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Larval Walleye Pollock, *Theragra chalcogramma*, Rearing Techniques Used at the Alaska Fisheries Science Center, Seattle, Washington

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Larval walleye pollock, *Theragra chalcogramma*, rearing techniques used at the Alaska Fisheries Science Center, Seattle, Washington

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INTRODUCTION

Many species of marine fish larvae have been reared in the laboratory, yet there are few documents describing basic small-scale laboratory rearing methods (Hunter 1984 and references therein) and fewer describing specific details for rearing a particular species. This report describes the procedures used by the Fisheries-Oceanography Coordinated Investigations (FOCI) program of the National Oceanic and Atmospheric Administration (NOAA) for small-scale rearing of walleye pollock, *Theragra chalcogramma*, larvae. FOCI is a joint program between the Alaska Fisheries Science Center (AFSC, National Marine Fisheries Service) and the Pacific Marine Environmental Laboratory (PMEL), located in Seattle, Washington. The program began in 1986 to study the biological and physical processes affecting the recruitment of walleye pollock in Shelikof Strait, Gulf of Alaska. Laboratory experimentation is used to understand how walleye pollock larvae respond to changing environmental conditions. Rearing larval pollock involves collecting spawning adults in Shelikof Strait, fertilizing eggs aboard a research vessel, and transporting the eggs to the AFSC where the larvae hatch and grow. Spawning in Shelikof Strait typically begins in early April (Kendall et al. 1994). Recently, eggs have been obtained similarly from pollock spawning in the Bering Sea.

Larval pollock studies conducted at the AFSC include early life history characteristics (Bailey and Stehr 1986), physiological rates (Yamashita and Bailey 1989, Oozeki and Bailey

1995) and calibrating condition indices (Theilacker and Shen 1993, Canino 1994, Theilacker and Porter 1995). Elsewhere pollock larvae have been reared to examine otolith structure (Nishimura and Yamada 1984), and behavior (Olla and Davis 1990). Eggs for these studies were obtained from a brood stock maintained in the laboratory (Nishimura and Yamada 1984) or from adults collected from Puget Sound, Washington (Olla and Davis 1990).

METHODS

Adult Collection, Spawning, and Egg Transport[1]

Spawning pollock are collected using trawls. Eggs from ripe females are stripped into a mixing bowl filled with 1 liter (l) of 5 micron (μm) filtered seawater and then milt from 2 to 3 males is added. The mixture is gently stirred and left alone for 10 minutes to ensure the maximum number of eggs are fertilized. The fertilized eggs are ladled into a PVC sieve, rinsed several times in a bowl of filtered seawater, then transfered into 4 l jars 3/4 filled with filtered seawater. Approximately 1,500 eggs are stocked in each jar and then incubated in the dark at 3^{0}C in a refrigerator. Dead eggs are removed with a pipet, and half of the water is exchanged with filtered seawater daily until transport.

The eggs are transported to the AFSC in 1 l insulated bottles filled with 5μm filtered seawater (a 1 cm air gap is left

[1]See Appendix for equipment list.

at the top) and stocked with 500 to 1,000 eggs in each[2]. Coolers containing the insulated bottles and frozen plastic refreezable blocks are flown to Seattle as baggage aboard commercial flights.

Egg Maintenance and Rearing Setup[3]

The temperature and salinity of the seawater in a few of the egg-containing insulated bottles is measured upon their arrival at the AFSC. Four-liter jars are then filled halfway with filtered seawater, adjusted to the temperature and salinity at which the bottles arrived (see "Seawater collection" for a description of how to adjust salinity). The jars are stocked with 500 to 1,000 eggs by slowly pouring in the contents of a bottle. Each day, dead eggs are removed; every other day, 50% of the water is changed by back-siphoning using a 1/8 inch inside diameter (ID) plastic tubing siphon hose placed inside a 48 μm sieve and replacing the volume removed with filtered seawater. Eggs are incubated at 6^{0}C in the dark with no aeration until developmental stage 20 (Blood et al. 1994) at which time they are stocked into tanks. This occurs approximately 11 days after fertilization and is 2-3 days before hatching. One-hundred-twenty liter circular, black fiberglass tanks containing 90 l of seawater are stocked with 5 to 10 eggs per liter, and a 16-hour

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[2]The information presented in this section was partially summarized from "Strip spawning of adult pollock" written by D. Blood, AFSC. In: FOCI Field Manual edited by L. Britt, A. Brown, and J. Clark, AFSC, manuscript in preparation.

[3]See Appendix for equipment list.

light cycle is started which emulates Shelikof Strait light conditions during the spring. Banks of overhead fluorescent lights illuminate the water surface at 3.0 to 3.5 μmolphoton m^{-2} s^{-1}.

Pollock larvae are reared in a closed (non-circulating) system and tanks are placed in a temperature-controlled water bath. The standard rearing temperature is 6°C, which is the average seawater temperature in Shelikof Strait when pollock larvae are present (Kendall et al. 1987).

Seawater

Seawater collection[4]

Seawater is transported to the AFSC because the facility is not located near a seawater source. Seawater pumped from Puget Sound at Mukilteo, Washington, is filtered through 25, 5, and 1 μm cartridge filters set up in series (Fig. 1). Plastic 55 gallon barrels are rinsed once with filtered seawater before filling. The salinity is checked after the first barrel is full. Pollock are reared using seawater with a salinity of 32–33 parts per thousand (ppt), which is typical in Shelikof Strait, Alaska. Puget Sound seawater averages 28 ppt, so marine saltwater mix is added to each barrel to reach the correct salinity. Two-hundred-twenty grams of mix will increase the salinity of a barrel about 1 ppt; usually 1 kg of mix is added to each barrel.

[4]See Appendix for equipment list.

To the rest of the barrels the mix is added when the barrel is about 1/4 full, so the turbulence from filling will dissolve the mix more quickly.

UV Treatment of Seawater at the AFSC[5]

UV treatment of the seawater eliminates organisms that could grow in it during storage. With the pump out-flow adjusted to 2.5 gallons per minute, seawater is recirculated through the UV unit and back to the barrel for 30 minutes (Fig. 2). At the end of treatment, which thoroughly mixes the water, the salinity is rechecked; if more marine saltwater mix is added, the water is recirculated another 10 minutes at maximum pump flow and the salinity checked again. The treated barrels are stored at 4°C.

Prey Maintenance

Algae

Algae, used to feed rotifers and copepods, are grown in 250 ml Erlenmeyer flasks, 2.5 l Fernbach flasks, 20 l glass carboys and 32 gallon garbage cans. Algae Growth Media consists of nutrient and iron solutions added to seawater (0.5 ml of each solution per liter seawater; see Appendix). To provide light for the cultures, two 40-watt fluorescent bulbs are used for flasks and carboys, and four 40-watt bulbs are suspended about 30 cm over the garbage cans. All cultures are grown using a 16-hour light cycle and only the carboy and garbage can cultures are

[5]See Appendix for equipment list.

aerated. *Isochrysis galbana* (obtained from University of Washington, School of Oceanography) and *Pavlova lutheri* (obtained from University of Washington, School of Oceanography) are grown at room temperature (20°C) and *Katodinium rotundatum* (obtained from University of British Columbia, Northeast Pacific culture collection) is grown at 6°C. *Isochrysis galbana* and *Pavlova lutheri* are used because they are high in unsaturated fatty acids (Nichols et al. 1989) required by larval fish (Watanabe 1982, Watanabe et al. 1983, Bell et al. 1986). *Katodinium rotundatum* was chosen because it grows at cold temperatures, utilizes ammonia (which is detrimental to fish larvae), and serves as prey for the copepods and rotifers in the fish tanks. To ensure a continuous supply of algae, cultures are inoculated 1 week apart so that as one population nears crashing the other is coming up (Table 1). Cultures last approximately 3 weeks from the time of inoculation to crashing.

250 ml Erlenmeyer flasks -- Algae cultures are maintained year-round in 250 ml Erlenmeyer flasks filled with 150 ml sterilized media. These cultures provide the stock to start all the cultures used for rearing, so care is used to avoid contamination. A microwave oven is used to sterilize the culture media (Keller et al. 1988) by heating 4 to 6 flasks at a time on "high" until they have boiled for 1 to 2 minutes. After the flasks cool to the temperature at which the algae is grown, 1 ml of each culture is transferred into them.

2.5 l Fernbach flasks -- One-and-a-half liters of Algae Growth Media is microwave-sterilized in each flask. After cooling, the flask is inoculated with about 50 ml of either *Isochrysis galbana*, *Pavlova lutheri* or *Katodinium rotundatum* from the stock cultures. One flask of each species is started 6 weeks prior to when the larvae are estimated to be at first feeding (Table 1).

20 l glass carboys -- Household bleach is used to sterilize seawater when volumes larger than 1.5 l are needed. In a carboy, 10 l of seawater, an air hose, and siphon hose are sterilized using bleach added at 0.2 ml per liter of seawater and soaked overnight. The bleach is neutralized using a 1% sodium thiosulfate solution (Appendix) added at 1 ml per liter of seawater and vigorously aerating the water. If a bleach odor persists after 4 hours, more sodium thiosulfate is added; if not, the bleach is considered neutralized. Next, nutrient and iron solutions are added at 0.5 ml per liter of seawater and the carboy is inoculated with 500 ml of either *Isochrysis galbana*, *Pavlova lutheri* or *Katodinium rotundatum* from the Fernbach flasks. A carboy takes approximately 1.5 to 2 weeks to become a dense, healthy culture. One carboy each of *Isochrysis galbana* and *Pavlova lutheri*, and two carboys of *Katodinium rotundatum* are grown (Table 1).

32-gallon garbage cans -- Following the same procedure used for the 20 l carboys, 20 gallons of seawater and an air line are

sterilized using bleach. Five liters of either *Isochrysis galbana* or *Pavlova lutheri* (one garbage can of each is grown) from a carboy is used to inoculate a garbage can. A garbage can culture takes approximately 1.5 to 2 weeks to come up (Table 1).

After harvesting algae from a carboy or garbage can, the same volume of microwave sterilized-media is added to the carboy and bleach-sterilized (de-bleached) media to the garbage can.

Rotifers

The rotifer *Brachionus plicatilis* is used to supplement the natural zooplankton prey fed to larval pollock. Rotifers are grown at room temperature (20°C) in aerated 4 l jars, 20 l containers, and 32-gallon garbage cans. Two garbage can cultures are staggered 1 week apart (Table 1). Two 40-watt fluorescent lights on a 16-hour light cycle are suspended over the cultures. Two liters of rotifers (with an approximate concentration of 20-30 per milliliter) are used to inoculate a 20 l container. Garbage can cultures are started by first growing a dense algae culture (*Isochrysis galbana* or *Pavlova lutheri*) in it and then adding 10 l of rotifer culture. After the water becomes clear (meaning the rotifers have "bloomed"), the rotifers are fed 5 l from each garbage can culture of *Isochrysis galbana* and *Pavlova lutheri* every day. Every other day, a long tube siphon is used to clean the bottom of the rotifer container. A 44 μm sieve and a 3/8 inch ID plastic tubing siphon hose are used to back-siphon water out to reduce the volume of the culture (as described in

Egg Maintenance and Rearing Setup). After 1 month a rotifer culture is discarded.

A 150 ml rotifer stock culture is maintained year-round in a 250 ml Erlenmeyer flask at room temperature (20°C). Twice a week about half the culture is discarded and algae is added. Once a month, 25 ml of the culture is transferred into a clean flask with 125 ml algae.

Procedure for Stocking Fish Tanks with Rotifers

Due to the large number of rotifers in a culture, a high ammonia concentration is produced. Ammonia is toxic to larval fish; thus, in order to avoid adding it to the fish tanks the rotifers must be "washed". The fish tanks are stocked with 5 to 10 rotifers per milliliter of seawater 2 days prior to the time the larvae will initiate feeding. This is a 5-step process (Fig. 3):

1. Concentrate the garbage can culture by back-siphoning 20 l of water out as described earlier and determine the concentration of rotifers in the culture.
2. Gently pour 1 l of rotifers into a 44 μm sieve (Fig. 3, No. 1).
3. Move the sieve containing the rotifers into the wash bath of clean seawater and partially submerge it. Move the sieve up and down in the bath three times to "wash" the rotifers (Fig. 3, No. 2). Change the wash water after sieving 10 l of rotifers.

4. Transfer the rotifers to a 20 l container which has 1.5 l *Isochrysis galbana,* 1.5 l *Pavlova lutheri* and 4 l seawater in it (Fig. 3, No. 3).

   Repeat steps 2 - 4 until enough rotifers have been sieved to achieve the desired concentration in the fish tank. To stock 10 rotifers per milliliter, approximately 900,000 rotifers are added to a 120 l tank.

5. Cool the rotifers to 6°C and use a small beaker to gently add them to a tank.

Zooplankton

All life stages of the copepod *Acartia* sp. and unidentified gastropod and polycheate larvae are collected from lagoons located just south of Fort Casey, Whidbey Island, Washington. A 93 μm, 0.5 m diameter, 2 m long plankton net is towed about 2 m below the lagoon surface behind a rubber raft. To remove jellyfish and other large organisms from the collection, the zooplankton is immediately sieved into a 5 gallon bucket about half full of seawater. Plankton that passes through a 202 μm sieve is used for first-feeding (ff) larvae, and for older larvae (about 2 weeks after ff) plankton that passes through a 333 μm sieve is used. One frozen refreezable plastic block is added to each bucket to maintain temperature during transport.

At the AFSC, the zooplankton is resieved and the concentration in each bucket is reduced by moving half the volume into an empty 5 gallon bucket and adding filtered seawater so

that each bucket is 3/4 full. Zooplankton is maintained at 6°C and lives for approximately 10 days. Maintenance involves cleaning the bottoms of the buckets with a siphon every 3 days and replacing about 1/3 of the water (using the same technique that is used with the rotifers; see Prey Maintenance Rotifers) with filtered seawater and 1 l of algae (either *Katodinium rotundatum* or previously cooled *Isochrysis galbana* or *Pavlova lutheri*). The material and water siphoned from the bottom of the buckets is saved because it contains zooplankton that can be used. After the material has settled, water containing the zooplankton is siphoned into another container, and the rest discarded.

Procedure for Stocking Fish Tanks with Zooplankton

Fish tanks are stocked with 1 to 3 zooplankters per milliliter of seawater, 2 days prior to the time the larvae will initiate feeding. The following procedure is used:

1. Add 1 l of 6°C seawater to a 20 l container. Use a 3/8 inch ID hose to siphon half a 5 gallon bucket of zooplankton into the 20 l container.
2. Back siphon about half the water out of the 20 l container using a 44 μm sieve and 3/8 inch ID plastic tubing.
3. Determine the number of zooplankton in the 20 l container. In a 5 ml subsample, count only nauplii, copepodites, and veligers; larvae do not eat polycheates. Stocking a 120 l tank at 3 zooplankton per milliliter requires 270,000 individuals.

4. If there is not enough prey in the container, add more zooplankton and repeat steps 2 and 3.
5. Add zooplankton to the fish tanks in the same manner as the rotifers (see Prey Maintenance Rotifers).

Fish Tank Maintenance

Every morning the temperatures in all the fish tanks are recorded. Every day, or every other day, 10% to 20% of the water in each tank is replaced by using the procedure described in Egg Maintenance. Filtered seawater is added to the tanks through a 1/8 inch ID siphon hose. A pipet is used to remove any egg chorions or dead eggs or larvae from the tank bottoms. Each tank containing prey receives 1 l of *Katodinium rotundatum* at least twice a week. Tanks initially stocked with 5 to 10 rotifers per milliter and 1 to 3 copepods per milliter, are maintained at 1 to 3 copepods per milliter; usually no more rotifers are required after the initial stocking. The prey concentration in the tanks is monitored at least 3 times per week. Three 1.5 ml subsamples are taken from the surface, mid-water and bottom of the tanks (9 subsamples total).

RESULTS AND DISCUSSION

Larval growth rates can be used to assess rearing conditions. The rearing system described in this report has produced growth rates ranging from 0.09 mm (G. Theilacker and S. Porter unpublished data) to 0.14 mm per day (Theilacker and Shen

1993). In other studies, growth rates of larval pollock reared at 6°C range from 0.065 mm (Nishimura and Yamada 1984) to 0.11 mm per day (Yamashita and Bailey 1989). Using otolith increments, values from 0.14 to 0.23 mm per day have been calculated for early larval pollock growth in the field (Bailey et al. 1996).

The rearing techniques described in this report provide an environment that allows pollock larvae to grow well. The slowest rates obtained are at the upper end of the range determined in other laboratory studies and the fastest growth equals growth in natural conditions.

Currently a biofilter is being incorporated into the rearing system. Using a biofilter requires a flow-through system. Water in the fish tanks drains to a sump, is pumped to the biofilter, treated, and circulated back to the tanks. The biofilter contains bacteria that remove ammonia from seawater as it circulates through the filter. By using the biofilter, daily fish tank water changes will be eliminated so the larvae will be disturbed less. Additionally, it will reduce the amount of water needed to be transported to the AFSC.

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APPENDIX

Equipment Needed for Various Larval Rearing Activities

Spawning and transporting pollock eggs.

- cartridge filter (5μm) and housing.
- 30 cm diameter by 15 cm deep plastic mixing bowls.
- 30 cm diameter PVC sieve with 1 mm mesh.
- ladle.
- 4 l jars.
- refrigerator.
- 5 ml pipet.
- 1 l insulated bottles.
- plastic, refreezable blocks.
- 48-quart coolers.

Egg maintenance and rearing setup.

- 4 l jars.
- 1/8 inch ID plastic tubing.
- 48 μm sieve.
- 5 ml pipet.
- thermometer.
- salinometer.
- 120 l circular, black fiberglass tanks.
- temperature-controlled water bath.

Seawater collection.

- plastic 55 gallon barrels and a truck to transport them.
- 25 μm, 5 μm, 1 μm cartridge filters and housings (set up in series).
- small electric pump.
- 3/4 inch ID plastic tubing.
- marine saltwater mix.
- salinometer.

UV treament of seawater.

- UV unit - 15 watt, in-line unit.
- electric pump.
- 3/4 inch ID plastic tubing.
- salinometer.

Collecting zooplankton.

- 93 μm, 0.5 m diameter, 2 m long plankton net, cod-end, and 8 m rope.
- rubber raft, oars, chest waders, life vest.
- 6 or 8, 5 gallon buckets and lids.
- plastic, refreezable blocks (one for each 5 gallon bucket).
- sieves with mesh sizes 202 and 333 μm.

Algae Nutrient Recipes

University of Washington Experimental Hatchery Nutrient Mix

1. Trace Metal Stock Solution

995 ml distilled water plus 1 ml of each of the following solutions:

Sodium molybdate	12.6 g/l
Zinc sulfate	44.0 g/l
Cupric sulfate	19.6 g/l
Cobalt chloride	20.0 g/l
Manganese chloride	360.0 g/l

2. Phosphate Stock Solution

Sodium phosphate (monobasic)	40.0 g
Thiamin	0.4 g
Vitamin B_{12}	2.0 mg
Biotin	2.0 mg
Distilled water	1 l

3. Nitrate Stock Solution

Sodium nitrate	150 g
Distilled water	1 l

Mix solutions 1, 2, and 3 in the following proportions: 2:1:2. This mixture is the Algae Nutrient Solution. Store all solutions in a refrigerator. Add the Algae Nutrient Solution at 0.5 ml/l seawater when making Algae Growth Media.

Algae Iron Solution

To 1.8 g Ferric chloride and 6 g EDTA add 1 l distilled water[6]. Add Sodium hydroxide to increase the pH up to approximately 7; this will keep the EDTA dissolved. Store this solution in the dark at room temperature. Add the Algae Iron Solution at 0.5 ml/l seawater when making Algae Growth Media.

To make Algae Growth Media add both the Nutrient and Iron solutions to filtered seawater.

Sodium Thiosulfate Solution (used to neutralize bleach)

Dissolve 10 g Sodium thiosulfate in 1 l distilled water. Store at room temperature.

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[6]From modified IMR culture media recipe; Perry, M.J., M.C. Talbot, and R.S. Alberte. 1981. Photoadaptation in marine phytoplankton: response of the photosynthetic apparatus. Mar. Biol. 62: 91-101.

Table 1. The staggered rotation used for algae cultures grown in 2.5 l Fernbach flasks, 20 l carboys or 32 gallon garbage cans, and rotifers grown in 32-gallon garbage cans. "XX" = a new culture started.

ALGAE	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Isochrysis galbana	XX			XX		
Pavlova lutheri		XX			XX	
Katodinium rotundatum	XX			XX		
Katodinium rotundatum		XX			XX	
ROTIFERS						
Brachionus plicatilis	XX				XX	
Brachionus plicatilis		XX				XX

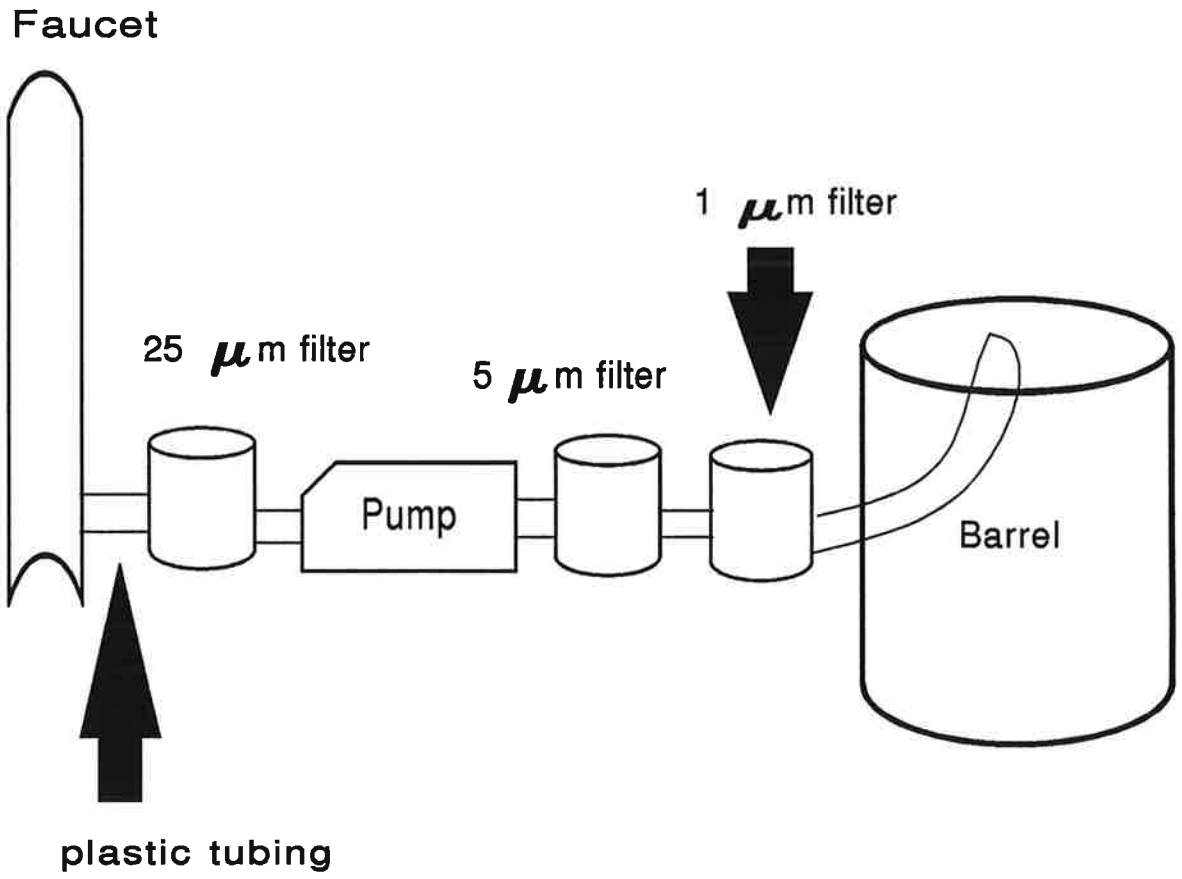

Figure 1. Seawater filtration system. The pump is used to reduce barrel filling time.

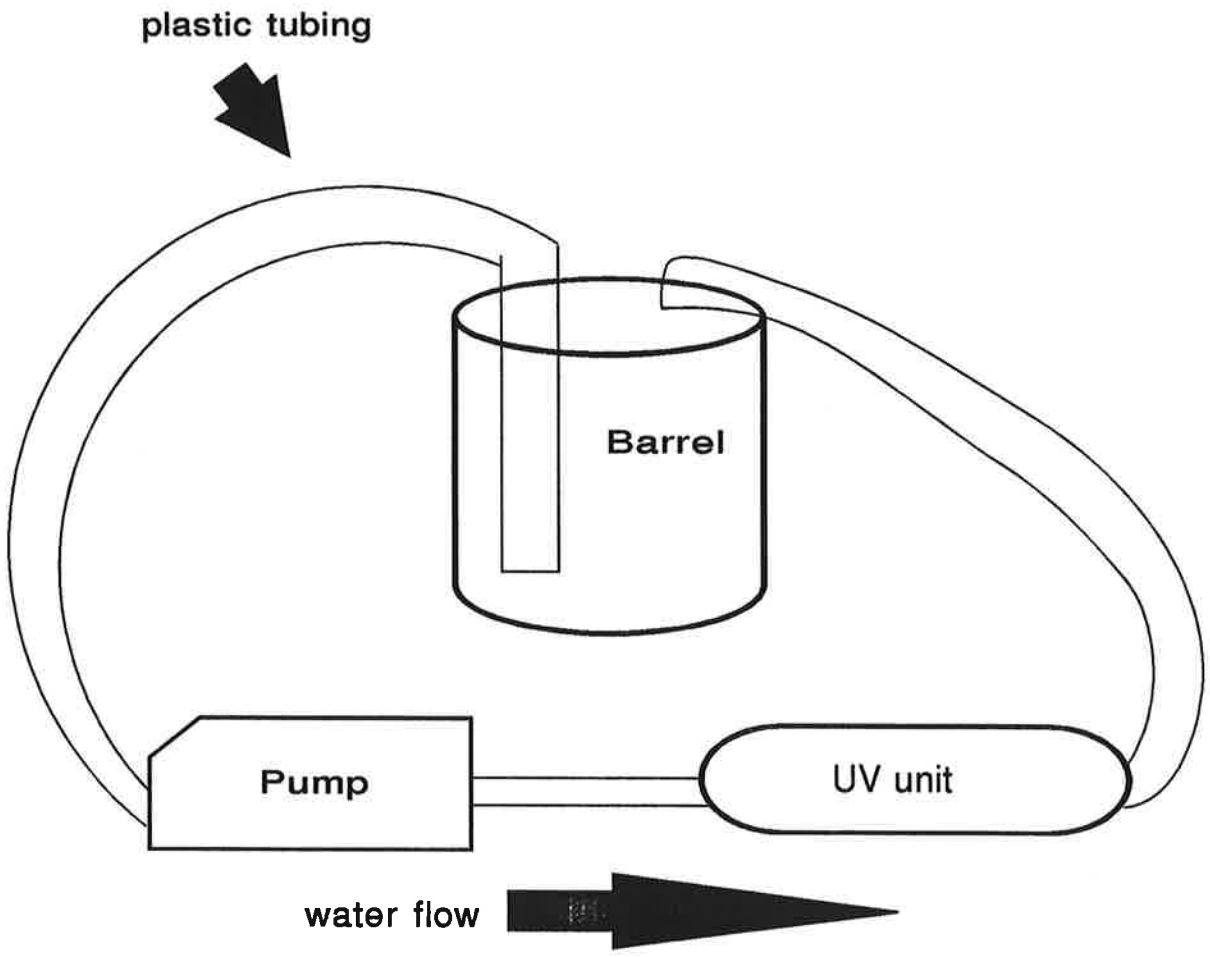

Figure 2. Seawater UV treatment system.

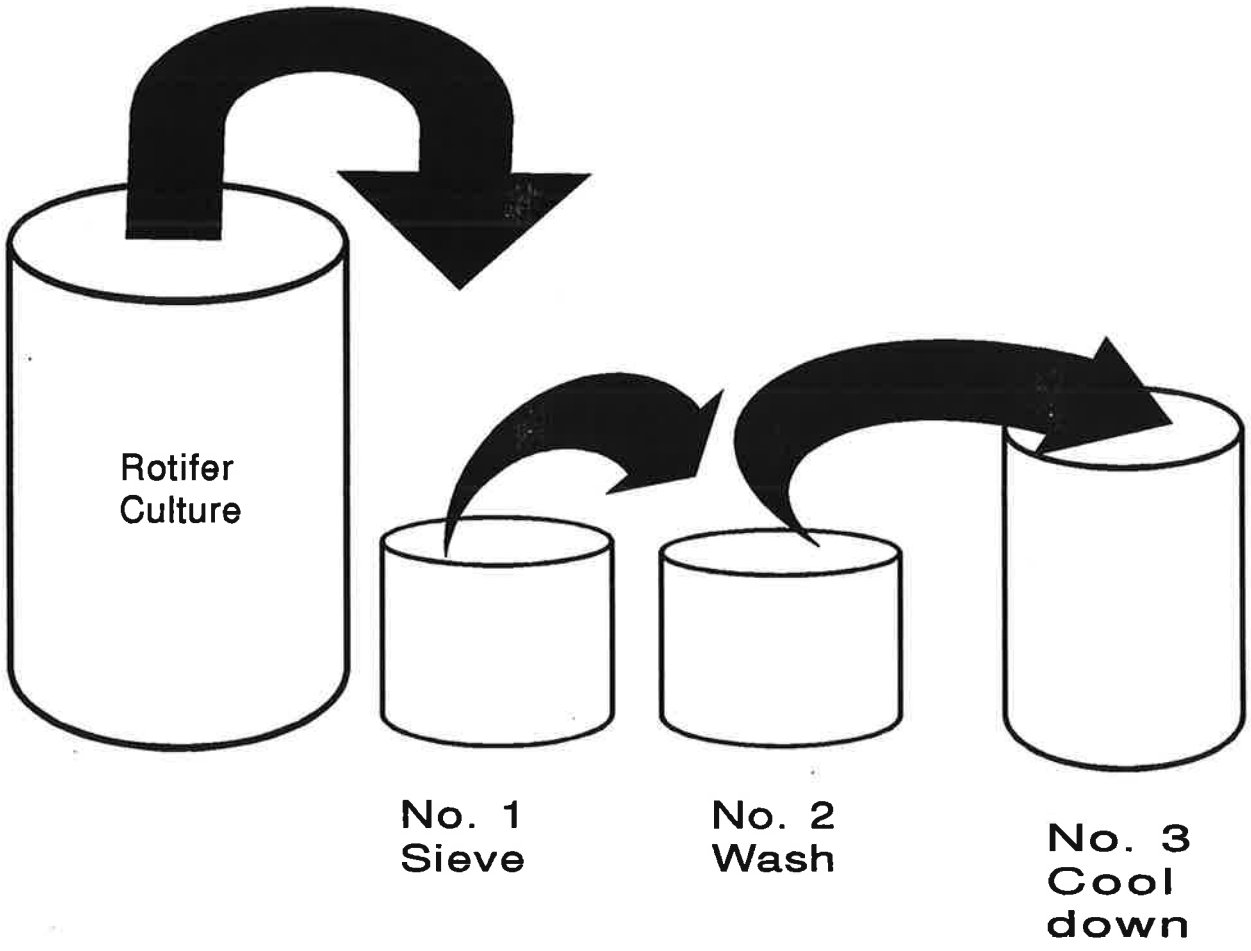

Figure 3. Rotifer washing procedure.