

Today's lab dissection burrows into the fascinating world of errant and tube dwelling polychaetes. You will be partnering up this week and observing your partners work as well as your own.

Nereis virens:

You should expect to accomplish the following in your dissection of *Nereis*:

1. observe and sketch the cephalic region, pygidium, and parapods (one anterior, one posterior)
2. observe and sketch a cross section including the coelom, musculature, nerve cord, blood vessels and, if possible, nephridia
3. internal anatomy (logitudinal section) identifying the digestive, circulatory and nervous system

Introduction: *Nereis virens* known popularly as the pile worm, clam worm or rag worm. It is an eastern species that is farmed commercially for bait. Small populations found locally began when anglars introduce them, loosing them from their hooks or releasing them after fishing. This errant polychaete is an active hunting predator with a **homonomous** body type with **bilateral symmetry**. The trunk segments are uniform and have the appearance of rings or **annuli**. The annuli are internally separated by intersegmental septa. Within each repeated segmented, you will find a portion of the nervous, circulatory and (usually) excretory system and a pair of (left, right) coelomic compartments. There is a pair of locomotory appendages on every segment except in the cephalic anterior region and on the posterior pygidium.

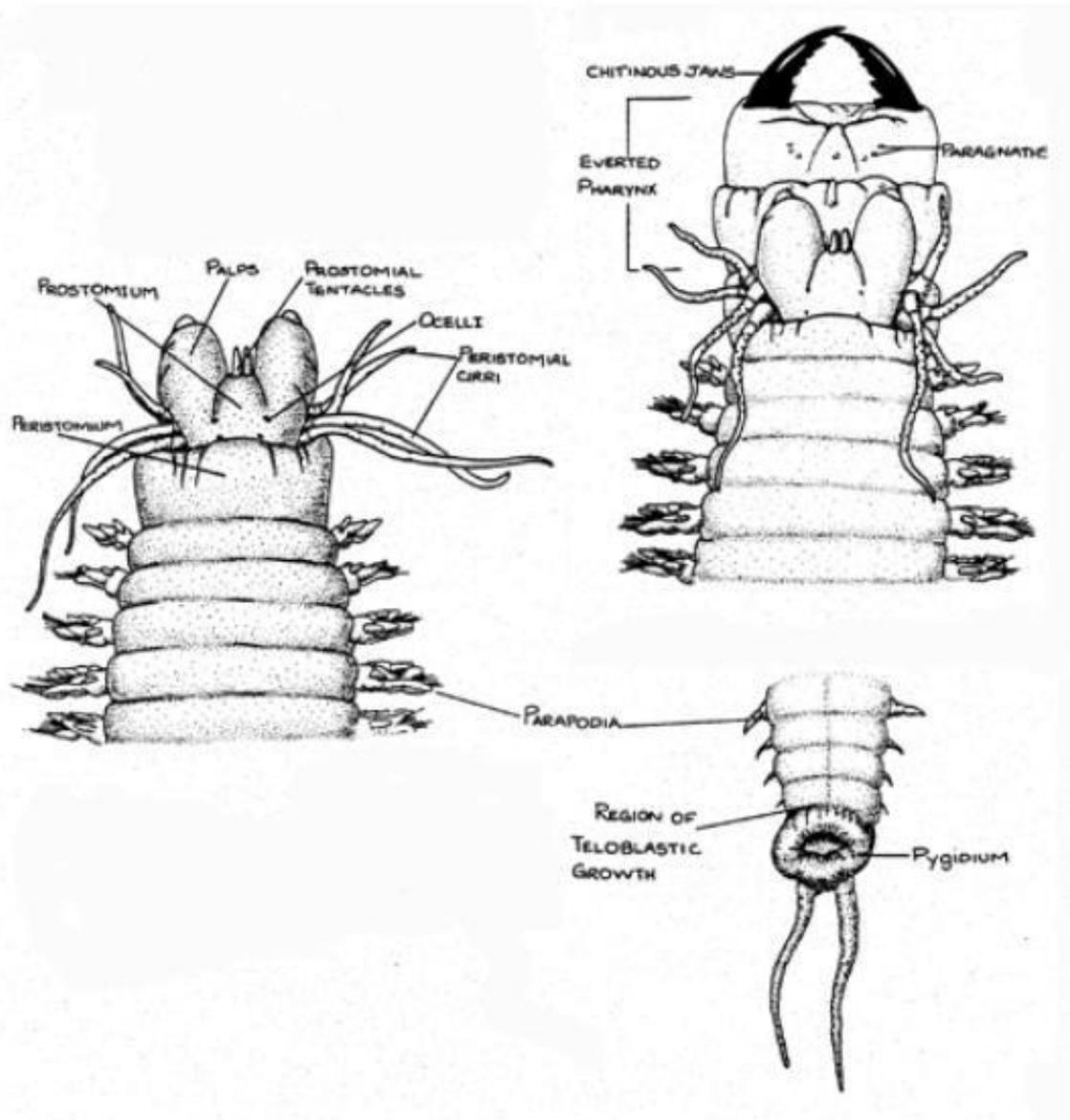
Watch a living worm crawl slowly and quickly and then put it in seawater to watch it swim. Which movement seems more efficient? Tease the animal a little to see if it will evert its proboscis, revealing its chitinous jaws.

External Anatomy

Cephalic Region: The cephalic region comprises the **prostomium**, where two pairs of ocelli, a pair of palps, and tentacles are located, and the **peristomium**, which has the mouth and cirri. Only a few animals will have their pharynx already everted to show the chitinous jaws and paragnaths. Share these with others. Most animals will have to be dissected to see the jaws at all.

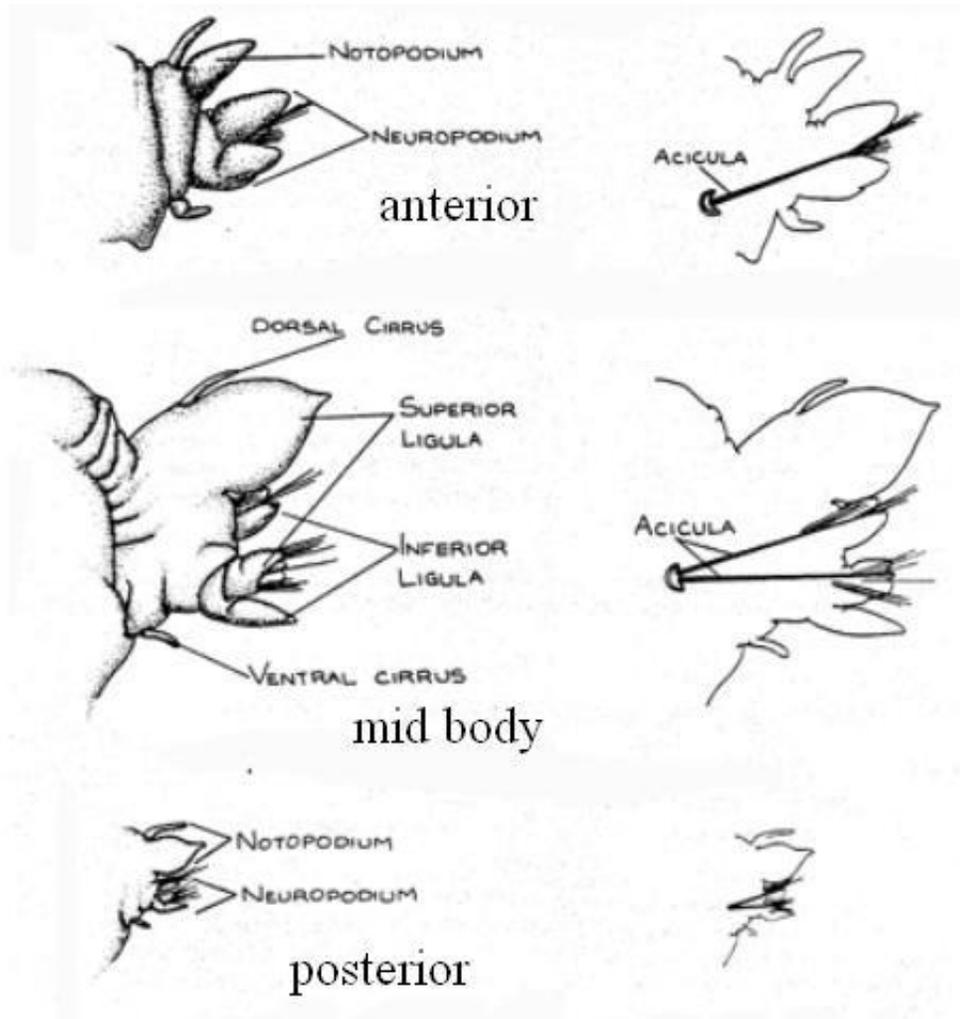
Pygidium: The trunk ends posteriorly at the elongated **pygidium**. **Teloblastic growth** adds segments to the worm only here next to the pygidium. The pygidium has a pair of long ventral cerci.

Fig 1 Nereis anterior and posterior regions



Locomotory Structures: Each trunk segment bares a pair of **parapodia**. These function as limbs (especially the ventral **neuropodium**) and gills (especially the dorsal **notopodium**). Detach an entire parapodium from the trunk for examination. The details of the notopodium and neuropodium, along with the parapodial **setae**, are used for keying out species. The first and second pairs of parapodia differ from the rest. Internally; the first two have only a single **acicula**, a modified seta which acts as a skeletal support. All other parapodia have two aciculae and two setal bundles per parapodium. The dorsal and ventral cirri are tactile organs. Actually find and draw these structures, opening parapodia if need be to look inside. The parapodia on the most posterior segments are smaller. So, despite the first appearance of parapodial monotony, there is a certain differentiation of them along the length of the worm.

Fig 2 Nereis parapods

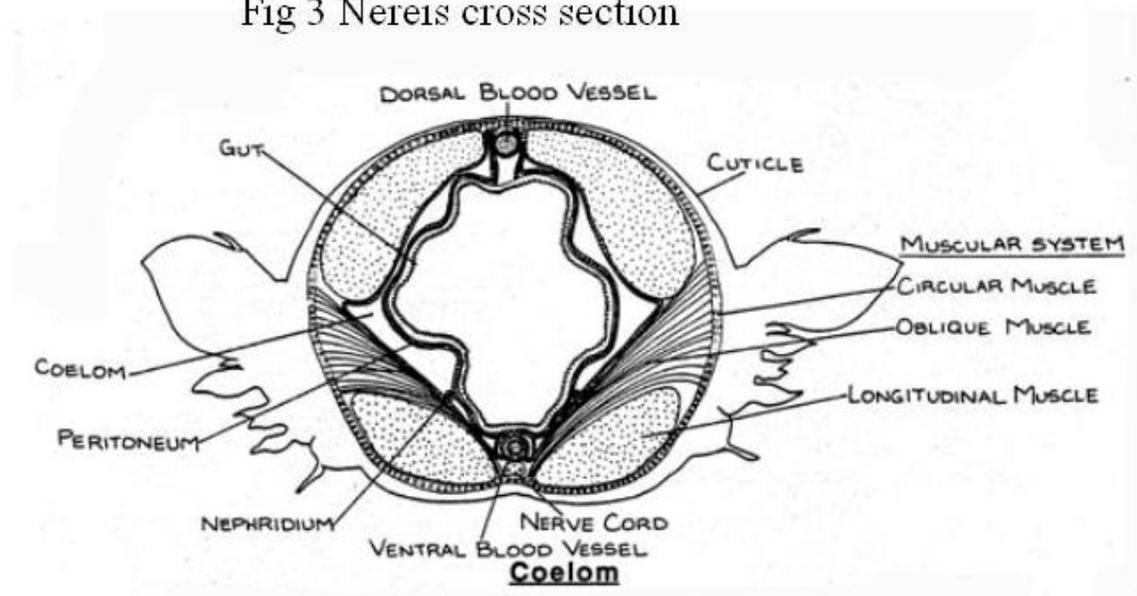


Cross Section : With the neat slices, make a thin cross-section of a mid-trunk at a septa. You may wish to use your scissors or alternatively a razor blade. Make sure you section your worm in the posterior half, as you will use the anterior portion for your next dissection. This cross-section will reveal the muscular system and the coelom & its lining of peritoneum, and possibly the segments' nephridia. Draw your prep, not the drawing! The pairs of **coelomic compartments** in each segment act as a hydrostatic skeleton for body support. The **peritoneum** surrounds coelomic compartments, coating the surfaces of internal organs.

Coelom: *N. virens* lacks permanent gonads. Instead, gametes form from the peritoneum, develop further in the coelom, and are released via the nephridia.

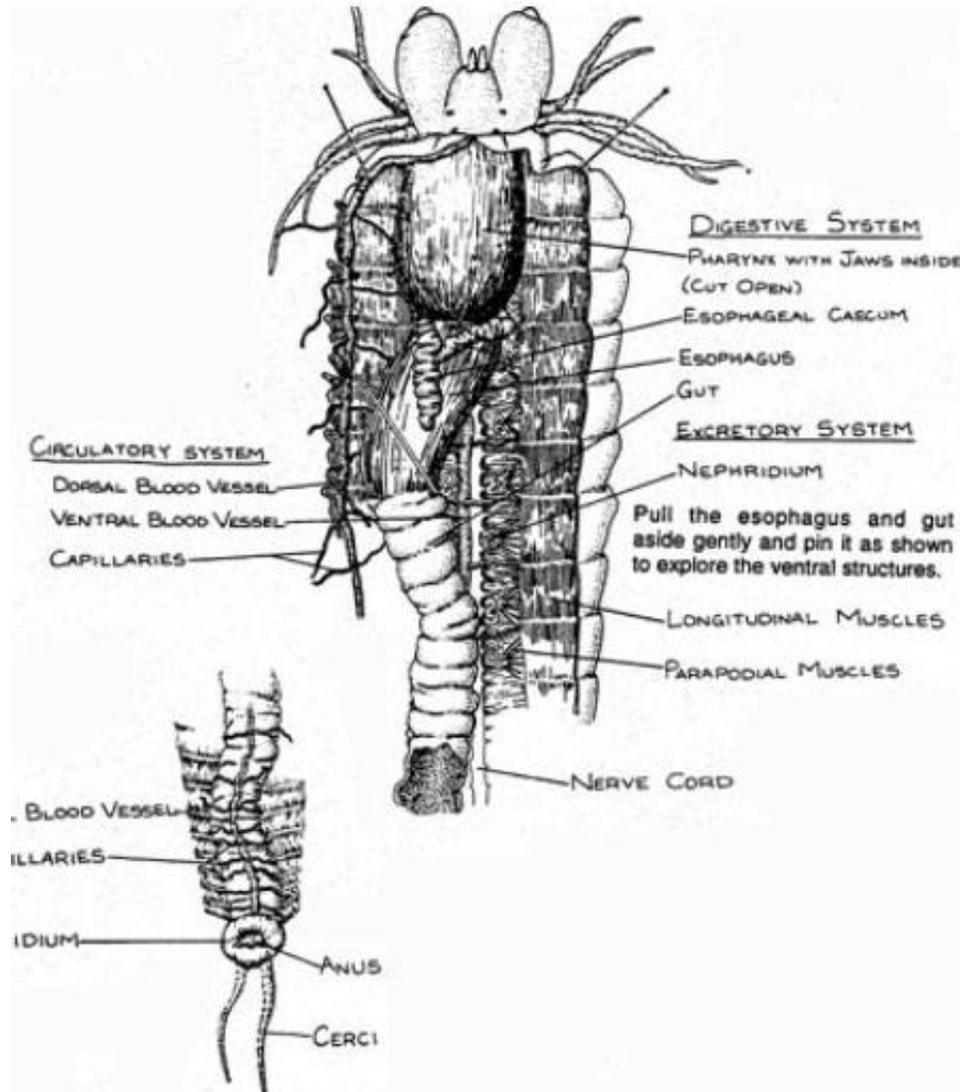
Excretory System : There is one pair of **nephridia** per segment. Each trunk coelomic compartment has a **nephrostome** that collects waste. From there, a ciliated tube penetrates the intersegmental septum, into the segment immediately behind. Waste is discharged from that segment at a pore below the base of the neuropodium. You probably won't be able to find any nephridia in the cross section but you may have more luck in the next dissection.

Fig 3 Nereis cross section



Internal Structures: For the longitudinal dissection of your specimen, pin the anterior half of the worm lengthwise and make a shallow lengthwise cut to one side of the dorsal blood vessel. Cut from the top of the peristomium towards the rear of the worm. Pin the worm open as wide as possible. Pull the esophagus and gut aside gently and pin it as shown to explore the ventral structures. You should be able to identify the ventral and dorsal blood vessels, the ventral nerve cord, septa separating the coelomic compartments and peripheral structures such as lateral blood vessels, oblique muscles, and acciculae.

Fig 4 Nereis digestive system

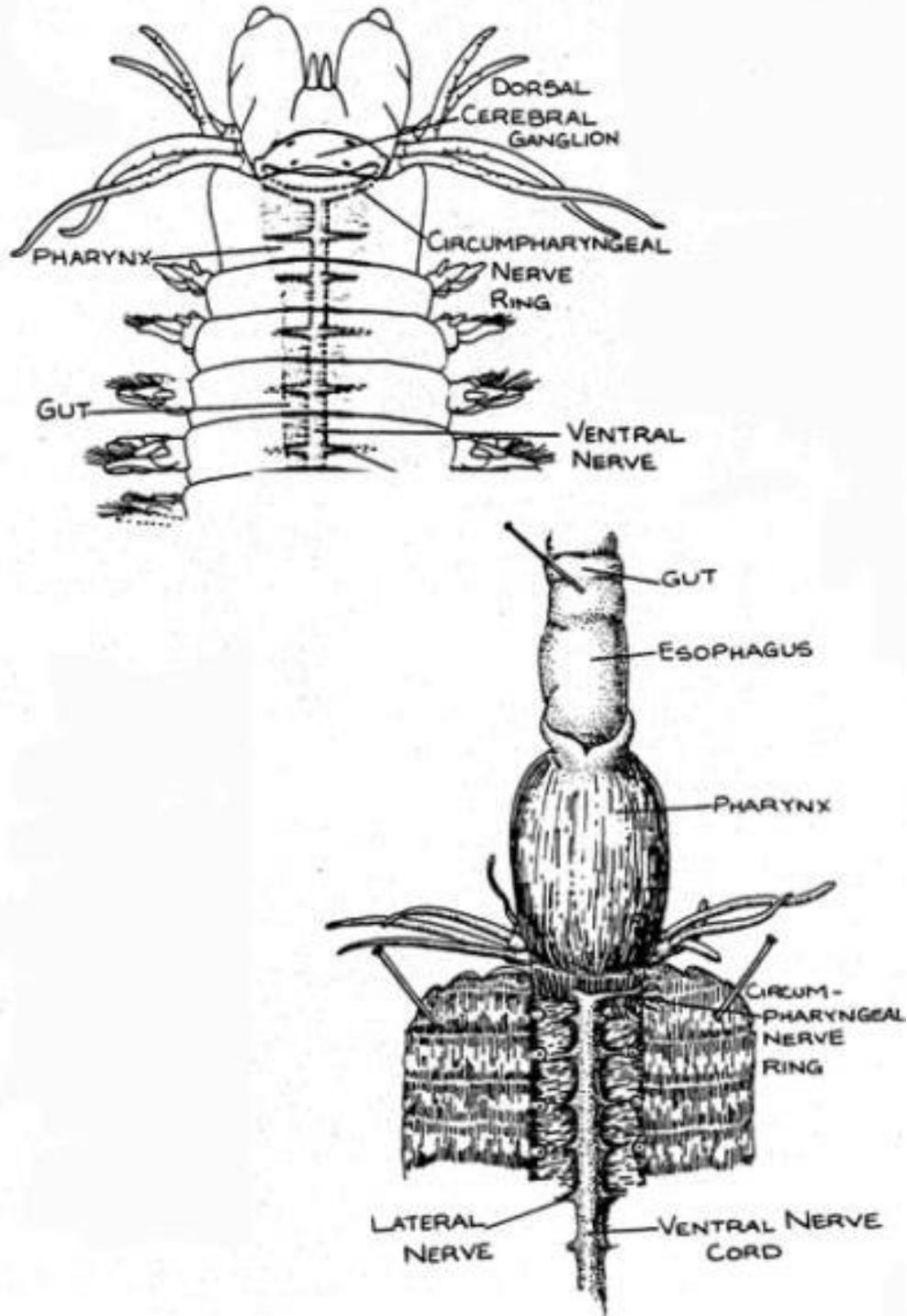


Nervous System

The nervous system of *N. virens* is simple and surprisingly easy to examine. It consists of a **dorsal cerebral ganglion**, which lies underneath the ocelli. A nerve ring surrounds the pharynx. It connects to the **ventral longitudinal nerve cord**. Nerves appear whitish and glisten. The ventral nerve cord below the digestive tract and the ventral blood vessel may look single-stranded but in fact is double-stranded. **Lateral nerves** arise from the swollen **ganglion** in each segment and run to the body wall musculature and the parapodia. You may elect to cut the esophagus and fold the gut over the worm's head. Follow the ventral nerve cord anteriorly. The nerve ring around the pharynx swells dorsally into a bilobed cerebral ganglion, within the prostomium. You will probably need to cut away tissue as

you follow the circumpharyngeal nerve ring. Nerves from the cerebral ganglion pass to the antennae, palps, tentacles, and cirri.

Fig 5 Nereis nervous system



Phragmatopoma californica:

Your goals for today's dissection of this reclusive annelid are as follows:

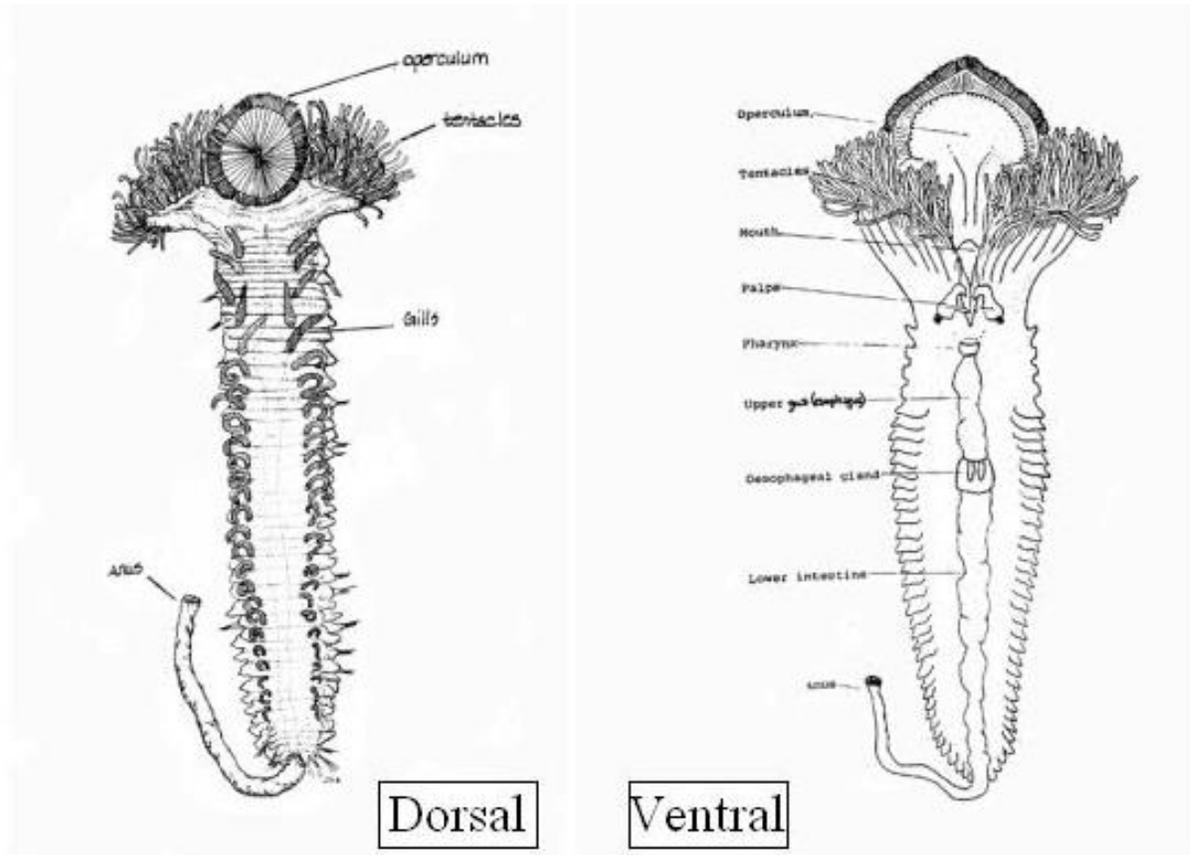
1. observe and sketch the external anatomy of a worm. Look for the circulatory system and the ciliary action associated with the gills.
2. observe and sketch the specialization of the parapodia in the different regions of the body (minimum of three different locations).
3. observe and sketch the specialized features associated with the cephalic region (tentacles, mouth, ciliary action)..
4. observe and sketch the internal organs in a longitudinal cut including the digestive tract, blood vessels, and possibly the nerve cord.

Introduction: *Phragmatopoma californica* ranges from the central California to Ensenada (Baja California). These worms are filter-feeders and live in mid-tidal habitats where there is plenty of tidal action and vigorous surf. Large colonies are obvious in mussel beds and below rocky outcroppings. The colonies appear as rounded honeycomb masses that range from 10 cm to 2 m across in size. Isolated honeycomb worms are also widely distributed among the mussels but are less prominent -- just twisted sandy tubes lying on surfaces here and there.

Special Adaptations: Because these worms are tube-dwellers, *Phragmatopoma* have many special adaptations that errant worms lack (and, of course, vice versa). The best way to observe these traits is to look at a live animal inside its tube. *Phragmatopoma* aren't very cooperative when it comes time to pull them out of their tubes; you have to coax them. Just grabbing the animal by its tentacles and pulling on them will most likely get you just half a worm. The best method we have found starts with cutting out individual tubes you want, not through them, so that you end up with a whole worm in a whole tube. Next, carefully cut a small vertical window in the side of the tube. This worm is now ready for live observation within its tube. To remove the worm then without harming it, carefully cut away pieces of the sand tube until the worm can be lifted out. Spend a while examining the tube itself during all this. Is there an orderliness to the size and distribution (by shape? by size?) of the tube's sand grains? What holds the grains together? Can you figure out, from its construction, how the tube was made by the worm? How it might be repaired by the worm? What dangers afflict the tube? That tube is the worm's house and home, and so it is highly suited to the worm's needs. What can you infer and conjecture about the worm's life and needs -- about its world -- by examining its tube?

External Anatomy, Parapodia, and Musculature: *Phragmatopoma* lives most of its post-larval life in a tube, but it is a surprisingly mobile animal within those confines. This portion of this guide introduces you to some traits that aid the animal in locomotion. Compare this design to that of the more mobile *Nereis*, *Phragmatopoma* also exhibits

Fig 6 Phragmatopoma external anatomy

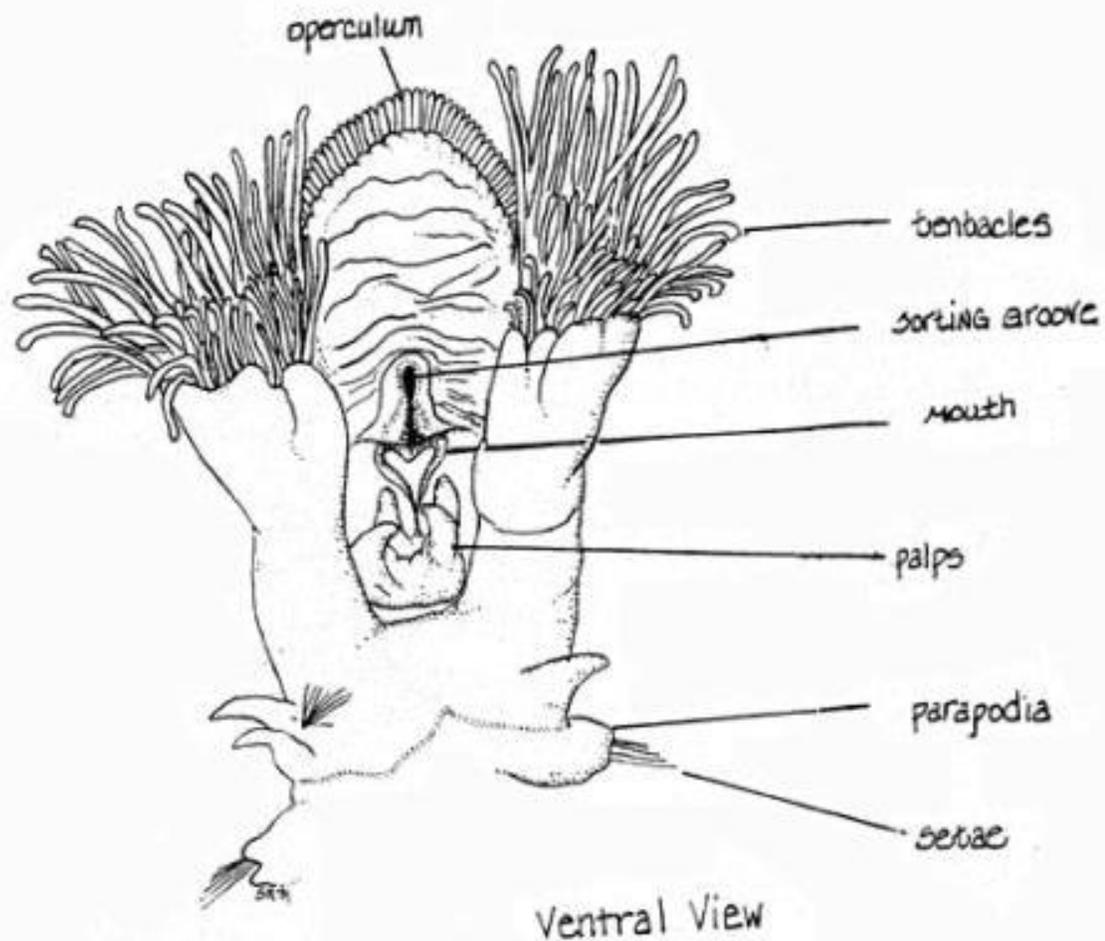


distinct body segmentation (metamerism). The series of body segments are bounded anteriorly by the operculum. Just posteriorly (and visibly ventrally) of the operculum is the oral area. Next are two thoracic segments and three parathoracic ones - marked by distinctive appendages. The numerous trunk segments follow next. These merge abruptly into the worm's tail, or caudum. Try and determine the location of the pygidium. To gain a sense of the external animal's segmental organization, lay a (preferably fresh) relaxed specimen on its side in a dissection pan, and hold it in place by pins at either end. Notice where the body segments each begin and end and the features repetitively present on each individual segment, especially the lateral parapodia. Most polychaetes have a rather undifferentiated trunk, from the head region to the anus. But Phragmatopoma has a modest differentiation of the most anterior trunk segments; it has a couple of "thoracic" segments immediately behind the head and then three "parathoracic" segments before the bulk of the trunk (the "abdomen") of the worm which extends posteriorly to the hind-most recurved rectal portion of the body. Each trunk segment (except the two thoracic segments) bears a pair of lateral parapodia. The setae of the parathoracic segments are quite different from those of the abdominal segments, and this distinction merits your attention. On the animal, they are fairly visible without detaching the parapodia from the body. Notice the robustness of the parathoracic setae (setigers, actually -- fused setae)

and their different orientation from the abdominal setae. Try to account for this parathorax-abdomen distinction in functionally ways -- what does the animal do with its parathorax that it does not do with its abdomen?

The Worm's Collar: Identify the various parts of the prostomium. Consider this entire specialized region as an integrated whole: take careful note of the region's structure and apparent modifications (Compare and contrast its traits with those of *Nereis*' prostomium). Look at the structure of the anterior part of the head leading to the operculum. Just above the mouth, do you see that ciliated groove? What does it do?

Fig 7 Phragmatopoma Tentacles and mouth



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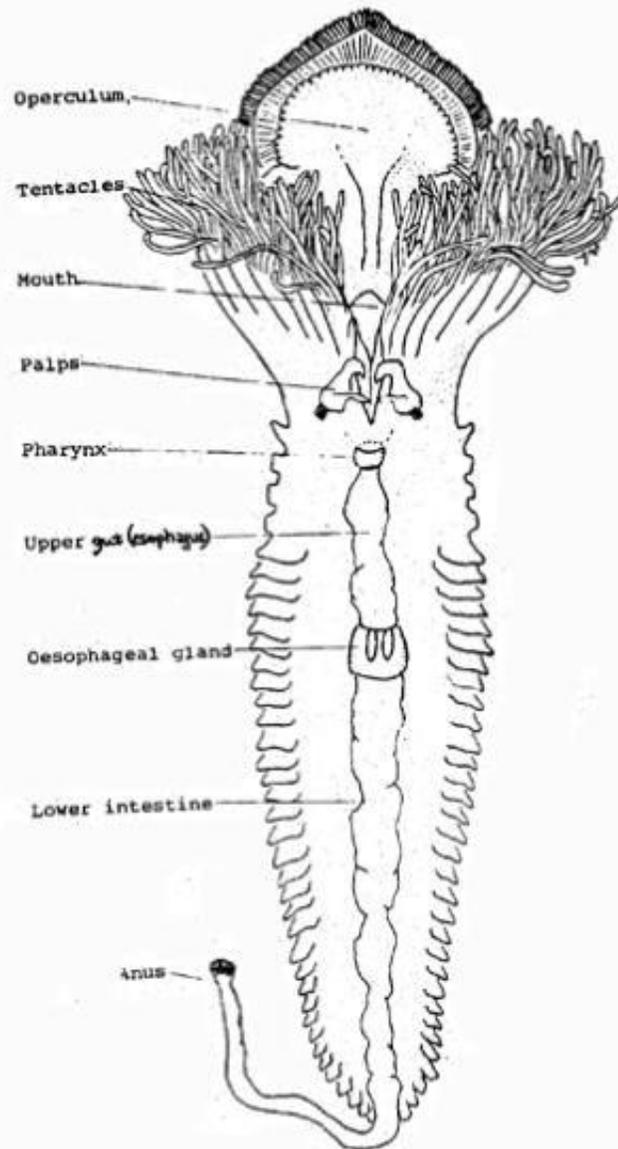
Digestion: The salient external features of *Phragmatopoma's* digestive system are the crown of purple feeding tentacles and the anus. The most rewarding observation of these features can be made by watching a living animal in cold sea water, relaxed a little with $MgCl_2$. Close scrutiny of the anus reveals the petal-like rim of the orifice and waves of peristaltic motion in the hindgut. The position of the anus prevents wastes from being trapped in the bottom of the worm's tube. Does the surface itself of the worm's trunk have traits that insure getting fecal wastes from the anus to the outside of the tube?

You may wish to examine the tentacles closely using a compound microscope. With a microscalpel and your forceps, slice off a tentacle and mount it on a slide with sea water and cover it with a coverslip. Examination this way reveals the tentacles' three-dimensional structure. The cilia whip particles through secondary food-grooves, into the mainstream of the primary food-groove, and then down into the mouth.

To study internal anatomy, pin a nicely relaxed specimen, palps up, at the base of the tentacles and between the last few abdominal parapodia. Cut between these palps and on down past the tenth pair of parapodia. Carefully spread the tissue open and pin it back gently. The anterior white organ is the pharynx, and the rose-colored organ located between the fourth and eighth abdominal parapodia is the esophageal gland. It may be involved in adjusting coelomic conditions or even excreting material by dumping mucus into the coelom. And it seems to be active digestively, because it secretes mucus into the gut. Note how convoluted the intestine is; this prevents its rupture when the worm elongates -- a simple solution to a big problem!

The Circulatory & Respiratory System: *Phragmatopoma* has two major circulatory vessels, one ventral and one dorsal, running the length of the worm. Posterior to the thorax and parathorax the dorsal vessel is a broad sinus bathing the gut. The vessel's anterior portion is contractile. There is a lot of action to see on the gills. Hemoglobin accounts for the blood's red color. Respiratory gills project dorsally in two rows all along the trunk. Vessels branch off from the dorsal vessel and irrigate the gills, then rejoin the dorsal vessel. Cilia cover each gill and encourage gas exchange by moving water over the gill-surfaces. Examine the gills. Notice the motion of their cilia and trace the small vessels (by the red blood) that are visible in the gills and that extend out on the surface distally from the circulatory loops there. Watch the contractile movements of the dorsal vessel as it pumps blood through the system.

Fig 8 Phragmatopoma Digestive System



Note below is a copy of the material that will be posted on the whiteboard at the start of the lab

Each numbered item must be a separate drawing on its own page; each item in a table must be labeled in the drawing.

Remember:

- Only draw on one side of a page.
- Put the phylum, class, genus, and species in the upper right of every page.
- Put a title on every drawing.
- Write orientation markers on every drawing.
- Number your pages and include this lab in your table of contents.

Nereis Virens

1. Observations and external anatomy (dorsal)
2. Close-up of pygidium and cephalic regions (dorsal) **45 minutes**

| | | |
|------------------------------|-------------|----------|
| prostomium | peristomium | palps |
| cirri | pygidium | cerci |
| region of teloblastic growth | ocelli | parapods |

3. Anterior, mid-body, and posterior parapods **30 minutes**

| | | |
|-----------------|-----------------|---------------|
| notopod | neuropod | dorsal cirrus |
| ventral cirrus | setae | acicula |
| inferior ligula | superior ligula | |

4. Cross section (don't forget to label the view!) **30 minutes**

| | | |
|----------------------------|---------------------|-----------------|
| cuticle | gut | coelom |
| circular muscle | longitudinal muscle | circular muscle |
| ventral blood vessel (VBV) | nephridium | peritoneum |

5. Longitudinal dissection **30 minutes**

| | | |
|--------------------------|---------------------------|------------------------------------|
| VBV | dorsal blood vessel (DVB) | capillaries |
| muscles | esophagus | circumpharyngeal nerve ring (CPNR) |
| dorsal cerebral ganglion | pharynx | gut |
| esophageal caecum | anus | cerci |
| pygidium | ventral nerve cord | septa |

Phragmatopoma californica

1. Observations and external anatomy (dorsal and ventral)
2. Close-up of cephalic region (ventral) **30 minutes**

| | | | |
|-------------------|----------------|-------------------|-----------------|
| gills | parapod | setae | thoracic area |
| parathoracic area | abdominal area | oesophageal gland | pharynx |
| palps | mouth | operculum | sorting groove |
| tentacles | anus | upper gut | lower intestine |

3. Parathoracic and abdominal parapods **15 minutes**

| | | |
|-----------------|-----------------|-------|
| notopod | neuropod | gills |
| inferior ligula | superior ligula | |

4. Longitudinal dissection (diagram blood flow) **45 minutes**

| | | | |
|--------------------|--------------------|-------------------|---------|
| tentacles | gills | capillaries | anus |
| muscles | lower intestine | oesophageal gland | VBV |
| esophagus | upper gut | CPNR | pharynx |
| oesophageal caecum | ventral nerve cord | | |

Conclusion: Consider how polychaetes are similar to and different from the ancestral annelid (modified versus preserved features, evolutionary adaptation, etc.). Compare and contrast features and functions of *Nereis* and *Phragmatopoma*.