# Protocols for Measuring Molting Rate and Egg Production of Live Euphausiids

Courtesy of the Peterson Lab at Hatfield Marine Science Center, Newport, Oregon, USA

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**The following information applies to both molting rate and egg production experiments.**

## Net tows for live euphausiids

Euphausiids are diel vertical migrators, so in most cases sampling will need to be conducted at night to catch enough animals for experimental use. We use a bongo net (0.6m diameter) with black 333µm mesh nets. We do an oblique tow to about 20m depth at ship speeds of 1.5-2 knots with a wire speed of about 30m/min. Using a solid codend (or taping over the holes of an existing codend with duct tape) keeps the animals in better condition since the water does not all drain out of the codend.

Fill a cooler about half full of seawater. For sorting purposes, it is helpful if the cooler is white inside. When the net comes on board, immediately pour the contents of the codend into the cooler. Do not rinse the net prior to emptying the codend. Set up live animal experiments as soon as possible after the tow comes out of the water, as the water quality in the cooler can quickly deteriorate when animals start to die. Use healthy, swimming animals for experiments. Do not use animals with obvious damage (missing eyes or appendages, whitish patches on body, not actively swimming, etc.). If there are a lot of jellies or salps in the tow, the animals are often too damaged to use for live experiments.

## General notes on conducting experiments

Check the temperature (and salinity if possible) of the water prior to starting an experiment. If you have the option, you can cool water that is too warm in the incubator or by filling a cooler with water and immersing jars filled with ice in the water. If your ship has a CTD with large enough bottles, you can also do a CTD cast down to a depth where the water temperature and salinity are appropriate, collect that water, and use it for your experiments. Do not set up an experiment if you cannot get water of appropriate temperature (and salinity) for your study area.

We use clear polycarbonate jars for our incubations. New jars are soaked for several days in seawater to remove any potential residue. Other plastics should also be fine, but we recommend that all new jars be soaked in seawater prior to use. Numbering the jars makes it easier to track individual animals during an experiment. The photograph on page 3 shows the jars we use, along with some of the supplies for setting up live experiments.

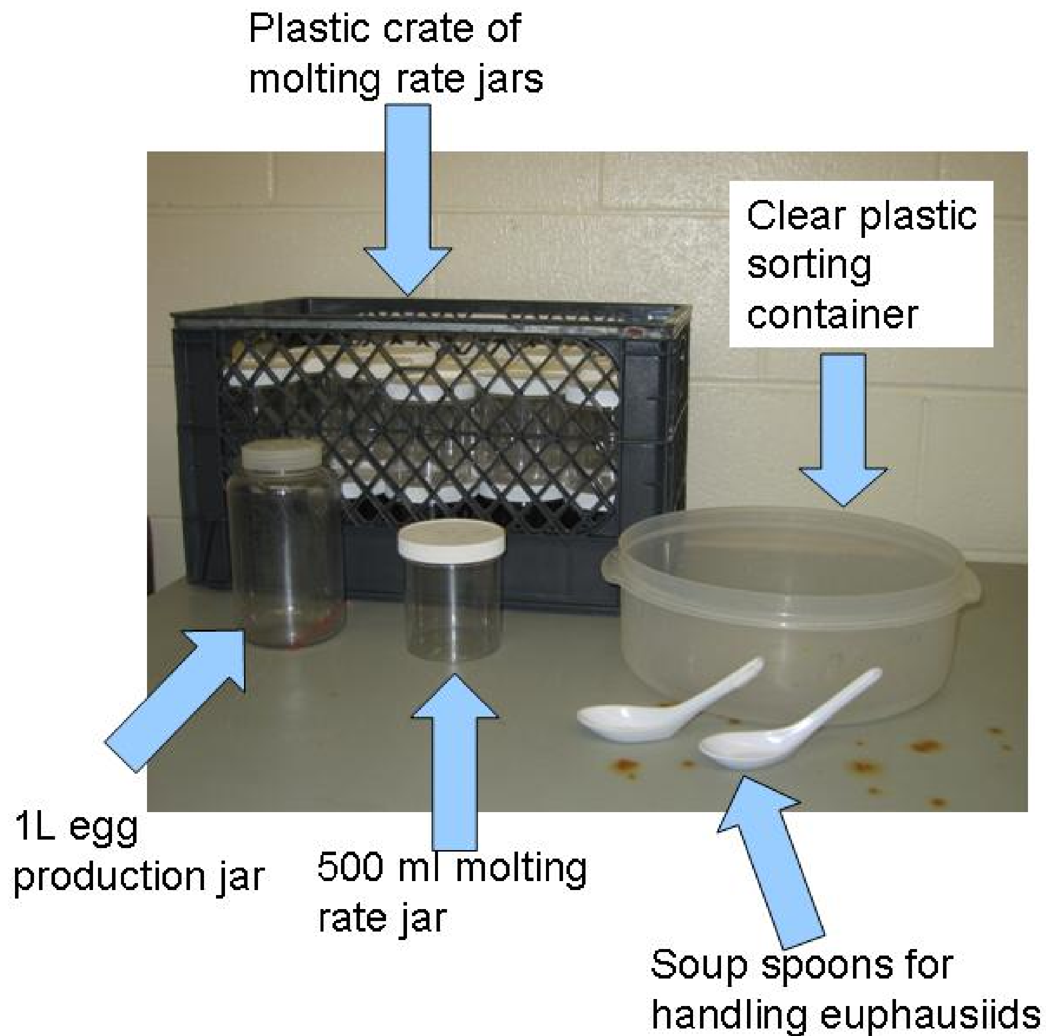
Experimental temperature: our experiments are conducted with the incubator set at 10.5°C as that is a typical temperature in our study area. Your experimental temperature should reflect a typical temperature encountered in your study area. Keep the animals in the dark except when you check the experiment.

For any live work with euphausiids, the goal is to have one euphausiid per jar. Moving the animals from the cooler to a sorting container prior to placing them in jars dilutes other plankton in the sample and makes it easier to keep other material (copepods, chaetognaths, siphonophore bracts etc) out of the incubation jars.

Record as much information as is available to you during the experiment. Station depth, temperature, salinity and surface chlorophyll are all useful to know when you are analyzing the data. Each time experiments are checked, record the date, time, water temperature in the jars, number of molters or spawners, number dead, etc.

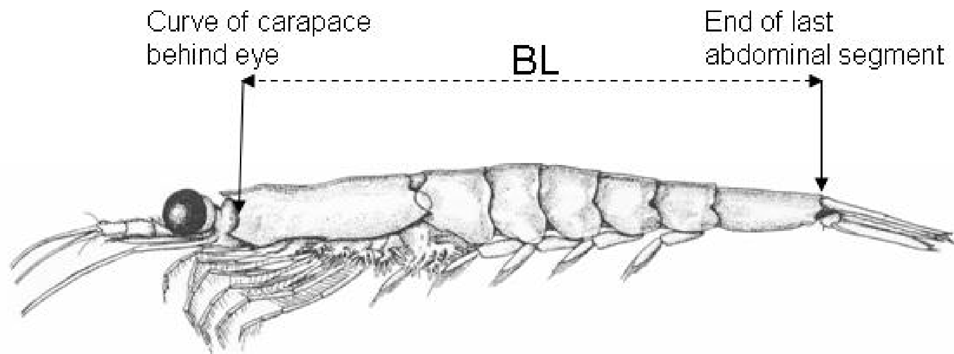
**The following pages contain specific protocols for molting rate and egg production experiments.**

# Some of the supplies for conducting live euphausiid experiments



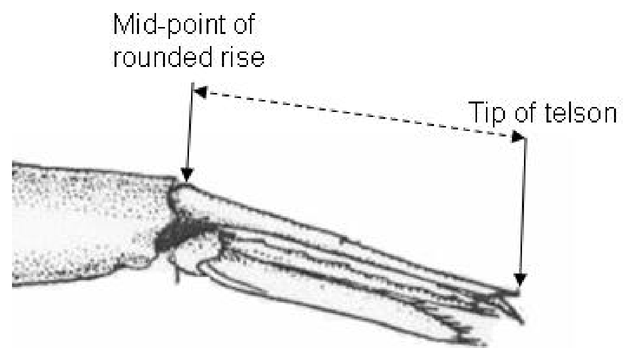
## Figure 1. Body Length measurement

**Measure from the curve of the carapace behind the eye to the end of the last abdominal segment.**




## Figure 2. Telson measurement

**Measure from the middle (highest point) of the rounded rise at the anterior end of the telson to the posterior end, excluding telson spines.**



Euphausiid Molting Rate Experiment Protocol

## AT SEA

Materials:

| Euphausiids | Incubator set at appropriate temperature |
|---|---|
| 500 ml jars (clear) with lids | Thermometer |
| Sieve with 200 µm mesh | Data sheet |
| Seawater (see notes on temperature & salinity) | Vials (7 ml and 20 ml) |
| Soup spoons (white) | 5% formalin |
| Sorting container (white or opaque plastic) | Forceps |

Euphausiids selected for experiments should be actively swimming and free from obvious damage (white/opaque areas on the body, missing eyes or appendages, etc). If there are a lot of molts floating in the cooler when you start sorting animals, do not use that catch for a molting experiment. It is best if all euphausiids in an experiment are the same species although smaller animals may be difficult to distinguish by eye. Our standard protocol for a molting experiment is 30 animals. If you will have multiple experiments running at the same time, it is easiest if all jars from one experiment are stored together in a container that can be labeled with experiment details (station name, start date, check time etc.). We use plastic crates (see photo on page 3), which are also useful for storing the jars when they are not in use.

**Method:** Experiments should be set up as soon as possible after a net tow. Fill the sorting container with seawater and wait for the bubbles to dissipate. Use the soup spoons to gently scoop healthy, swimming euphausiids from the cooler into the sorting container. If you accidentally squish an animal with the spoon, do not use it in the experiment.

Fill 500 ml jars with 200 µm filtered seawater. Use the soup spoon to place one animal from the sorting container into each jar. Make sure the jar contains only one animal and that the animal is not stuck in the surface tension before you put the lid on the jar. When all jars contain animals, label the crate and put it in the incubator. Keep the animals in the dark except when you are checking the experiment.

Record station, date, time and number of jars on the data sheet at the start of the experiment. You may also want to record station lat/long and depth, temperature, salinity and chlorophyll if available. Check every 12 hours for molts. You can usually see the molts pretty easily by gently swirling the jar or tipping it upside down. Molts will usually be on the bottom of the jar, but sometimes get stuck in the surface tension and will be floating at the top. Make sure everyone who will be checking the experiment knows how to identify that a molt is present. Each time the experiment is checked record the time, number of molts and number of dead animals on the data sheet. If an animal molts and dies, preserve it like any other molter (see below). If an animal dies without molting, discard the animal and note it on the data sheet. Dead non-molters are not included in any experimental calculations.

When an animal molts, preserve the animal and molt together in a small vial (we use 7 ml glass scintillation vials). Carefully remove the molt from the jar using the forceps. Pick up the molt near the front to avoid damaging or detaching the telson. Check the molt in the vial to see if there is a telson attached. If not, try to find it in the jar since the telson is measured to obtain the growth increment for each animal. Sieve the animal out of the jar and use the forceps to place it in the vial.

The best way to pick up the animal is by the clump of antennae right in front of the eyes. This minimizes damage to the body (which is soft after molting) and to the telson. Check in the sieve for any molt parts. Fill vial with 5% formalin.

We label our vials with the information shown in the box. An explanation of each item is shown next to it in parenthesis.

| Label | Explanation |
|---|---|
| PaCOOS | (cruise name) |
| 6/29/04 | (date molt preserved) |
| NH25 | (station name) |
| 12 hr | (experiment check time when molt was found) |
| #1 | (sequential # of all molters from this experiment) |

**Non-molters**: at the end of the experiment, preserve all living animals that did not molt together in one vial (we use 20 ml scintillation vials). Label as above except with "non-molters" instead of the hour and animal number. At the end of the experiment, rinse all the jars with warm fresh water and let them dry.

**Note about vials:** Glass scintillation vials work well for preserving the euphausiids from these experiments. They are clear, so it is easy to see that the molt and animal are both in the vial and the storage trays the vials are packaged in make it easy to keep preserved animals organized.

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## IN THE LAB:

### Measuring molters:

Use a dissecting probe to dip the animal and molt out of the vial. Put the animal and molt in a Petri dish full of water and place the dish on the stage of a dissecting microscope. It is easier to see the telson when you measure the animals in water. Only work with one molter at a time to keep from confusing which animal goes with which molt.

Measure the length of the animal and note the life history stage/sex. We measure body length (BL) – from the curve of the carapace behind the eye to the end of the last abdominal segment (Fig. 1). We can't measure the length of the whole molt so we use the change in length between the telsons of the animal and the molt to determine a growth interval. Measure the lengths of the animal telson and the molt telson (Fig. 2). Measure both telsons using the same magnification on the dissecting microscope. We also measure the length and record the stage/sex of all the non-molters. There is no need to measure telsons of non-molters. The same person should do all the measurements within an experiment. The molting rate data sheet (p. 8) is designed so that we can record all data from shipboard experiment checks and laboratory measurements on the same data sheet.

**Calculations from molt data:**

1. Calculate Intermolt Period (IMP)

   IMP = 1 / (# molts / total # in expt) / #days incubated

   - # molts = number of animals that molted during the experiment
   - total # in expt = total number of animals in experiment (molters + non-molters). Do not include animals that die without molting during the experiment in this total.
   - # days incubated = total days animals were held in experiment (usually 2)

2. Calculate total length of post-molt and pre-molt euphausiids from telson length. The equation shown below is given as an example. This equation is from a regression of body length vs. telson length of juvenile and adult *Euphausia pacifica* using measurements made in our lab. You may need to do a similar regression for your target species.

   TL from telson length (mm) = 5.426*(telson (mm))-0.9869

3. Subtract the pre-molt length from the post-molt length to get the change in length (note that *Euphausia pacifica* can shrink so the change in length may be negative):

   $TL_{post\text{-}molt} - TL_{pre\text{-}molt}$ = mm growth

4. Divide change in length by IMP to get a growth rate (mm/day) for each individual euphausiid that molted during the experiment:

   mm growth/IMP=growth rate in mm/day

Molt Expt Data Sheet

| Cruise & Ship: | |
|---|---|
| Station: | |
| Net: | |
| # bottles: | |
| Sorted by: | |
| Temp/Conditions: | |

| Molt Per. | Date | Time | Temp. | # molts | # dead |
|---|---|---|---|---|---|
| start | | | | | |
| 12 | | | | | |
| 24 | | | | | |
| 36 | | | | | |
| 48 | | | | | |
| TOTAL | | | | | |
| *Measured by:* | | *Date:* | *Scope:* | | |

| | | | | **ANIMAL** | | **Animal Telson** | | **Molt Telson** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| An # | Molt Date | Molt Time | Species | BL (div.) | Scope Mag. | Tel. (div.) | Stage /Sex | Tel. (div.) | Molt stage | Mag for telsons | Notes on individual animals |
| 1 | | | | | | | | | | | |
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Experiment notes:

Egg Production Experiment Protocol

# *Euphausia pacifica*

Note that egg production experiments do not yield an egg production **rate** but rather an estimate of **brood sizes** for individual females. Use healthy, swimming females with purple ovaries (see photo at left). Do not use animals with obvious damage (missing eyes or appendages, whitish patches on body, not swimming, etc.).

| | |
|---|---|
| Euphausiids with purple ovaries | Incubator set to appropriate temperature |
| 1L bottles (clear) | Data sheet |
| Sieve with 200 µm mesh | Wash bottle of filtered seawater |
| Seawater (see notes below about temperature and salinity) | Forceps |
| Soup spoons (white) | Dissecting scope |
| Sorting container, white or opaque plastic | 2 oz jars or small Petri dishes |
| 120 µm or smaller sieve | Lab counter |
| Thermometer | Petri dish with grid |

**Setting up the experiment:** Fill sorting container with seawater. Using a soup spoon, gently scoop healthy, swimming females with purple ovaries into the container. Try to minimize the amount of other material added to the container along with the female. **NOTE**: check the temperature and salinity of the water prior to starting an experiment. Do not set up an experiment if you cannot get water of appropriate temperature and salinity.

Fill 1L bottles to overflowing with 200 µm filtered seawater. Place one purple female in each bottle using the soup spoon. Put the lid on the bottle and check to be sure it contains only **one** animal. When all bottles contain animals, label the crate (station and check time) and put it in the incubator. Record header information and bottle numbers on data sheet. Remember to note how many bottles are in the experiment. Keep the animals in the dark except when you are checking the experiment.

**Checking the experiment:** Check every 12 hours for eggs. All females, regardless of whether or not they lay eggs, are kept for the whole 48 hour experiment and checked every 12 hours. Check to see if the ovary is still purple or if eggs are visible in the jar. Note that females will sometimes release eggs and still have a purple ovary, so a purple ovary does not guarantee that the female has not released any eggs. If the ovary is no longer purple, or if eggs are visible in the jar, the next step is to collect the eggs. Swirl the bottle gently and pour the water through a sieve (120 µm or smaller). Catch the sieved water in another bottle. Try to keep the female in the bottle (this takes some practice). If she lands in the sieve, immediately rinse her back into the bottle using the wash bottle of filtered seawater. As soon as you've poured all the water through the sieve, pour it back into the bottle with the female to keep the time she is without water to the absolute minimum. Rinse the eggs from the sieve into a 2oz jar or small Petri dish, and count them using the dissecting microscope. Placing a gridded Petri dish under the dish of eggs will help you keep track of where you are while counting. Record the number of eggs and their condition on the data sheet. Discard the eggs unless you would like to use them for additional experiments such as embryo development, hatching success etc.

Each time you check the experiment record the time, water temperature, number of spawners and number dead. For each individual female, record whether the ovary is purple and the total number of eggs released during the past 12 hours. Note if the female seems unhealthy or is not actively swimming.

If an animal dies, write DEAD at that check period, and note the color of the ovary. If she spawned during the experiment, measure her body length and record in the column labeled "BL & mag." Be sure to note which dissecting microscope you are using. Discard the animal.

**Ending the experiment:** At the end of the experiment, measure all females that spawned, thank them for participating, and let them go. If any females molt during the experiment, measure the telson of the molt (when molt is seen) and animal (at end of experiment). Animals that did not spawn or molt during the experiment do not need to be measured.

**Preserving eggs:** It is much easier to count the eggs during the experiment, but if this is not possible they can be preserved for counting later in the lab. We use 60ml plastic bottles to preserve eggs. The volume of material in the bottle is not that high, but you will need room for a lot of rinse water. Pour all the eggs into the sieve. Rinse them down gently in the sieve until they are all together, then rinse them into the preserving bottle. You may want to use a small funnel. If the female can't be measured live at sea, you can preserve her along with the eggs. Add enough 100% formalin to get a concentration of 5% formalin in the bottle. Label the bottle with cruise name, station, experiment date, female number and whatever other information you need to identify the experiment. If you preserve the female in the same bottle, some eggs are likely to get stuck to her. Be sure to check for this when you count the eggs.

# *Thysanoessa spinifera*

Note that egg production experiments do not yield an egg production **rate** but rather an estimate of **brood sizes** for individual females. We developed this protocol for *Thysanoessa spinifera* because the eggs of this species are very sticky. They adhere to the incubation jars and cannot be rinsed out without damaging them, resulting in low brood size estimates. This species is only found in the eastern north Pacific ocean, but we include this method in case you encounter other species with sticky eggs. We do not know if other species of euphausiids have sticky eggs. *Thysanoessa spinifera* have blue ovaries when they are ready to spawn. Use healthy, swimming females with blue ovaries. Do not use animals with obvious damage (missing eyes or appendages, whitish patches on body, etc.).

| Euphausiids with blue ovaries | Incubator |
|---|---|
| 500 ml (16 oz) jars | Data sheet |
| Sieve with 200 µm mesh | Wash bottle of filtered seawater |
| Seawater (see notes below about temperature and salinity) | Forceps |
| Soup spoons (white) | Dissecting scope |
| Sorting container, white or opaque plastic | Gridded Petri dish, large |
| 120 µm or smaller sieve | Lab counter |
| Thermometer | |

**Setting up the experiment:** Fill sorting container with seawater. Using a soup spoon, gently scoop healthy, swimming females with blue ovaries into the container. Try to minimize the amount of other material added to the plastic container along with the female. **NOTE**: check the temperature and salinity of the water prior to starting an experiment. Do not set up an experiment if you cannot get water of appropriate temperature and salinity.

Fill 500 ml jars with 200 µm filtered seawater. Place one blue female in each jar using the soup spoon. Put the lid on the jar and check to be sure it contains only **one** animal before returning it to the crate. When all jars contain animals, label the crate (station and check time) and put it in the incubator. Record header information on data sheet. Remember to note how many bottles are in the experiment. Keep the animals in the dark except when you are checking the experiment.

**Checking the experiment:** Check every 12 hours for eggs. All females, regardless of whether or not they lay eggs, will be kept for the whole 48 hour experiment and checked every 12 hours. At each experiment check, record the time, the water temperature in the jars and the number of spawners. Note if the female does not seem healthy or is not actively swimming.

If the female is no longer blue, or if eggs are visible in the jar (they will usually be stuck to the bottom), assign the female a number and write it on the lid. Carefully remove the female from the jar using the soup spoon. Try not to touch the bottom of the jar as this may damage eggs that are stuck there. Sieve most of the water from the jar into a new jar. Place the female in this jar with the numbered lid and return her to the experiment. You may need to add some more filtered seawater to the new jar with the female.

Counting the eggs: Rinse the contents of the sieve into a dish and count any eggs you see there. Check for any eggs that may be stuck to the sieve. To count the eggs in the jar, place a gridded Petri dish under the jar. Jar and dish should fit under the microscope at low magnification. Count the eggs in the jar using the microscope. Record the total number of eggs and color of the ovary on the data sheet.

Total number of eggs = eggs in jar + eggs in dish + eggs in sieve

If an animal dies, write DEAD at that check period, and note the color of the ovary. If she spawned during the experiment, measure her body length and record in the column labeled "BL & mag." Be sure to note which dissecting microscope you are using. Discard the animal.

**Ending the experiment:** At the end of the experiment, measure all females that spawned, thank them for participating, and let them go. If any females molt during the experiment, measure the telson of the molt (when molt is seen) and animal (at end of experiment). Animals that did not spawn or molt during the experiment do not need to be measured.

**Note:** There is no good way to preserve these sticky eggs to count later in the lab since they cannot be rinsed out of the incubation jar without doing extensive damage to the eggs. However, being sticky does make them easier to count at sea since they do not move around under the microscope.

Egg Production Data Sheet

| Cruise: | |
|---|---|
| Station: | |
| Net: | |
| # bottles: | |
| Sorted by: | |
| Temp/Conditions: | |

| Check | Date | Time | Temp. | # spawn | # dead |
|---|---|---|---|---|---|
| start | | | | | |
| 12 | | | | | |
| 24 | | | | | |
| 36 | | | | | |
| 48 | | | | | |

| | Start Time: | | 12 hr: | | 24 hr: | | 36 hr: | | 48 hr: | | BL & mag | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bottle # | Species | Purple? | Eggs? | Purple? | Eggs? | Purple? | Eggs? | Purple? | Eggs? | Purple? | scope: | Comments |
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**A brief explanation of our cruise data sheet (shown on p. 14):**

We sample the Newport Hydrographic (NH) line off of Newport, Oregon, every two weeks (weather permitting) from the 55' R/V ELAKHA. Stations are located 1, 3, 5, 10, 15, 20 and 25 miles from shore. Cruises are scheduled so that the first half of the cruise is in the daylight and the second half is in the dark. The data sheet is divided into five sections: Header, Daytime data, Nighttime data, Notes and Wildlife sightings.

**Header:** The header section of the data sheet contains the general information about the cruise: date and time, weather conditions, personnel on board and whether we operated the flow-through CTD during the cruise.

**Daytime data:** This is where we record the sampling we do from inshore to offshore in the daylight. The station names are in the left-hand column. We record our arrival and departure times at the station and the readings for temperature, salinity and fluorescence from the flow-through CTD. Daytime sampling consists of a Secchi depth, CTD cast, ½ m vertical net cast and surface chlorophyll samples. At stations NH05, NH15 and NH25 we also collect surface nutrient samples. Shaded boxes indicate that we do not take that sample at that station.

**Nighttime data:** Bongo tows are conducted at stations from offshore to inshore, so the numbering is reversed. We do not do a bongo tow at NH01. We use a 0.6m diameter bongo frame with black 333µm mesh nets. Bongos are towed obliquely from the surface to 20m depth. We record arrival and departure times at the stations and the temperature, salinity and fluorescence readings from the flow-through CTD. Bongo tows are usually done at night, but we record whether the tow is at night or during the day. Before the net goes into the water we record the starting reading of the General Oceanics flowmeter (GO Start) and after the tow we record the ending reading (GO End) in order to calculate the volume of water filtered during the tow. Any live experiments are recorded in this section also.

**Notes:** This is where we record any details about the cruise that will be helpful when we are analyzing the data – changes in weather conditions, equipment malfunctions etc.

**Wildlife sightings:** Most of these species are commonly seen in our study area. A few are of interest because they are rare.

| DATE: | | Weather conditions etc: | Personnel on board: |
|---|---|---|---|
| Leave dock: | | | |
| Clear jetty (depart): | | | |
| Clear jetty (return): | | | |
| Arrive dock: | | Flow-through system on: | |

| Station | Arrival Time | Flow-thru Temp. | Flow-thru Salinity | Flow-thru Fluor. | Secchi depth | CTD | 1/2 m # Revs | CHL total | CHL <10 | NUT | Lugol | Departure Time |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NH01 | | | | | | | | | | | | |
| NH03 | | | | | | | | | | | | |
| NH05 | | | | | | | | | | | | |
| NH10 | | | | | | | | | | | | |
| NH15 | | | | | | | | | | | | |
| NH20 | | | | | | | | | | | | |
| NH25 | | | | | | | | | | | | |

* CTD and 1/2 m net to within 2-3 m of bottom depth. MAX DEPTHS: CTD = 250 m. 1/2 m net = 100 m.

| Station | Arrival Time | Flow-thru Temp. | Flow-thru Salinity | Flow-thru Fluor. | Bongo (D/N) | GO Start | GO End | Expts (type & time) | Departure Time |
|---|---|---|---|---|---|---|---|---|---|
| NH25 | | | | | | | | | |
| NH20 | | | | | | | | | |
| NH15 | | | | | | | | | |
| NH10 | | | | | | | | | |
| NH05 | | | | | | | | | |

Notes:

| Wildlife sightings: | Location & # | Species | Location & # | Species | Location & # |
|---|---|---|---|---|---|
| Common Murre | | Gray whale | | Auklets | |
| Black-footed albatross | | Humpback whale | | Songbirds | |
| Sooty Shearwater | | Harbor porpoise | | | |
| Northern Fulmar (gray & white, come to boat) | | Northern right whale dolphin | | | |
| Pink-footed Shearwater (white under wings) | | Sharks | | | |
| Buller's Fulmar (rare) (big w/lots of white) | | Jellies | | | |
| Phalaropes | | Pelicans | | | |