# **Nematodes**

Vinegar Eel <u>Mixed Nematodes</u> <u>C. elegans</u>

**Species:** varies **Species:** elegans Species: aceti **Genus:** varies Genus: Caenorhabditis **Genus:** Turbatrix Family: varies Family: Rhabditidae Family: Panagrolaimidae **Order:** Rhabditida **Order:** varies **Order:** Rhabditida **Class:** varies **Class:** Secementea **Class:** Secementea **Phylum:** Nematoda **Phylum:** Nematoda **Phylum:** Nematoda Kingdom: Animalia Kingdom: Animalia Kingdom: Animalia



### Conditions for Customer Ownership (per USDA Permits)

We hold permits allowing us to transport these organisms. To access permit conditions, click here.

Never purchase living specimens without having a disposition strategy in place.

There are currently no USDA permits required for this organism. In order to protect our environment, never release a live laboratory organism into the wild.

### **Primary Hazard Considerations**

• Always wash your hands thoroughly after you handle any of these organisms.

# **Availability**

• Nematodes are cultured in our labs and are available year-round.

### How Will Animals Arrive and Immediate Requirements

- Vinegar eels and mixed nematodes will arrive in a 2 or 8 oz. plastic jar with media. Upon arrival, the lid on the jar should be loosened to allow for gas exchange.
- C. elegans will arrive on Nematode Growth Medium in a Petri dish. C. elegans does not have any immediate requirements.
- We over-pack each order of nematodes. It is normal to have some deceased nematodes in the container. You will receive at least the quantity of live nematodes stated on the container.

# Captive Care

#### **Habitat:**

- Vinegar eels can live in the jar they were shipped in for about one week. If you would like to subculture them, fill a 2,000 mL flask 3/4 full with a 50/50 solution of apple cider vinegar and distilled water. Add a few pieces of peeled apple and pour in the rest of your vinegar eel culture. It can be kept at room temperature. Add more media as it evaporates. You should subculture once per year.
- Mixed nematodes can live in the jar they were shipped in for about one week. If you would like to subculture them, pour soil water media into a culture dish until it is about 3/4" deep. Add 3-4 wheat seeds to the culture dish. Pipet at least 100 nematodes into the culture. You can expect it to peak in 4-5 weeks. Add more soil water media to the culture as it evaporates. You should subculture once per month to keep cultures going. It can be kept at room temperature.
- *C. elegans* is cultured on Nematode Growth Medium (NGM) at room temperature. If you would like to subculture *C. elegans*, you will need to pour more plates of NGM. Refer to the pouring plates literature for more information on how to pour your plates. Once you have more plates prepared, you can transfer *C. elegans*. One method is to remove a chunk of agar from an older, uncontaminated culture with a sterilized scalpel or spatula and place it onto a new plate. The agar will contain hundreds of *C. elegans*, which will then crawl out of the old agar and spread out into the new plate. Another method is to lay a strip of sterilized filter paper on an older culture, where it will pick up *C. elegans*. When the filter paper is saturated with moisture from the culture, touch it to a new plate, where *C. elegans* will be deposited. *C. elegans* should be subcultured every 2 weeks.



#### Care:

- Vinegar eels do not require special food; the vinegar media and apples are sufficient.
- Mixed nematodes do not require special food; the soil water media and wheat seeds are sufficient.
- *C. elegans* requires E. coli OP50 (Ward's 86-0615) as food. When subculturing *C. elegans*, you should streak E. coli across the NGM plate. Be sure you are using E. coli from an uncontaminated source.

### **Information**

- Method of reproduction:
  - Vinegar eels reproduce sexually. The male transfers sperm to the female and the ova are then fertilized. The female can also store the sperm in the seminal receptacle for future fertilization.
  - Mixed nematodes typically reproduce sexually.
  - C. elegans reproduces sexually.
- Determining sex:
  - In both vinegar eels and mixed nematodes, the males are smaller than females. A curved posterior end also identifies the male nematode.
  - C. elegans exists either as a hermaphrodite or a male. The predominant sexual form of C. elegans is the hermaphrodite.

### Life Cycle

Nematodes and Vinegar Eels

- Nematodes go through six stages of development including: egg, four larval stages, and adult. For vinegar eels, this cycle will
  occur completely in the vinegar medium. The eels will take five weeks to reach the adult stage. Their total life span is about 10
  months.
- Temperature and moisture levels determine when the egg hatches, and the larva will not emerge from the egg until the environment is one that is favorable for survival.
- The newly hatched larva, called the L1 stage, feeds and grows until it must molt. The molting process will occur four times before the nematode reaches the adult stage.
- In many types of parasitic nematodes, the larva will infect its host following its second molt, L2. The infection occurs when the nematode is in the L3 stage of its development.
- The fourth and final molt, L4, will take place within the host in most species. That host may be an insect, plant, or animal. Following the final molt, the nematode will be an adult but not yet capable of reproduction.
- A final phase of growth makes the adult capable of sexual reproduction, and it is only at this final phase in the nematode life cycle that genders are established.

### C. elegans

• The life cycle of *C. elegans* is temperature-dependent. *C. elegans* goes through a reproductive life cycle (egg to reproductive adult) in 5-1/2 days at 15°C, 3-1/2 days at 20°C, and 2-1/2 days at 25°C. Eggs hatch and larva proceed through 4 stages, each of which ends in a molt. When they reach adulthood, they produce about 300 progeny each. They live a total of about 2 weeks.

### Wild Habitat

- Vinegar eels are free-living nematodes which inhabit the byproducts of fermentation and feed on bacteria. They are preyed upon by fish.
- Nematodes have successfully adapted to nearly every ecological niche from marine to fresh water, from the polar regions to the tropics, as well as the highest to the lowest of elevations. They are present in freshwater, marine, and terrestrial environments.
- *C. elegans* lives in temperate soil environments. In the wild, it feeds on bacteria that develop on decaying vegetable matter.

# Special Notes

- *C. elegans* can adopt an alternative life form, called the dauer larval stage, if plates are too crowded or if food is scarce. Dauer larvae are thin and can move but their mouths are plugged and they cannot eat. Dauers can remain viable for three months. Dauer larvae can roam around for months and then reenter the L4 stage when they encounter a food source and live about 15 more days.
- Nematodes make interesting study specimens because they are among the lowest group of animals to possess a complete digestive tract, including mouth and anus.

### Disposition

• Please dispose of excess living material in a manner to prevent spread into the environment. Consult with your school to identify its preferred method(s) of disposal.

#### Vinegar Eels:

- You can safely use one of the following disposal methods:
  - Feed them to fish.
  - Autoclave them in a beaker at 121 °C for 15 minutes.
  - With plenty of tap water running, pour them down the drain.

#### Mixed Nematodes:

- You can safely use one of the following disposal methods:
  - Treat culture with a 10% bleach solution for 24 hours (1 part bleach to 9 parts culture medium or water culture medium removed). Then rinse bleach solution down the drain with water until you can no longer smell bleach. Rinse remaining materials and containers with water and dispose of them in a general garbage container.
  - Carefully wrap specimens and their containers in a biohazard bag (make sure the bag does not contain anything sharp that might puncture the bag) and tie closed (a twist tie works well). Autoclave the bag for 30 minutes at 121 degrees C and at a pressure of 15 lbs. per square inch. Dispose of autoclaved bag as your school recommends.

#### C. elegans

- You can safely use one of the following disposal methods:
  - Use a 20% bleach solution for 10 minutes (make sure the culture does not open until it is submerged in solution in order to the organisms are not released into the environment).
  - Place the organism in 70% isopropyl alcohol for 24 hours (make sure the culture does not open until it is submerged in solution in order to the organisms are not released into the environment).
  - Autoclave the organism @ 121°C for 15 minutes in an autoclavable bag. The Petri dish it is contained in will melt in an autoclave, so be sure to bag your organism and close it securely before autoclaving.

