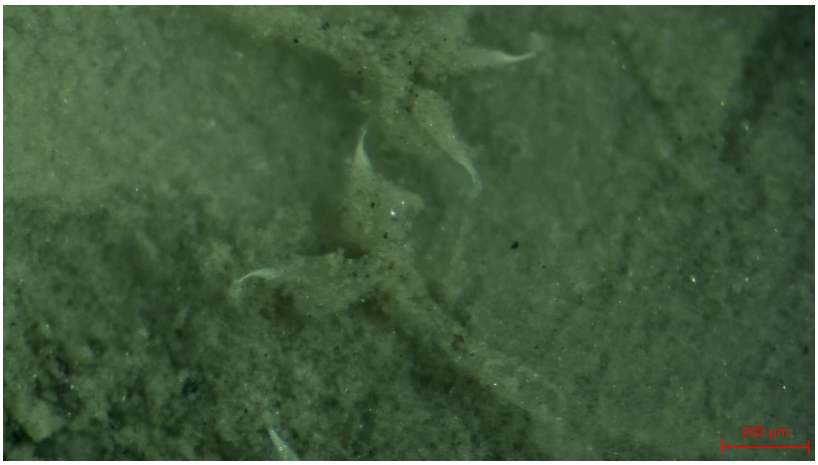

Image 26: Spermatophores on the surface of the mud.

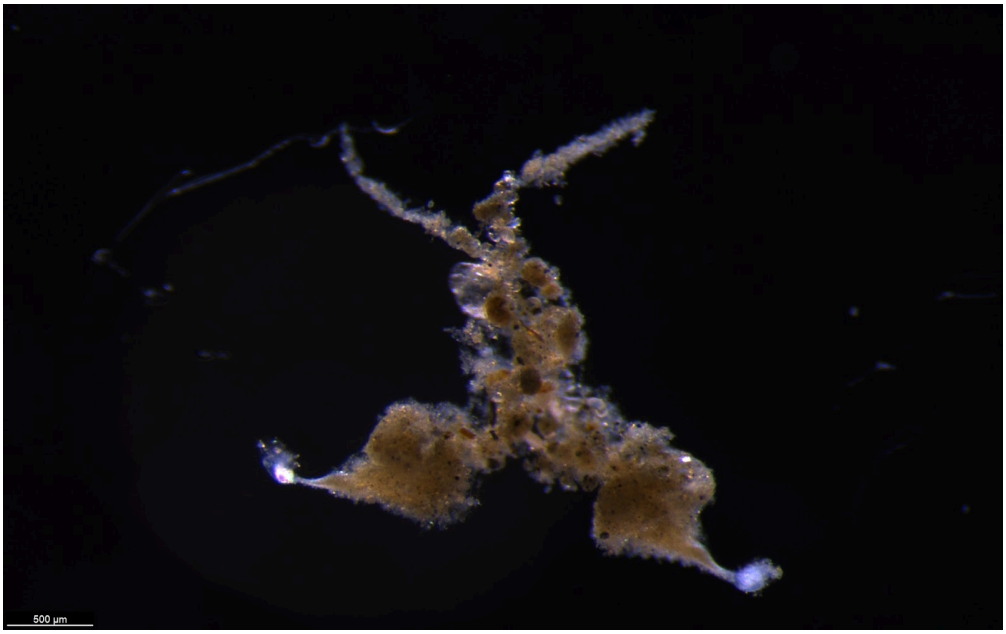

Image 27: Spermatophore structure outside of mud.

Cleaning Bowls

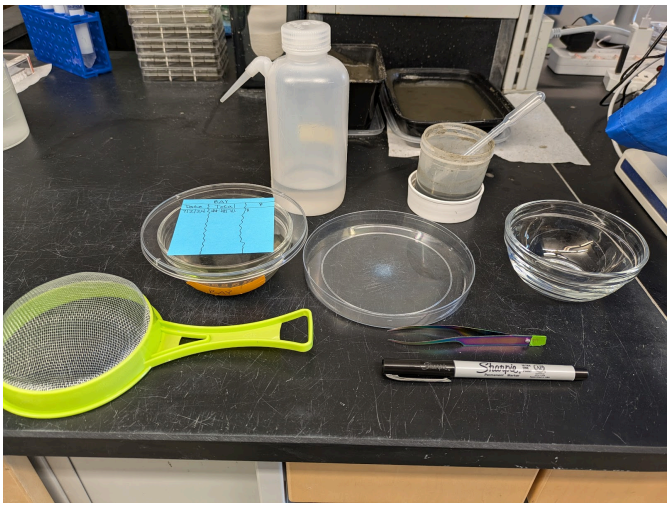

Image 28: Supplies for cleaning bowls of Streblospio.

- Organize bowls to be cleaned by the population.
- Get a small flour sieve for each population to be cleaned.
- Take your first bowl to be cleaned. Remove the label from the side of the bowl and place it on a clean bowl. On the bowl's lid, update the cleaning date with today's date.

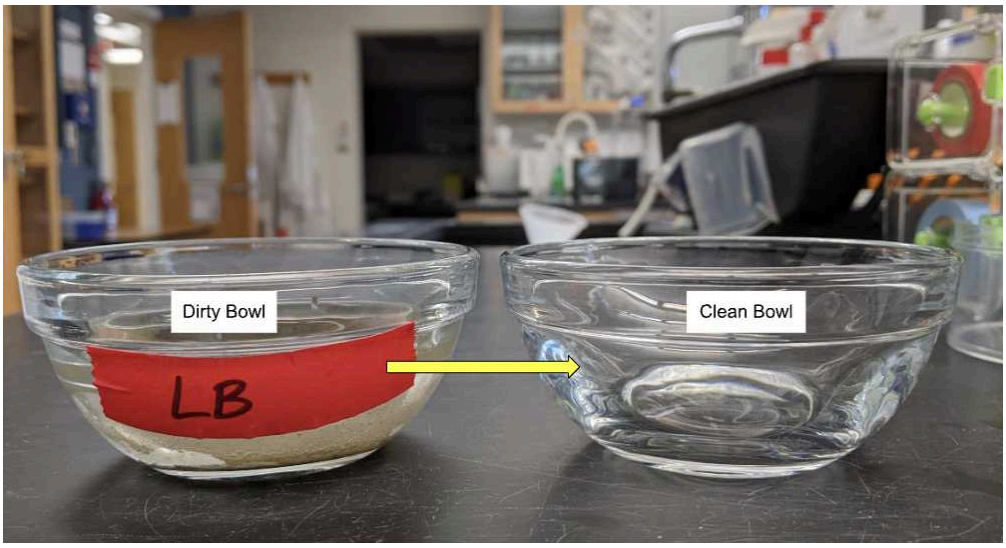

Image 29: How to move the label from the original bowl to a clean bowl

- Slowly decant the ASW from the top of the bowl into a sink or secondary container. See this video for clarification if needed:

https://www.youtube.com/embed/eyg6VbKR53o?si=FjHYMztfZxqnW7zn

Video Credit: Bryana Bynum

- Over the sink, dump the mud into your sieve.
- If needed, use an ASW squirt bottle to get all of the mud out of the original bowl and into the sieve.

- Lightly spray the sieve with more ASW to get off excess mud. Spray until there are mostly mud tubes in the sieve
- Dump the sieve contents into a large petri dish. Use the spray bottle to get all of the tubes off of the sieve

https://www.youtube.com/embed/7JyiYz4r6i8?si=HtXCDue0CxF2yUPq

Video Credit: Bryana Bynum

- Use forceps to find the worms in the large petri dish and transfer to the new, clean, labeled bowl. Inspect worms for gametes or broods if needed.
- Use a disposable pipette to add approximately 5-10 ml of mud to the bowl. There should be no fewer than 4, no more than 12 worms per bowl.
- Put the lid on the new bowl. Adjust the total number of worms tally if there were any missing, dead, or removed for brooding.

Sexing Worms

Female

- At a light microscope, focus on your worm of interest's well or plate.
- Pause and look for movement. Notice any irregular movement (i.e. other than the water moving).
- Using soft insect forceps, scoop underneath the area of movement that you suspect is the worm.
- Use the forceps to tap the end of the tube that you think the worm is in to scare it out of its tube enough to confirm there are palps and branchia.
- Use forceps to move the worm, perhaps in its tube, momentarily to another plate or tray. Add a small amount of ASW to the temporary well. If the worm is not fully out of its tube yet, scare the worm out by gently tapping the posterior end of the worm's tube. See this video for an example

https://www.youtube.com/embed/ElUNYfgtfQs?si=Gs4SO6azxiF3Ldtg

- Once the worm is out of the tube, wait for it to steady (it will likely thrash and swim in an infinity/figure-eight symbol pattern once out of the tube).
- Once settled, zoom in on the worm's body wall to look for eggs.

Image 30: Female Lecithotroph

Image 31: Female Planktotroph

Image 32: Example highlighting the female brood pouch

- Make sure you can count individual eggs and the body wall is not just whitish. Male worms will have a sparkly white hue on their body wall sometimes.

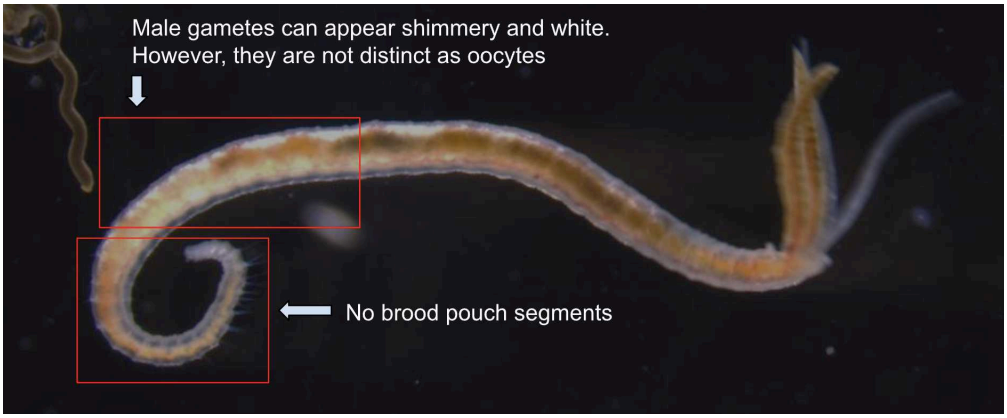

Image 33: Example of male Steblospio; absence of brood pouch and presence of male gametes highlighted.

- If there are distinct eggs on the worm, the worm can be moved back to its original well, and the lid marked with a female symbol.

Male

- At a light microscope, focus on your worm of interest's well or plate
- First, scan the surface of the mud for spermatophores. They should look like the structures below. You might have to zoom in 3-5x to properly see them. See Image 26 & 27 for examples.

- Once you have located spermatophores, you can mark the worm's lid with a male symbol.

If unsure whether your worm is male or female but it looks to be mature (i.e. you see a shiny white body wall, but didn't see any spermatophores, or you think you saw a brood pouch but no eggs) you can mark the worm with a "$" to signify it is a mature worm, but you could not 100% say it was male or female.

How to Ship Worms

- Test salinity of the seawater from your sample
- Take samples collected from the field. Scoop approximately 1 tablespoon of collected mud into a large petri dish and fill with seawater at the appropriate salinity.
- At a microscope, search for Streblospio in the subset of your sample.
- Put worms into a well in a 6-well plate with a little bit of mud (just enough to cover them) and fill the well with seawater. Repeat until you've gone through your entire sample. (Note: set aside brooding females if desired)
- Put 5 worms in each well (30 total per plate).
- Let worms sit for about a day. Revisit the wells and remove any dead worms.
- Put worms of 1 plate (~30 worms) into a 50 ml falcon tube.
- To the tube, add about 15-20 ml of mud and 20 ml of seawater. Close the lid. Wrap lid with lab tape or parafilm.
- Label the tube with the location, date collected, salinity of the water the worms are currently in, and any other pertinent information (i.e. females were noticed).
- Pack tubes in a styrofoam shipping box in a cardboard box. If warm outside, pack with an ice-pack. Put a piece of plastic or packing material so the tubes do not sit directly on the ice-pack.
- Ship worms to the desired lab!

Supplies List

	Item	Supplier	Catalog #	Purpose
	Bowls (Glass finger bowls)	Amazon	N/A	Storing worms with no need for family history, making large crosses with no family history
	Acrylic tray	Amazon	N/A	Store plates and bowls of worms on.
	Nalgene carboy (5 gal)	Amazon	N/A	Mixing artificial seawater in
	Refracto meter	Amazon	N/A	Used to measure salinity of
	Instant Ocean (160 gal)	Amazon	N/A	Sea salt used for making seawater
	Glass bottles (1000 ml)	Amazon	N/A	Storing seawater

	Pipetman (ONiLAB)	Amazon	N/A	Pipetting samples, removing mud from well plates,
	Disposable pipettes	Amazon	N/A	Moving mud
	Forceps	Amazon	N/A	Entomology forceps for moving worms
	Sieves (coarse and fine)	Amazon	N/A	Typical flour sieves of coarse and fine mesh - used for sieving mud samples from the field. Larger metal sieves preferred for field, small plastic sieves preferred for cleaning bowls in the lab.
	5 gallon bucket	Amazon	N/A	Collecting mud
	Garden spade	Amazon	N/A	Collecting mud
	Thin sharpies	Amazon	N/A	Writing on 6-well and 24-well plates
	Aquarium tubing	Amazon	N/A	Used to decant water and mud during field collection
	Rhodomonas stock	Carolina Biological	153597	Create flasks of lab Rhodomonas algae stock
	Tetraselmis stock	Carolina Biological	152610	Create flasks of lab Tetraselmis algae stock
	Alga Grow	Carolina Biological	153751	Media for growing algae
	Squirt bottle	Fisher Scientific	03-409-15	Store and distribute artificial seawater
	40 um cell strainer	Fisher Scientific	22-363-547	Strain algae before feeding to larvae
	6-well plates	Genesee	25-105MP	Storing individual worms, making crosses of 2-4 worms, holding larvae (~30/well)
	24-well plates	Genesee	25-107MP	Storing individual juvenile worms
	Falcon tubes	Genesee	28-108	Store autoclaved algae, water samples, and shipping worms
	Serological pipettes	Genesee	25 ml: 12-10610 ml: 12-104	Attach to pipetman. 25 ml ideal for clearing 6-wells, 10 ml needed for clearing 24-wells.

	Vacuum filter	Genesee	25-235	Filtering artificial seawater
	Large petri plates	Genesee	32-106	Used for looking for worms from field samples or bowls
	Small petri dishes	Genesee	32-105	Good for making a single cross, or spreading out mud when trying to find a single worm.
	Nalgene containers (125 ml)	Thermo Fisher	2118-0004PK	Used to autoclave mud, used at lab bench
	200 ul pipette + pipette tips	USA Scientific,Genesee	Pipette: USA Scientific: 7100-2200Pipette tips: Genesee: 23-150SPL	Moving larvae and juveniles
	Glass pipette	VWR	14673-010	Moving specimen or seawater. Can be pulled and broken for removing broods from female worms.
	Stereo Microscope	Zeiss	Stemi 508	View worms
	Microscope camera	Zeiss	Axiocam 208 (color)	Capture images for population data, behavior, and other projects during animal care

Protocol references

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