



Video transcript

How to collect and study nemertean larvae

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- Like most benthic marine invertebrates, nemerteans have planktonic larvae.
- In any particular location around the world some species of nemerteans may be easier to find as planktonic larvae than as benthic adults.
- This is especially true of parts of the world where there is not much in the way of intertidal zone.
- So much of the collecting of adults has to be done either by dredging or by SCUBA diving.
- Planktonic larvae may be difficult to identify because all of the species descriptions are based on the internal or external anatomy of adults.
- But larvae can be matched to adults based on DNA sequence data.
- In this video I will demonstrate how to collect, examine, and preserve nemertean larvae for DNA-barcoding.
- To collect nemertean larvae you'll need a plankton net with a mesh size of about a 100 to a 150 or 200 microns.
- Gently lower the net in the water and drag it slowly through the water.
- You can do this pacing up and down a dock, or by towing the net behind a kayak or a boat.
- It may be useful to have a little weight attached to the mouth of the net to help sink it down.
- If you are using a motorboat, be sure to go as slowly as is possible, going in and out of gear.
- Now we are pulling up the net. Raise it out of the water and dip it in a few times to rinse the plankton into the cod end.
- Unscrew the cod end and unclip it, and pour the sample into a jar.
- You can place the sample into a cooler or a bucket to take it back to the lab.
- You may want to dilute dense samples. And, in general you will want to keep the samples cold and sort it in the next few hours so that the animals remain alive.
- To sort the plankton samples, you will want a bowl, a turkey baster, a glass pipette, some small dishes and some filtered seawater.

- Use the turkey baster to move a small amount of your sample into the bowl. You don't want too much, maybe 1 or 2 cm deep, and examine it under the dissecting microscope.
- Once you find your larva, use a glass pipette and separate it into a small dish with filtered seawater.
- Keep sorting the sample, separating interesting specimens until you are done with it.
- Once you finish sorting your sample, you are ready to look at some of your selected specimens.
- This is the iconic nemertean larva called the pilidium. It looks like a hat with earflaps. The earflaps are called the lappets; there's also these two lobes, you can see a ciliated band spanning the lobes and lappets, an apical tuft at the anterior end, and inside the pilidium you can see it's gut, which is this dark brown area, and a little juvenile worm riding around.
- Nemertean larvae range in size from about 100 microns to over a millimeter but most of them are fairly small, so you will need to make a slide prep, and examine each larva under the compound microscope.
- You are going to use a glass slide and pipette the larva onto the slide in a small drop of seawater.
- Then you will take a cover slip and use a piece of modeling clay to create little feet to support the cover slip over the slide so you don't squish your specimen, and gently lower the cover slip into the slide.
- You may need to compress the specimen just a little bit to trap it and prevent it from swimming.
- For this you will put the slide back under the microscope, and gently compress the clay at the corners, while looking at it through the microscope to make sure that you don't damage the specimen.
- Now you are ready to examine it under the compound microscope.
- Note the shape, color and developmental stage of the larva and, if at all possible, photograph it.
- Pilidia come in many different shapes. Some look like hats or helmets, others look like socks.
- Most pilidia are planktotrophic but about 20 species are lecithotrophic.
- They have very much reduced larvae that superficially resembles cnidarian planula larvae or trochophore larvae of some spiralian like annelids.
- The pilidium larva is only found in one clade of nemerteans, called the Pilidiophora
- Other groups including Hoplonemertea and Paleonemertea have the so-called planuliform larvae which essentially are little planktonic juveniles.

- Once you have looked at the live larva and photographed it, you will want to preserve this individual for DNA-barcoding.
- For this you will want to use a pair of forceps to carefully lift the cover slip off the larva, open a tube and fill it with preservative such as 95% ethanol.
- And then pipette your larva with as little water as possible into the tube.
- So, pipette it up and down, to make sure the larva gets into the tube for sure, and then check to see that the larva is in the tube.
- You might want to examine the tube under the dissecting microscope, just to verify that the larva is really in it, and close the tube and label it in such a way that you can match the specimen to the photographs.

