A Practical Hatchery Manual

Production of Southern Flounder Fingerlings

by Harry V. Daniels and Wade O. Watanabe

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Introduction

Introduction

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Previous page photo: Harry V. Daniels displays southern flounder fingerlings.

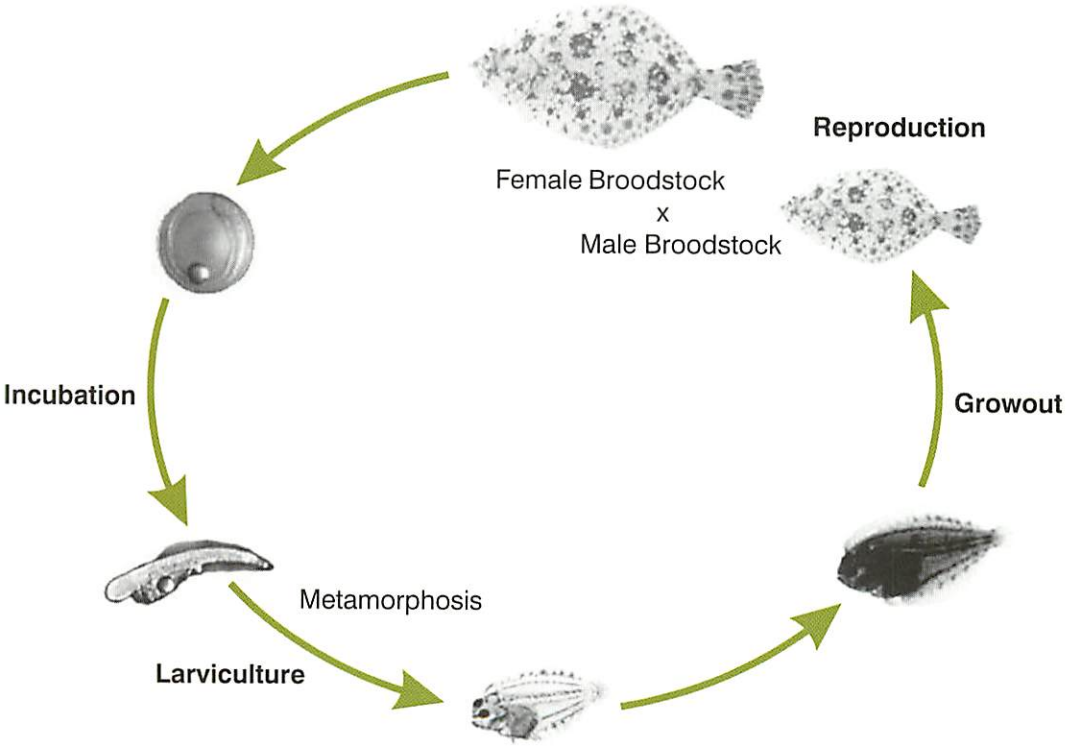
Practical information on flounder fingerling production is difficult to find. Much of the literature on hatchery methods for culturing flatfish is reported in the scientific journals and describes the methods used in small-scale experiments for research purposes.

This manual is designed to provide a comprehensive, hands-on reference to the procedures used to produce southern flounder (*Paralichthys lethostigma*) fingerlings. It is meant to be a benchtop handbook for use in a working southern flounder hatchery, with step-by-step descriptions of hatchery procedures from egg production through the process of weaning the fingerlings to dry feeds, when the flounder reach a size of 3.5 cm, or 1.5 in. (*See Figure 1.*) This is the size at which they are considered fingerlings and can be transported to growout tanks or sold to other growers.

Southern flounder is a high-value species with strong economic potential for aquaculture. The information described here has been assembled from our years of experience growing southern flounder larvae at North Carolina Department of Agriculture Tidewater Research Station (TRS) Fish Hatchery in Plymouth, N.C., and at the University of North Carolina at the Wilmington Center for Marine Science (CMS) at Wrightsville Beach, N.C.

The TRS hatchery's water treatment system is a completely closed recirculating seawater system. Therefore, these procedures have been developed for

Figure 1. Hatchery production of flounder.



recirculating water treatment systems, but are equally applicable to hatcheries with flow-through water.

Many of the methods described in this manual are commonly used in all marine finfish hatcheries and are extensively documented in other books or manuals. For those interested in more detailed descriptions of particular methods, see Appendix C. Complete materials lists for the different production areas and activities are provided in Chapter 4 on economics.

This manual represents the combined work of many people over the past eight years. We thank our graduate students and technicians for their dedication and hard work during the development of these production methods. Their enthusiasm and insightful comments have improved the practicality of these methods immensely. We are particularly grateful to Rebecca Dunning of the N.C. Department of Agriculture for her detailed economic analysis. Ryan Murashige of North Carolina State University provided many helpful suggestions from his years of experience managing a Japanese flounder farm. Ronald G. Hodson, director of North Carolina Sea Grant, provided technical advice.

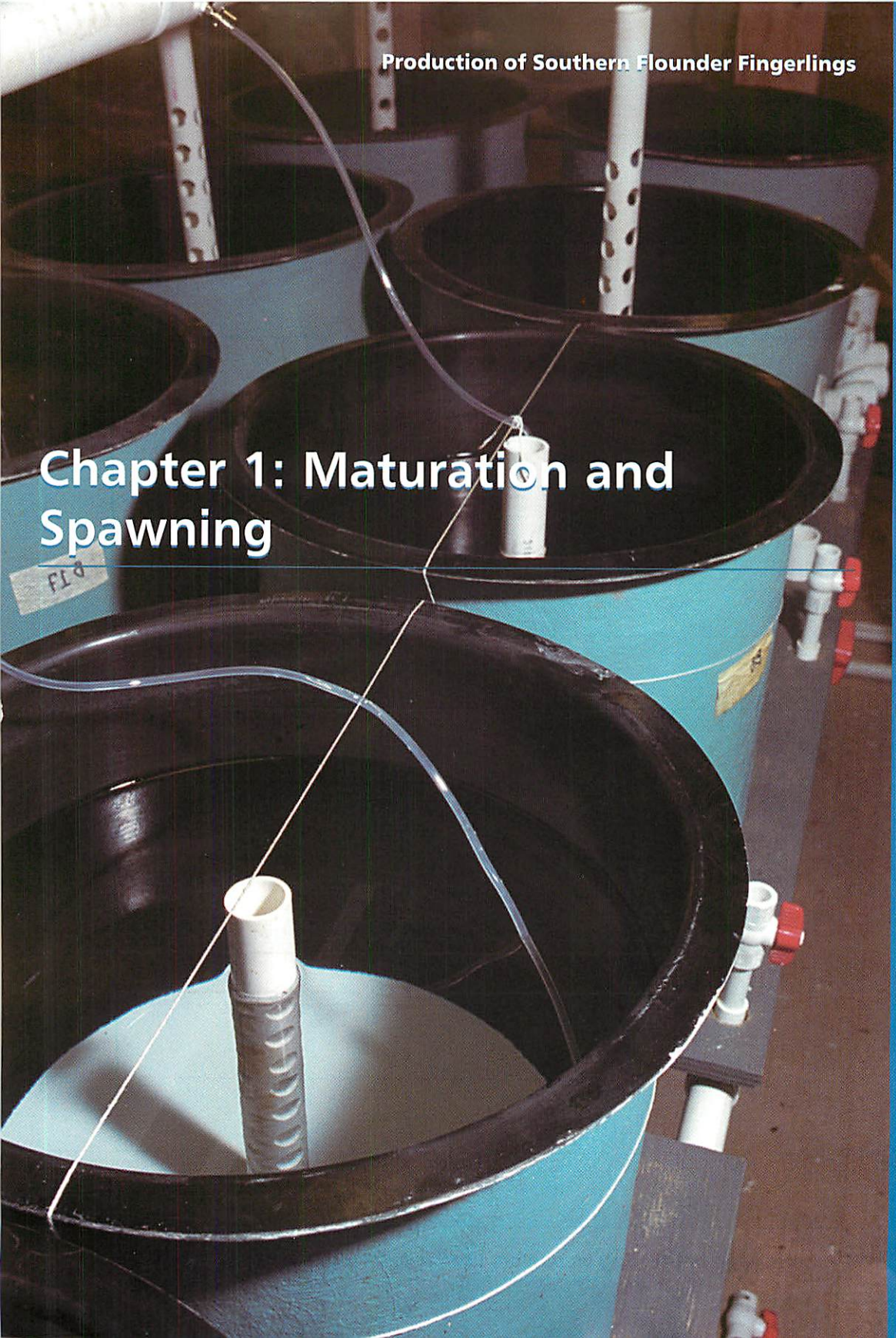
Biology of Southern Flounder

Southern flounder are found in rivers and estuaries along the Atlantic Coast from North Carolina to northern Florida, and from Tampa Bay, Fla., along the Gulf coast into southern Texas. These flounder are found in a wide range of salinities. Adults have been captured in waters from 0 ppt to 36 ppt salinity.

It is not uncommon to catch southern flounder by hook and line far inland in coastal rivers. Mature adults migrate offshore during the fall to spawn in marine waters.

The spawning season begins in December at the northern extreme of their natural range, and in late January to February in the southern extreme. Immediately after spawning, the fish return to estuaries and rivers.

Larval flounder feed on zooplankton in offshore waters for the first few months. Then, metamorphosis begins and the larvae are washed through inlets into estuaries where they settle to the bottom and begin moving up into the rivers.



Chapter 1: Maturation and Spawning

Notes

Previous page photo: Egg incubators used in larviculture process.

Source of Broodstock

Wild-caught fish are suitable for use as broodstock if they are handled carefully. Fish that are caught in pound nets are the highest quality as they are less stressed or have the lowest chance for damage from other capture methods, such as gill nets or hook-and-line fishing.

Fish can be spawned during their first year in captivity if they are caught during the early fall (September to mid-October) and placed immediately into a room with temperature and lighting control. Egg quality will be marginal during the first year, but should improve as fish become acclimated to the confines of the tank, the periodic presence of humans and the type of feed. Highest egg quality likely will be achieved during the second and third years of captivity.

Wild-caught fish should be quarantined for six weeks before being introduced to the hatchery. They are initially given a formalin bath (25 ppm for 30 minutes) and an intraperitoneal (IP) antibiotic injection of oxytetracycline before being placed into the recirculating quarantine system. Prospective broodstock are fed cut fish for two weeks and then gradually switched to a commercial dry pellet feed — approximately a two-week transition. Although it is sometimes very difficult to train wild-caught flounder to eat pelleted feed, it is possible to train fish by the end of the six-week quarantine. At this time the fish can be introduced to the brood tank system.

Hatchery-reared fish are a good source of broodstock because they are already conditioned to the tanks, trained to eat pelleted feed, and are accustomed to the presence of humans. Theoretically, the biggest fish of a particular batch represent the fastest growers and should produce fast-growing offspring. Males are smaller than females, so be sure to collect a sufficient number of medium-size fish when selecting broodfish.

System Requirements

Location

Sudden loud noises or bright lights are stressful to broodstock, and will result in poor performance. Isolate the broodstock system from all other hatchery activities to minimize noise (slamming doors, loud music, telephones) and other disturbances (people walking by).

Flounder spawn according to day length (photoperiod) and temperature. To control and coordinate spawning activity, broodstock systems must have some means of isolating the fish from outside light and temperature fluctuations. These controls also will allow fish to be spawned out of season at any time of the year.

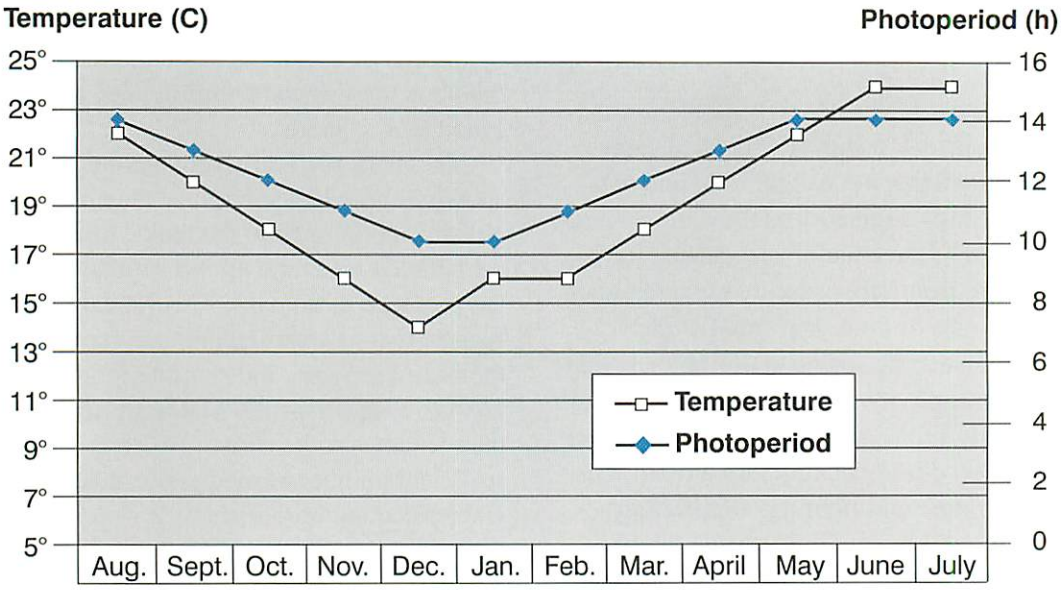


Figure 2. Photothermal conditioning of southern flounder broodstock for January spawning.

Design

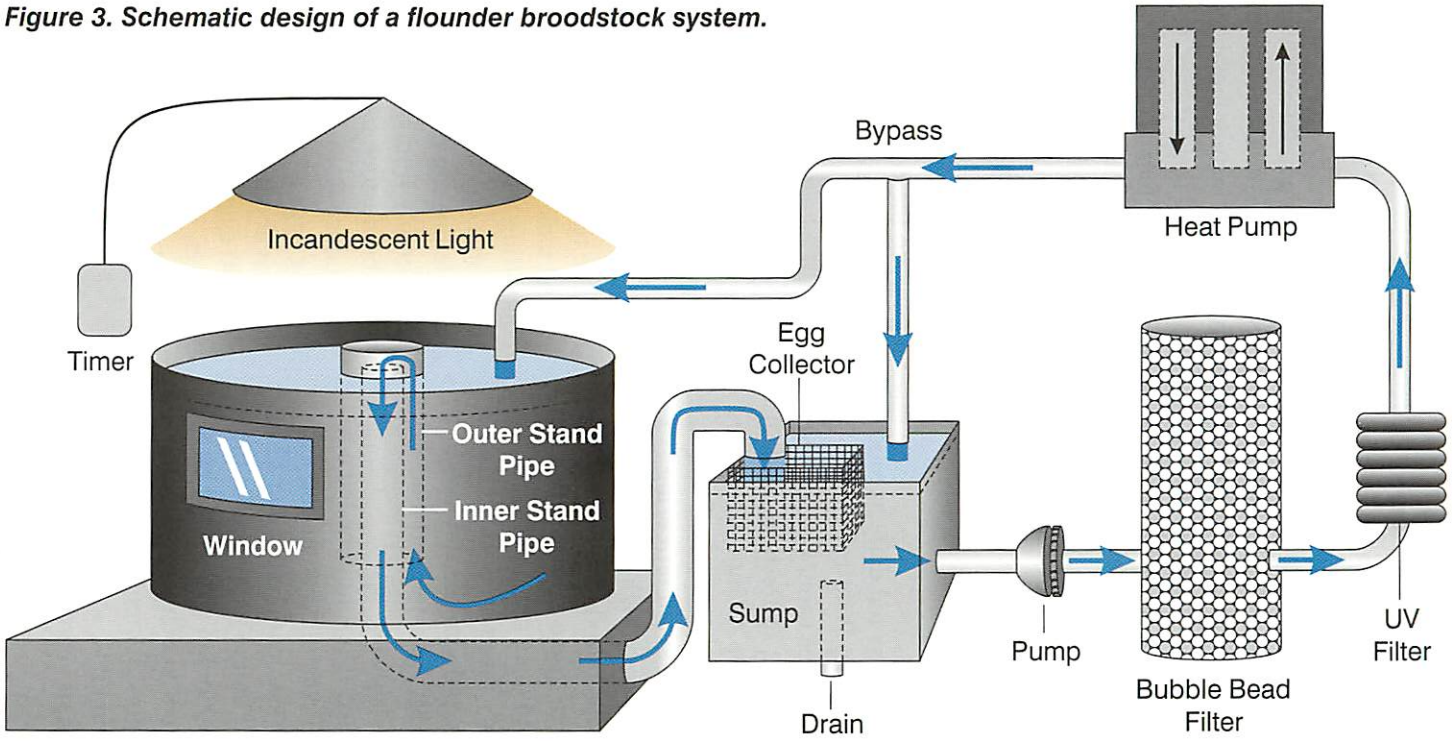
Optimum day length for spawning is 10 hours of light. (*See Figure 2.*) Simple timers wired directly into the lighting system are adequate to control photoperiod. Some hatcheries use 25-watt bulbs to initially illuminate the broodstock area before the 100-watt bulbs come on. At dusk the 100-watt lights are turned off first, then the 25-watt bulbs. The gradual change of light from dawn and dusk keeps the fish much calmer.

Water temperature should be maintained with a heat pump with sufficient capacity to hold water temperatures at 13° C for at least one month at any time of the year and below 25° C to avoid problems with high temperatures. This will allow adequate control to coordinate the actual release of eggs, which is helpful for the planning and coordination of larviculture activities.

Tanks should be circular with a minimum 6-ft. diameter and 4-ft. depth. (*See Figure 3.*) Tanks up to 14 ft. in diameter, or even larger, can be used, but they are more difficult to clean and make it harder to capture the fish. A center drain makes cleaning easier. A side drain can be used and the tanks kept clean by siphoning the bottoms.

A typical recirculating system for broodstock includes a sand filter, an ultraviolet (UV) filter and a biological filter, such as a bubble bead filter or a fluidized bed sand filter. Properly conditioned fish will spawn directly into the tank water, so the drainage system must be routed through an egg collector — a tank containing a simple 500-micron filter basket — before it returns to the water treatment system.

Figure 3. Schematic design of a flounder broodstock system.



Maturation and Spawning

Water flow should be sufficient to cause a noticeable circular movement of the water. Design the pumping system to provide enough pressure to pass through the various filters and still provide a strong flow of water in the tank. Flow rates should be sufficient to affect a 100 percent exchange of the water every 3 hours. A typical broodstock system with two, 6- to 8-ft. diameter broodstock tanks requires a 1- to 2-hp pump to push the water through all the filters and still achieve the necessary water flow in the tanks.

Feeding

The fish should be fed daily as much as they will consume. Wild-caught broodstock sometimes cannot be trained to eat a pelleted feed, so fresh frozen fish, such as Lake smelt or Atlantic silversides, should be used. Be sure to keep the fish frozen until use and avoid refreezing uneaten fish. Use only high-quality frozen fish to prevent the introduction of bacteria that could lead to disease. Frozen fish can be supplemented once per week with vitamins. Simply insert an over-the-counter vitamin pill into the stomach of a recently thawed Lake smelt and feed the fish to the broodstock as usual.

Domesticated fish should be fed a commercial pelleted diet containing a minimum of 55 percent protein and 12 to 15 percent fat. Use the largest pellet the fish will eat. The pellet size should be at least 12 to 16 mm in diameter. Commercial diets contain sufficient amounts of vitamins and minerals, so additional supplementation of vitamins is not needed.

Stocking Density and Egg Production

Stock two to three males for each female. An 8-ft. diameter tank should hold about 4 to 6 mature females (1.5 to 2 kg each) and 10 to 12 males (0.4 to 0.75 kg each). Once females begin to swell up with the ripening eggs, spawning likely will begin within a few weeks. Eggs are small, 1 mm in diameter, transparent with a single oil droplet, and float close to the water surface. Nonviable eggs have a cloudy whitish appearance and sink rapidly to the bottom of the tank.

The first few times that eggs are released into the tank, they will probably have low fertility — less than 10 percent. Fertility rates should improve within a week — greater than 50 percent — and remain high for about one month. Then, both egg production and fertility will begin to decline.

The timing of egg production from natural tank spawns is unpredictable. By controlling the photoperiod and temperature, some coordination of spawning is possible but egg production still varies considerably from day to day.

A broodstock tank with 3 to 4 females can produce from 10 mL to 300 mL of floating eggs a day. Because each mL contains about 1,000 eggs, 10,000 to 300,000 eggs can be produced daily. However, it is not unusual for one or two days to pass without egg production.

Hormone-Induced Spawning

Hormone implants can be used to accelerate egg development in females. Commercially available implants are small (approximately 1 cm long and the diameter of a dried spaghetti noodle) and are inserted into the muscle of the fish using a large gauge needle. These implants allow a slow, sustained release of the hormones into the bloodstream of the fish, resulting in more predictable and higher-quality egg production.

The implants themselves are a compressed mixture of cellulose and cholesterol that holds the powdered form of the hormone (usually Gonadotropin-Releasing Hormone analogue or GnRHa). Only eligible fish should be implanted. Eligible females have soft, swollen abdomens. Their eligibility can be confirmed by placing the fish on a clear plexiglass sheet over a 40-watt light bulb. (*See Figure 4.*) The gonads can be clearly seen with this backlighting technique. Using this method, we have established an informal grading scale where fish are given a score of 1 to 4 based on their gonadal development.

A score of "1" means little or no discernible development; "2" is discernible development and slight abdominal swelling, but the gonads only extend less than half way back to the caudal fin; "3" means that abdominal swelling is easily noticeable and gonads extend greater than three-fourths of the way back to the caudal fin; and "4" means gonads are fully extended with pronounced abdominal swelling and a small clear area near the oviduct.

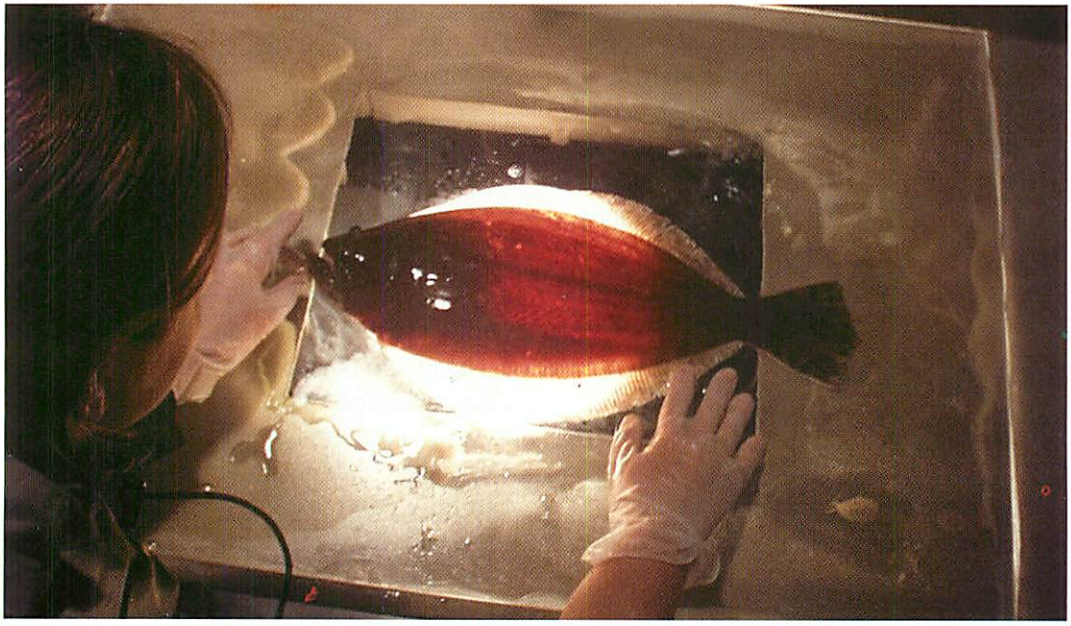


Figure 4. Backlighting technique reveals female flounder's fully extended gonads (horn-shaped shadow extended to caudal fin).

Figure 5. Collecting sperm from a male flounder.

Only females that score 3 or 4 should be implanted. Once the fish are implanted, they will begin producing eggs within about 48 hours. At this point, the females can be left in the tank and allowed to spawn with the males — called a tank spawn. Or, the fish can be removed daily and stripped of their eggs — called a strip spawn.

If the strip-spawning technique is used, the males also must be taken out of the tank and the sperm removed with a pipette and saved in a vial on ice.

Strip-Spawning Protocol

Males are squeezed first and the sperm is stored in a small vial on ice. (*See Figure 5.*) The motility can be checked on a glass slide using a compound microscope at high magnification (1000 X magnification) before use. A small drop of seawater must be added to the sperm sample on the slide to activate the sperm. The sperm is collected using a 1-mL tuberculin syringe, without the needle. Syringes are stored on ice until ready to be used.

Three to four female fish are removed from the tank using long-handled, deep-dip nets, and placed in a bath with a tranquilizer (MS-222) and slime coat. After several minutes to allow the tranquilizer to take effect, one fish is removed by hand and placed on the light table. (*See Figure 6.*) Fish that score 3 or 4 are then moved to a foam pad on top of a stainless steel table, and the abdominal area is carefully dried off. One person stands at the front side of the table holding the fish, using the foam pad and table to stabilize the fish. A second person stands behind the table holding a large-mouthed glass jar

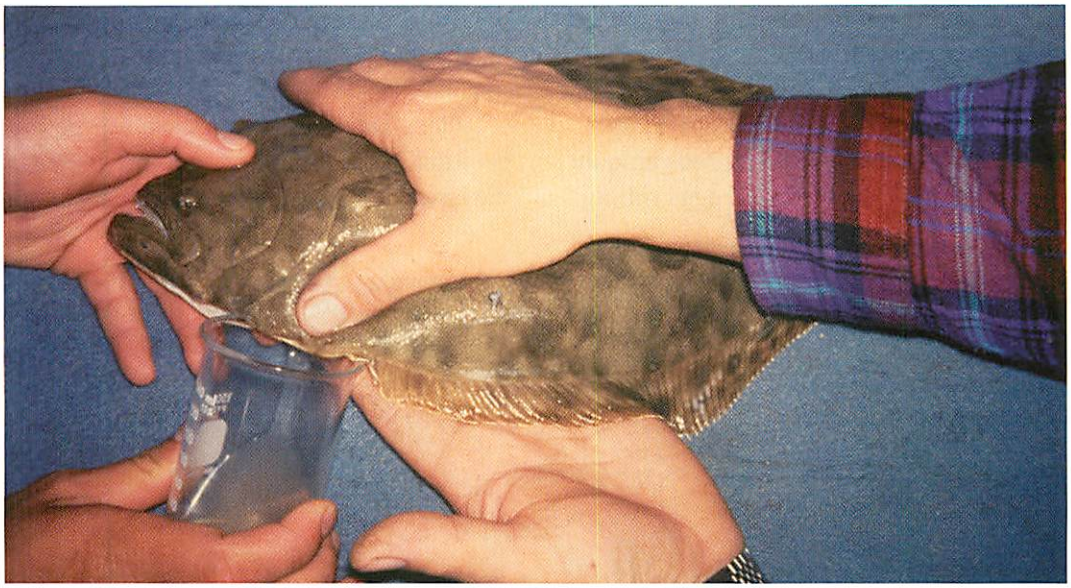


Figure 6. Collecting eggs from a female flounder.

or beaker. To make the collection of eggs easier, a hole can be cut in the top of the table. The fish is held above the table and the glass jar below. The fish is carefully squeezed to release eggs from both the ovaries. Once the eggs are collected, the jar is covered with a paper towel and set aside. The fish is moved to a recovery tank with strong aeration before being returned to its original tank.

After the eggs are collected from three or four fish, several drops of sperm from at least two males are added to each of the jars of eggs. The jars are swirled gently for approximately one minute before a small volume of water is added (5 to 10 mL). The jars are swirled for 90 seconds, an additional 100 to 200 mL of water is added, and the fertilized eggs are allowed to sit for another 90 seconds.

The eggs are carefully transferred to graduated separatory funnels. The volume of floating, sinking and total volume of eggs are measured and recorded. Eggs are given a 10-minute bath in 50-ppm iodine. After rinsing, the eggs are transferred to 70-L incubators with water temperature at 16° C and salinity at 33 ppt. For each incubator, the number of eggs stocked, and the identity of the females and males spawned are recorded.

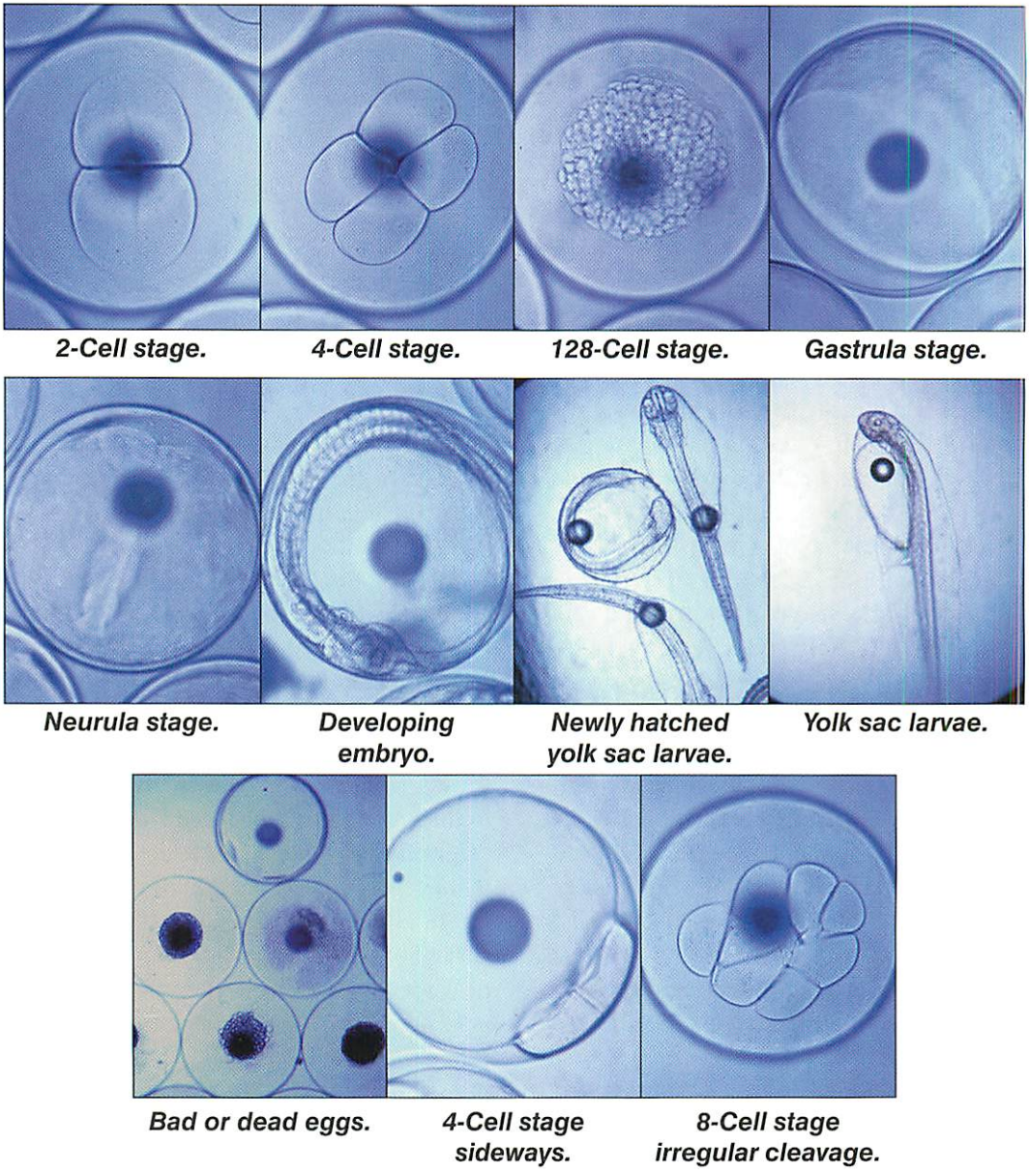
After 24 hours, or the next day, percent fertility is determined. (*See Figure 7.*) The volume of eggs that have sunken to the bottom are subtracted from the original volume to estimate the number of viable eggs and the percent of good eggs left floating in the incubator. If the survival is low (< 50 percent), the batch is discarded at this time. After 48 hours, the eggs are again evaluated and the same data collected. The eggs are then stocked into 1,000-L larviculture tanks.

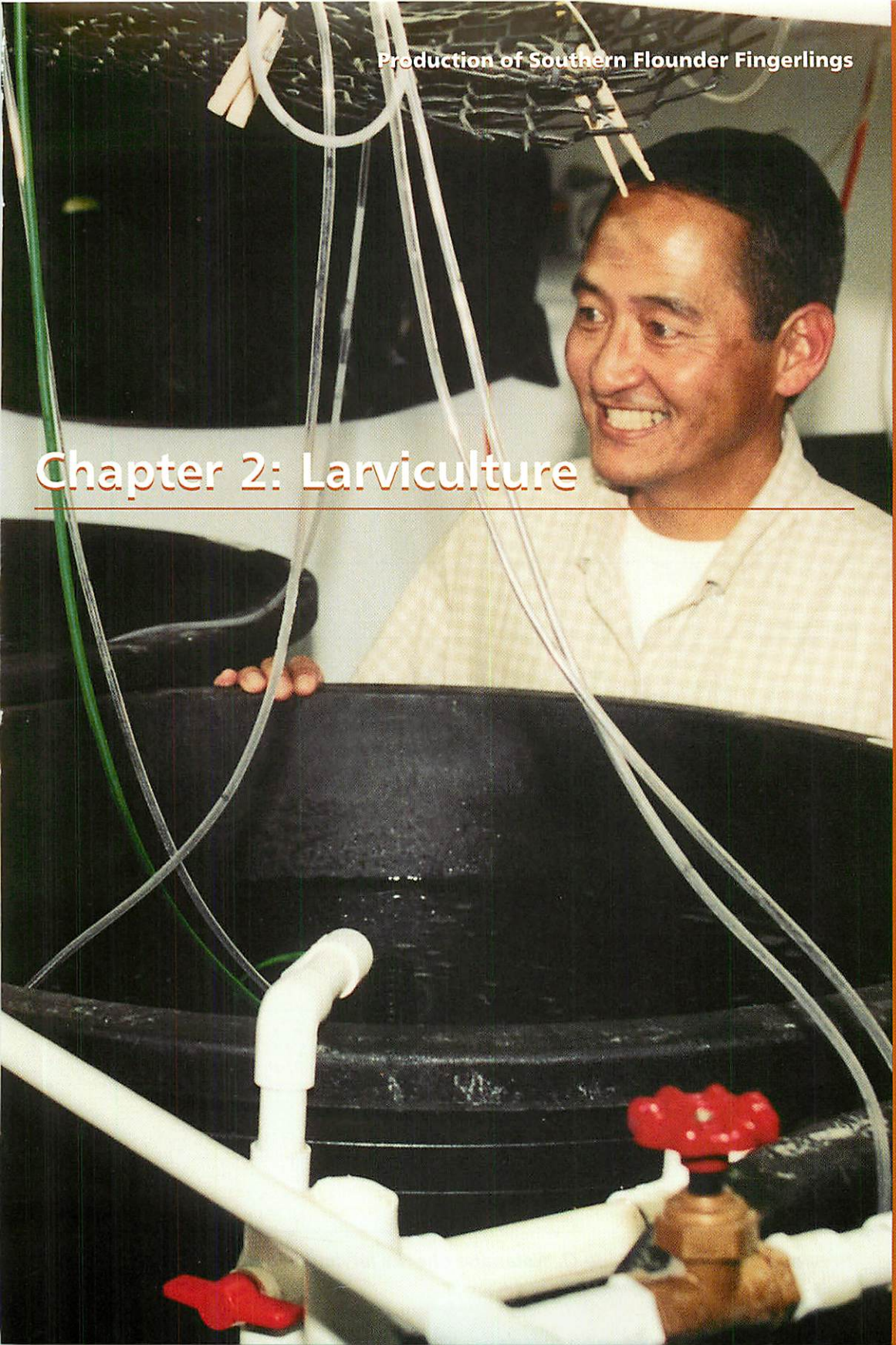
Many hatchery managers prefer to

minimize the amount of handling of eggs and the work involved with incubating eggs in separate containers and instead opt to stock fertilized eggs directly into the larviculture tanks. Use a vigorous amount of aeration to keep fertilized eggs rolling gently in the water column. Just prior to hatch, even the fertilized eggs will begin to sink.

Once hatching is complete, reduce the aeration to the minimum required to keep the larvae gently rolling in the watercolumn.

Figure 7. Egg and larval development.





Chapter 2: Larviculture

Notes

Previous page photo: Wade O. Watanabe checks larviculture tanks.

Introduction

The culture of flounder larvae is difficult and time consuming. Larvae require small, slow-moving, live food with an adequate nutritional composition. Rotifers (*Brachionus plicatilis*) are the first live food given to larval flounder. Brine shrimp (*Artemia*) are the second live food. Rotifers and *Artemia* alone lack many essential nutrients for the larvae, so they must be enriched before they are fed to the fish.

Coordination of live food production to correspond to the needs of developing larvae requires planning, flexibility and experience. In this section, detailed, step-by-step procedures for the management of feeding flounder larvae are provided.

Procedures for culturing rotifers and *Artemia* are found in Appendices A and B. References for detailed instructions on algae culture are provided in Appendix C.

Because recently hatched larvae are small and transparent, they are difficult to see when stocked in a large tank. Mortality of small fish is sometimes sudden and difficult to explain by the hatchery manager. However, with proper training and experience, consistent and reliable production of fingerlings is possible. Experienced hatchery managers are patient, pay attention to detail, and resist the temptation to make sudden and extreme changes in management.

Water Filtration Systems

Water filtration systems for flounder larviculture should focus on the physical removal of solids and the destruction of disease organisms, such as bacteria, viruses and protozoans. Because the amount of feed applied to hatchery tanks is very low compared to other production systems, biological filtration to remove ammonia and nitrite is of lesser importance than mechanical filtration and sterilization of the water.

The filtration system at TRS — in order of assembly — consists of a 2-hp centrifugal pump, commercial sand filter, foam fractionator, and ultraviolet (UV) sterilizer. An ozone sterilizer may be used instead of the UV sterilizer, but care must be taken to ensure that excess ozone is removed from the water with activated carbon before it enters the larviculture tanks. Header tanks are used to maintain constant pressure in the water pipes.

Excess water is circulated continuously through the filtration system via a large sump. Continuous filtration in this loop allows the filter components to be smaller and less costly than filters that are designed to clean the water in a single pass. In addition to these filters, bags made of 10-micron mesh are placed directly under the water flowing into the larviculture tanks to provide further solids removal.

From Egg Hatching to Metamorphosis

Preparation of Larvae Tanks

Fill the larviculture tanks with full strength seawater — 33-ppt salinity — that has been passed through a 10-micron filter and sterilized either with UV light or ozone. Use a vigorous amount of aeration and 300 percent daily water exchange before eggs are stocked. Immediately prior to stocking eggs, reduce water exchange to 100 percent per day and adjust the aeration accordingly.

Stock 25 eggs/liter into a 1,000-liter tank. Larger tanks can be used. (*See Figure 8.*) The aeration and mixing of these tanks is less uniform and they are more difficult to clean.

Hatching

Eggs will hatch after 48 to 55 hours of incubation at 16 to 18° C. Once the hatch appears to be finished, the amount of aeration should be reduced to avoid harming the delicate larvae with too much turbulence. Maintain 100 percent daily water exchange during egg hatch and immediately after so that the tank water is clear.

Figure 8. Large scale tanks for larvae culture.

When eggs hatch, oils and other materials are released to the water giving it a "milky" appearance. This residue will suffocate the larvae if not flushed out of the tank. A 250- to 300-micron filter screen should be placed over the standpipe to keep larvae from being flushed out. (*See Figure 9.*) Air stones should be placed next to the standpipe so the turbulent action of the rising bubbles will scour the filter clean.

The day the eggs hatch out is termed "Day 0," and is the start of the timeline leading to metamorphosis. At this time, it is very important to momentarily reduce the aeration and siphon off the dead eggs and eggshells to minimize protozoan contamination and bacteria growth.

Use a photoperiod of 12-hour light with standard 40-watt fluorescent lights placed 2 to 3 feet above the tanks. The lights will help maintain the algae bloom and will assist the fish in seeing and eating the live food. After egg hatch, water temperature can be gradually raised to 21 or 22° C and kept at this level throughout the rest of the larviculture cycle.

From "Day 0" to "Day 3," skimming the surface of the tank water is very important. Gently place a paper towel directly on the surface of the water, and slowly drag it and lift it to remove the oil and dirt that have accumulated. Left to build up, this surface film will trap the larvae and can kill a significant number of fish. The movement of the air bubbles will increase after the surface is cleaned of this surface scum. This is a sign that the water surface is sufficiently clean. There is no set schedule for skimming, but it should be done as needed at least four to five times per day.

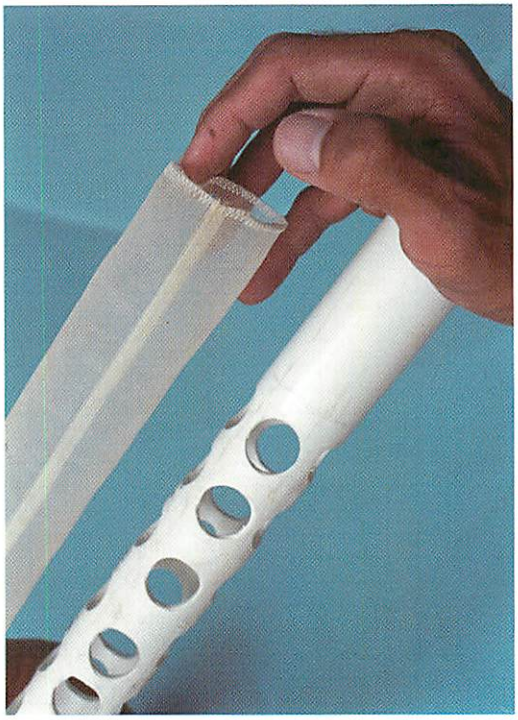


Figure 9. Standpipe and filter screen.

Feeding and Maintenance

The feeding schedule is shown in *Figure 10.* Live algae are added to the tank to condition the water and to provide a food source for the rotifers. Add enough algae to give the water a light green color, but still allow easy observation of fish near the bottom of the tank. Excessive amounts of algae make it difficult for fish to see the feed and for people to see the fish.

Unenriched rotifers are a poor food for fish but they are easily caught by the larvae and will continue to filter feed while in the tank. The algae are used to boost the nutritional profile of the rotifers. Algae can be added slowly by siphoning through a small diameter airline tube placed in a bucket perched above the tank.

Figure 10. Feeding timetable.

Day Count	Time of Day									
	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600
1	Algae									
2	Algae			Rotifers				Rotifers		
3	Algae			Rotifers				Rotifers		
4	Algae			Rotifers				Rotifers		
5	Algae			Rotifers				Rotifers		
6	Algae/1			Rotifers	1			Rotifers		1
7	Algae/1			Rotifers	1			Rotifers		1
8	Algae/1			Rotifers	1			Rotifers		1
9	Algae/1			Rotifers	1			Rotifers		1
10	Algae/1			Rotifers	1			Rotifers		1
11	Algae/1		Artemia	Rotifers	1			Rotifers	Artemia	1
12	Algae/1		Artemia	Rotifers	1			Rotifers	Artemia	1
13	Algae/1		Artemia	Rotifers	1			Rotifers	Artemia	1
14	Algae/1		Artemia	Rotifers	1			Rotifers	Artemia	1
15	Algae/1		Artemia	Rotifers	1			Rotifers	Artemia	1
16	Algae/1		Artemia	Rotifers	1			Rotifers	Artemia	1
17	Algae/1		Artemia	Rotifers	1			Rotifers	Artemia	1
18	1		Artemia		1				Artemia	1
19	1–2		Artemia			1–2			Artemia	1–2
20	1–2		Artemia			1–2			Artemia	1–2
21	1–2		Artemia			1–2			Artemia	1–2
22	2		Artemia			2			Artemia	2
23	2		Artemia			2			Artemia	2
24	2		Artemia			2			Artemia	2
25	2		Artemia			2			Artemia	2
26	2–4	4	Artemia	4	4	2–4	4	4	Artemia	2–4
27	2–4	4	Artemia	4	4	2–4	4	4	Artemia	2–4
28	2–4	4	Artemia	4	4	2–4	4	4	Artemia	2–4
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37	4	4	4	4	4	4	4	4	4	4
38	4	4	4	4	4	4	4	4	4	4
39	4	4	4	4	4	4	4	4	4	4
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47	6	6	6	6	6	6	6	6	6	6
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51	6	6	6	6	6	6	6	6	6	6
52	6	6	6	6	6	6	6	6	6	6
53	6	6	6	6	6	6	6	6	6	6
54	6	6	6	6	6	6	6	6	6	6
55	6–8	6–8	6–8	6–8	6–8	6–8	6–8	6–8	6–8	6–8
56	6–8	6–8	6–8	6–8	6–8	6–8	6–8	6–8	6–8	6–8
57	6–8	6–8	6–8	6–8	6–8	6–8	6–8	6–8	6–8	6–8
58	6–8	6–8	6–8	6–8	6–8	6–8	6–8	6–8	6–8	6–8
59	6–8	6–8	6–8	6–8	6–8	6–8	6–8	6–8	6–8	6–8
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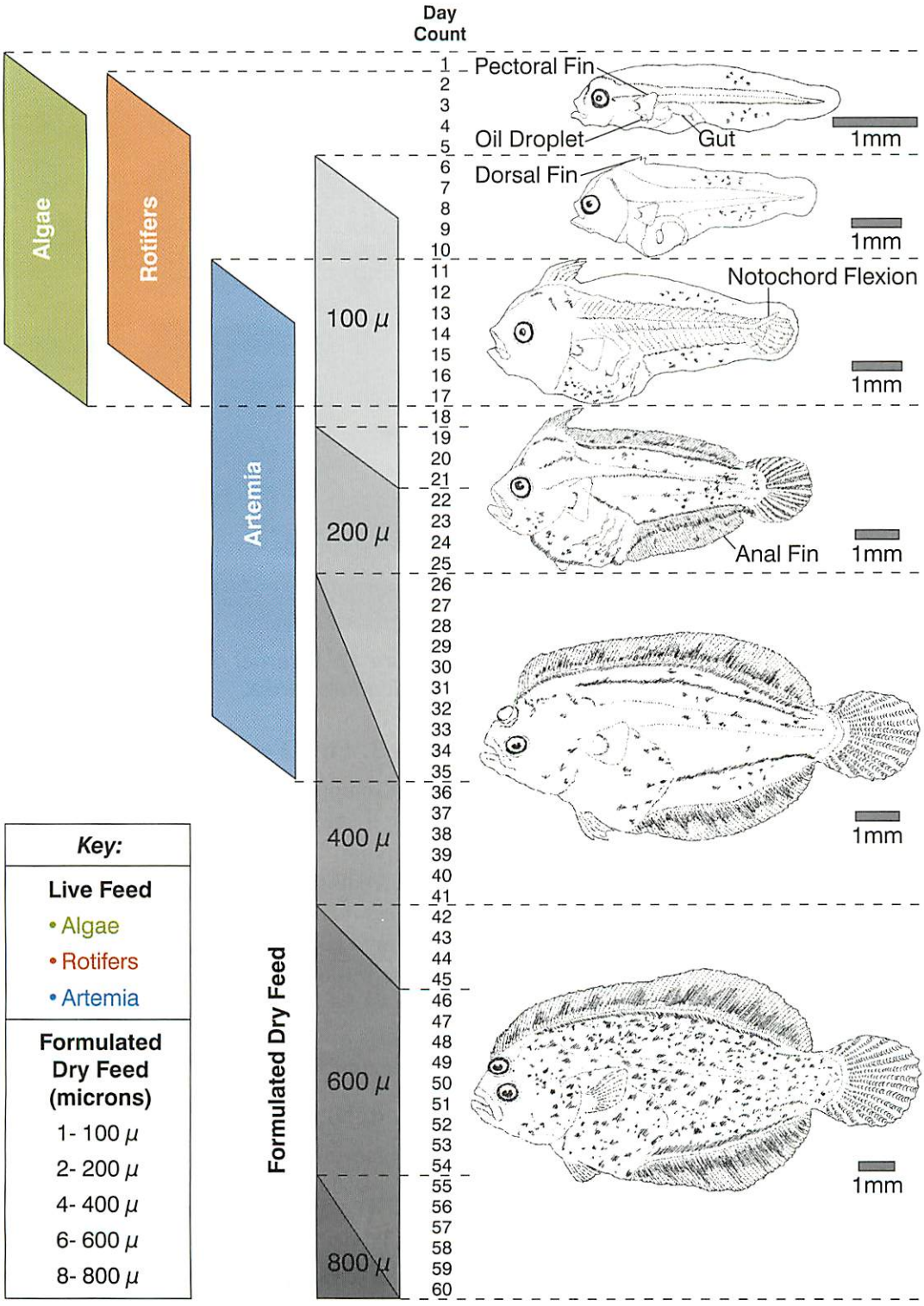

Day Count
Algae
Rotifers
Artemia
Formulated Dry Feed
100 μ
200 μ
400 μ
600 μ
800 μ
Pectoral Fin
Oil Droplet
Gut
Dorsal Fin
Notochord Flexion
Anal Fin
1mm
Key:
Live Feed
• Algae
• Rotifers
• Artemia
Formulated Dry Feed (microns)
1- 100 μ
2- 200 μ
4- 400 μ
6- 600 μ
8- 800 μ

In this manner, the algae are added slowly, and the environment of the tank is not changed abruptly.

Siphon the tank bottom every other day into a 5-gal. bucket. Move the siphon slowly so the solids are not resuspended into the water column. We use a ½-inch PVC "T" that has been sawed in half lengthwise and glued to a 3-ft. long, ½-inch pipe. A threaded female fitting is glued to the end, and an adapter is inserted to attach 3 to 4 feet of clear, flexible tubing that is placed into the 5-gal. bucket. Some people glue small strips of common kitchen scouring pads to the edges of the "T" to improve the scrubbing of the tank bottom.

Once the tank has been siphoned, allow the solids to settle to the bottom of the bucket before gently pouring any larvae back into the tank. You may need to spot siphon the tank between the scheduled siphoning.

The sides of the tank should not be cleaned, as the cleaning action suspends some of the algae and clouds the water, which is harmful to the fish. Instead, let the algae grow as the fish will clean the side on their own as the production cycle proceeds.

Day 2: S-Flex

Reduce water exchange to a trickle, about 50% per day. (*See Figure 11.*) Start adding rotifers at 20 rotifers/ml even though the fish will not be ready to feed. Be sure to maintain a slight green color in the tank by adding algae. This should correspond to an algal density of around 300,000 to 500,000 cells per mL. Fish will start making "S" shaped flex motions of their body in preparation for striking at the rotifers. This is normal and signals that feeding will begin soon. (*See Appendix A for Rotifer culture.*)

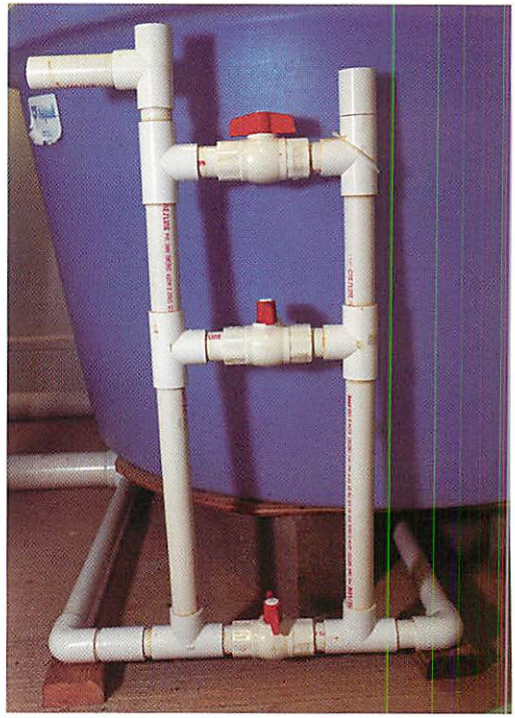


Figure 11. External drain pipes for larviculture tanks.

Day 3: First Feeding, Stage B

Count the rotifers twice daily, first thing in the morning and later in the afternoon. The first four to five days after first feeding, rotifer counts may exceed 20 rotifers/mL because the fish have not yet begun to keep pace with the reproduction of the rotifers. Don't worry about excessive rotifer numbers at this stage because the fish will begin to reduce the numbers in the next few days.

Day 6: Dry Feed

Start introducing a light amount of dry feed — 100-micron size — three times daily. Use an automatic feeder and be sure to skim the tank surface before feeding, or the dry feed will get trapped on the surface of the water and will not reach the larvae.

Day 10: Formalin Treatment

Treat each tank with 50 ppm of formalin for 30 minutes to control parasitic protozoans. Resume normal water exchange after 30 minutes. In water reuse systems, the water containing the formalin may have to be discarded or it will harm the biological filter.

Day 11: *Artemia* Feeding

Change the filter screen on the center standpipe to a 500-micron size. Begin feeding *Artemia* in addition to the rotifers. At first, add 200,000 *Artemia* for every 1,000 liters of tank volume. As the fish grow and gradually consume *Artemia* exclusively, by "Day 17" or so, increase feeding to 1,000,000 *Artemia* per 1,000 liters. Increase water exchange to 100% per day. (*See Appendix B.*)

Day 17: Stop Feeding

Stop feeding the rotifers on "Day 17." The numbers will gradually decline as they are eaten. Be sure to plan the hatching and enrichment of *Artemia* far enough in advance so that they are ready to be fed on the day they are needed.

Days 19 to 21: Beginning of Metamorphosis

Thoroughly siphon the bottom of the larviculture tanks. At this stage, the right eye of the larvae will begin to migrate over to the left side of the head, and the fish will begin to settle on the tank bottom. Some fish will appear to rest on the bottom even though their eyes are still in their original position.

Many fish in the water column will appear to be swimming at a 45-degree angle. This is normal behavior, as the fish are changing their swimming motion along with the migration of the eye.

At this time, the fish density will have to be reduced by "splitting" the tank. There are several options for splitting the tank depending on the number of available tanks. Fish will settle on the tank bottom at different rates. The slower-developing larvae will remain near the water surface, while the faster developing fish will settle quickly on the bottom of the tank. Remove the smaller fish with a beaker or other small container and place them into one or two clean tanks, depending on number of fish and tank availability.

The splitting process takes time, but try to complete the transfer within one week. More than 80 percent of the larvae should complete metamorphosis by Day 31. During this time, gradually reduce the *Artemia* densities from 1,000,000, to 500,000, to 200,000 *Artemia* per 1,000 liters (from 1,000/L to 200/L), in three-day increments.

At the same time, increase the amount of dry feed applied to the tank but maintain the frequency of feeding. It will help to use automatic, vibratory feeders on timers to dispense the dry feed throughout the day. Use a 200- to 250-micron dry feed during this stage. The larvae will not be able to consume all the dry feed, and much of it will accumulate on the bottom of the tank. It will appear that the feed is being wasted, but this is a necessary step to ensure that the fish train to dry feed.

With experience, some hatchery managers can eliminate the feeding of *Artemia* and train the fish to dry feed before the start of metamorphosis. *Artemia* are expensive, so reducing the amount fed to the larvae will help reduce production costs.

Cannibalism

The faster-growing fish will begin to eat fish that are about half their size around the beginning of metamorphosis. Cannibalism is unavoidable but can be managed by removing (grading or splitting) the smaller fish with a small beaker and placing them in a separate tank. Continue to follow the feeding, skimming and siphoning schedule until harvest during Days 45 to 50. The dry feed size can be increased to 400 to 500 microns.

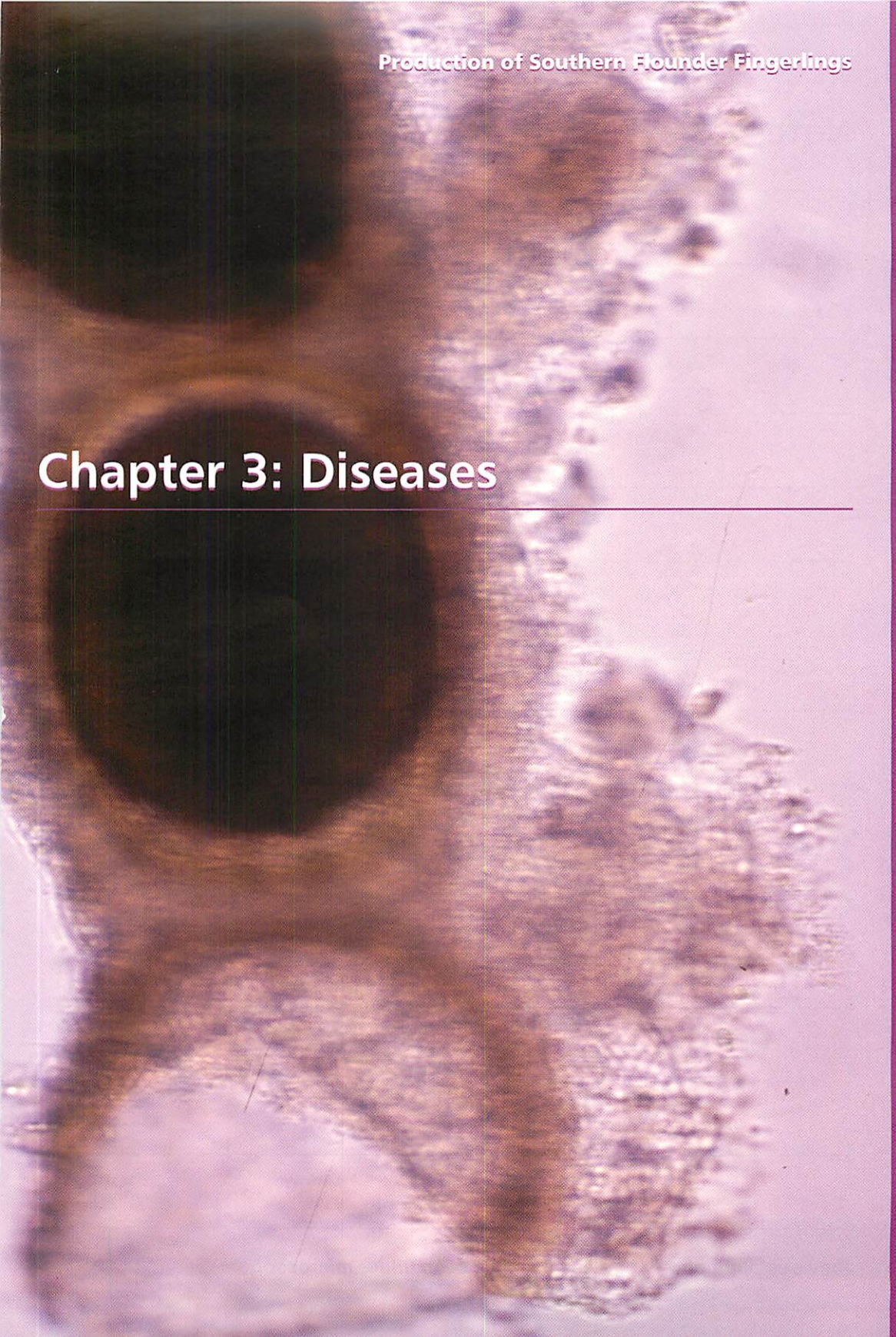
Nursery: Weaning to Dry Feed

Set up net pens constructed with a small-mesh screen material, similar to mosquito screen, in a larger tank. Pens should be 6- to 8-feet square, 18- to 24-inches high, made with knotless nylon netting, and stretched tight on a PVC frame. Be sure to leave enough space between the pens and the tank walls to allow you to siphon uneaten feed and other wastes that accumulate beneath the pens. (*See Figure 12.*)

Stock each pen with about 6,000 to 8,000 juveniles. Feed the penned fish five to six times daily or more, depending on demand. Keep a close watch on the feeding behavior of the fish and gradually add 700- to 800-micron feed to the other dry feed as the fish grow. At this point, siphon the tank bottom every other day and scrub the tank walls with a brush. Keep the fish in the pens until they reach 3.0 to 3.5 cm in length (about 1.5 inches). Maintain the temperature at 23° C. This should take about 2 to 3 weeks.

At this stage, fish should be moved to growout tanks or sold as fingerlings to other flounder producers.

Figure 12. Net pen for juveniles.



Chapter 3: Diseases

Notes

Previous page photo: Egg cases of lice seen under microscope.

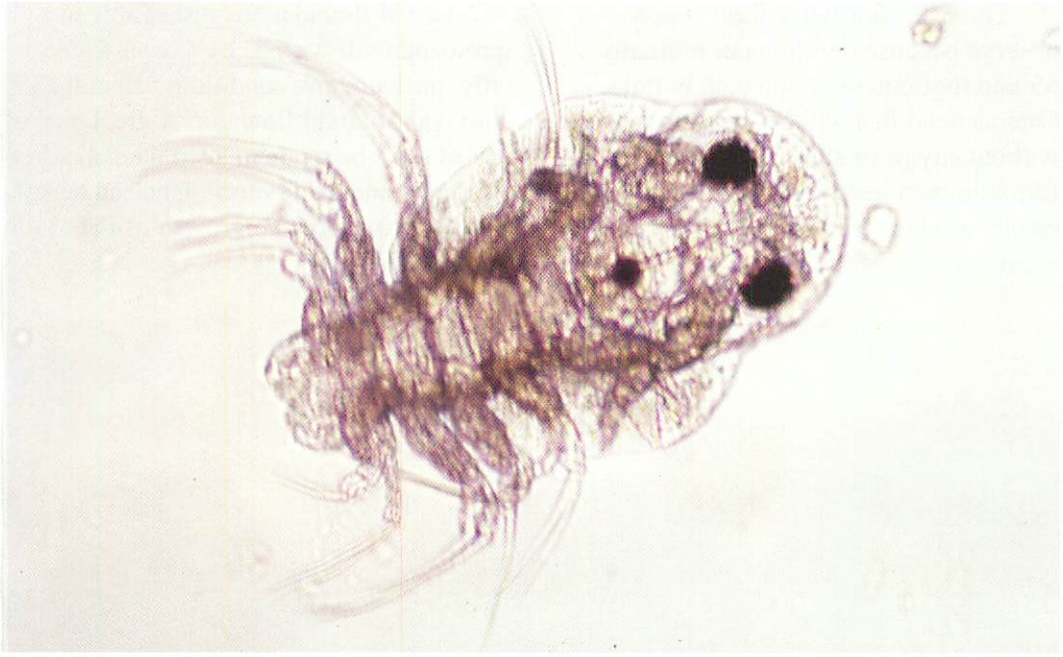


Figure 12. Fish lice.

Wild broodstock often enter the hatchery with a variety of diseases, including sea lice (*Argulus sp.*), Marine Ich (*Cryptocaryon sp.*), parasitic copepods (*Ergasilus sp.*), and intestinal worms. Many of the external parasites can be effectively treated with a formalin treatment (25 ppm for 30 minutes). Sea lice are stubborn and may require several treatments or manual removal of the lice in extreme cases. (*See Figures 12 and 13.*)

An indoor quarantine tank with a separate water system is needed to treat wild broodstock for the six-week period before they are stocked into the regular system. Domesticated fish are generally cleaner because of their origin, but they are equally susceptible to the same diseases as wild fish.

Bacterial diseases (*Aeromonas*, *Staphylococcus*, *Vibriosis*, etc.) are commonly seen but rarely affect the entire population of fish in one tank. *Edwarsiella tarda* is a persistent pathogen of Japanese flounder and may affect southern flounder as well. Viruses such as *epidermal hyperplasia* (herpes virus) and *nervous necrosis* (striped jack nervous necrosis virus) are also found in cultured Japanese flounder and may cause similar problems in southern flounder. Often the only sign of a bacterial infection is when a fish becomes darker in color or changes the color of only the back half of its body.

There are few behavioral cues to observe because the flounder normally remain motionless on the tank bottom. Often a dead fish will be found in the tank without any prior sign of disease. The formalin bath treatment described above can be used to treat individual fish or the entire tank if needed.

Larval flounder are susceptible to protozoan infestations by *Costia*. Generally, unclean tank conditions affect the survival of larval flounder. A great deal of effort must be made to keep the tanks free of debris and excess feed. Unclean tank conditions favor the growth of the protozoans.

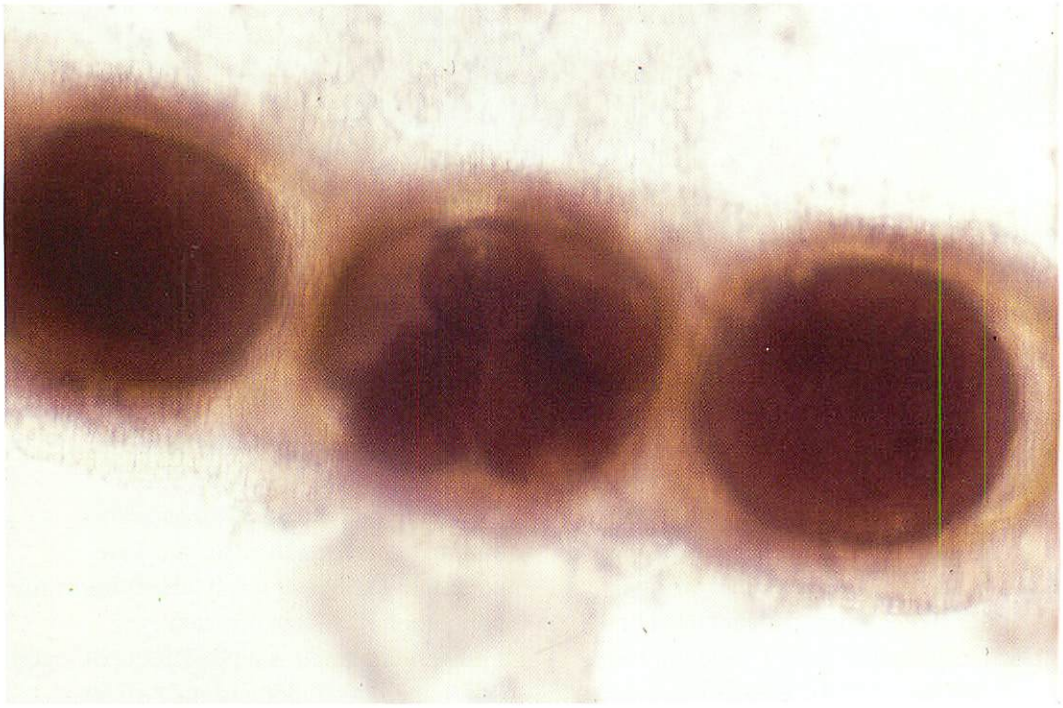
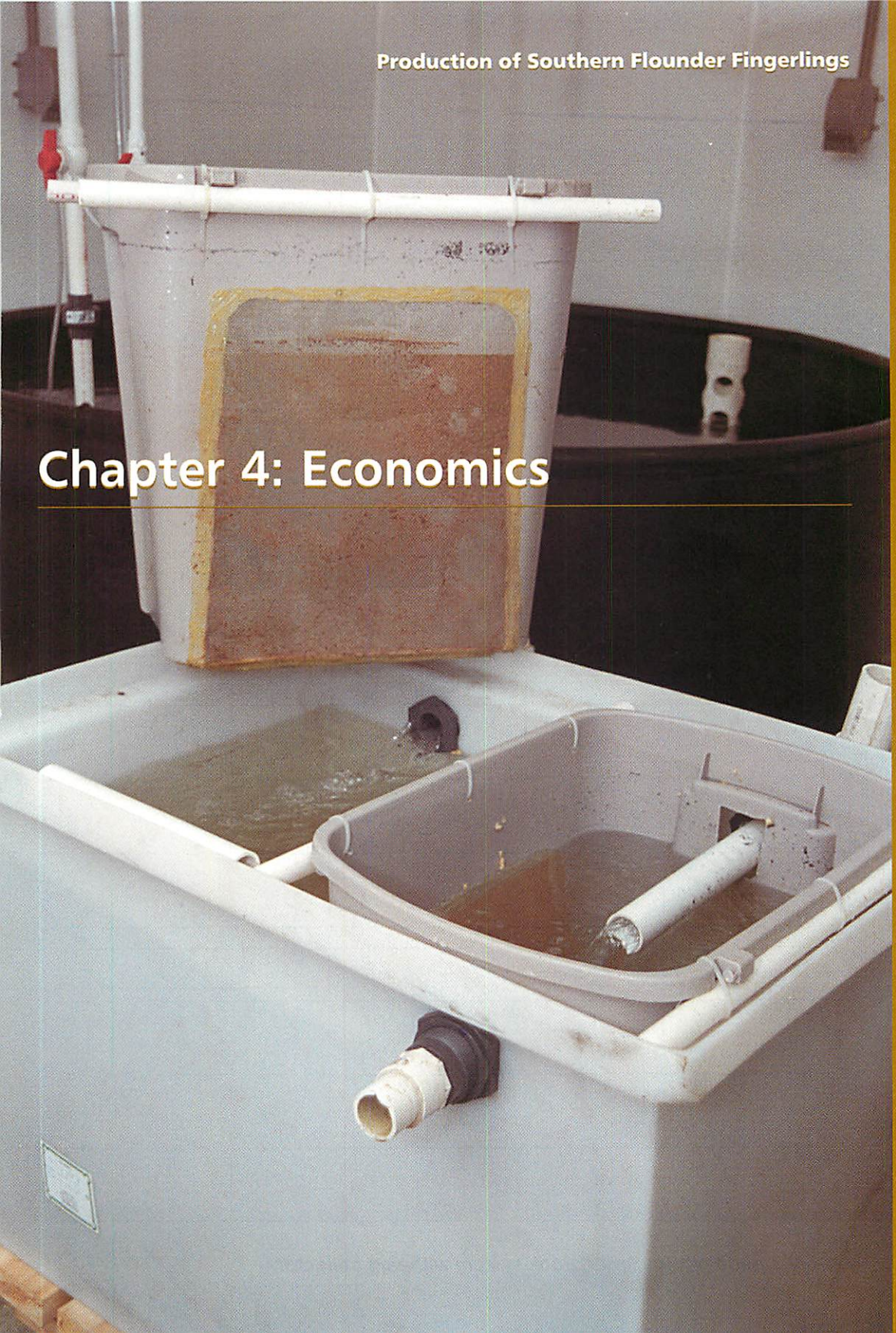


Figure 13. Fish lice egg cases.



Chapter 4: Economics

Notes

Previous page photo: Broodstock system with egg collector.

Sample Budgets

The sample budgets shown estimate the cost of building and operating a hatchery capable of producing 96,000, 3.5 cm southern flounder fingerlings per year.

The system is based on the flounder hatchery at the Tidewater Research Station in Plymouth, N.C. The hatchery consists of five separate systems — broodstock, larvae, nursery, algae and rotifer — each with its own set of tanks, pumps and water filtration equipment.

The systems are housed in a 30-ft. by 60-ft. insulated metal building with a concrete floor. Two acres are allowed for the building and half-acre settling pond and composter for waste management.

The total cost of the initial investment is $182,521, which includes $40,000 in construction labor and equipment set-up. (*See Figure 14.*)

Table 1 illustrates the total cost of the investment which is financed over 10 years at 7% interest. *Table 2* provides a detailed equipment list for the systems.

Tables 3a and 3b present the operating costs and estimated returns per year. As of 2003, there is no commercial production of southern flounder fingerlings, so a market price has yet to be established. However, fingerling prices for other cultured flounder, in the U.S. and worldwide, typically range from $2 to $3 per fish for a 3.5 cm fingerling, with higher prices as fingerling size increases.

The sample budget assumes a price of $2 for each 3.5 cm fingerling. We assume six spawns each year, with 25,000 fry stocked in each of the four larvae-system tanks. After 60 days in the larvae and nursery systems, 16,000 fingerlings are available for sale. At $2 each, this results in gross receipts of $192,000 annually.

More than half of the annual operating costs of $91,206 arise from labor — management labor at 29 percent of total cost; part-time labor at 14 percent of total cost; and electricity at 13 percent of total cost. Debt service to a creditor is about 20 percent of total cost.

The total variable cost — all costs excluding the fixed costs of debt service on the investment, property taxes and insurance, and rental of the oxygen tank — is $0.95 per fish, or $91,206 for the hatchery per year. The debt service, property taxes and insurance, and oxygen tank rental adds an additional $0.34 in cost per fish, for a total cost of $1.29 per fish or $123,993 for the facility per year. Thus, the hatchery must receive a price of at least $1.29 per fish to cover all costs of growing the fish. With a sale price of $2 per fish, the hatchery has returns of $68,007 to the owner's management.

Table 1. Flounder hatchery investment costs.

Investments	Unit	Price($)/Unit	# of Units	$ Cost	% of Total
Land	acre	4,000.0	2	**8,000**	**4.4%**
Waste Removal					
• Settling Pond	acre	10,000.0	0.5	5,000	2.7%
• Aerator (½-hp)	unit	2,500.0	1	2,500	1.4%
• Composter	unit	7,500.0	1	7,500	4.1%
Subtotal				**15,000**	**8.2%**
Building					
• Building, 30' x 60' (insulated metal building with concrete floor)	sq. ft.	14.6	1800	26,360	14.4%
• Electrical	unit	3,697.5	1	3,698	2.0%
• Plumbing	unit	3,600.0	1	3,600	2.0%
• HVAC (heating and cooling)	unit	2,600.0	1	2,600	1.4%
Subtotal				**36,257**	**19.9%**
Well and ¾-hp Pump (35 gpm)	unit	4,000.0	1	**4,000**	**2.2%**
System-Specific Equipment (see Table 2)					
• Broodstock System				37,582	20.6%
• Larval System				11,604	6.4%
• Algae System				1,850	1.0%
• Rotifer Rearing System				1,050	0.6%
• Nursery System				10,544	5.8%
Subtotal				**62,630**	**34.3%**
System-Wide Equipment					
• Generator	unit	4,200.0	1	4,200	2.3%
• Oxygen Monitor	unit	5,234.0	1	5,234	2.9%
• Misc. Harvest Equipment (nets, baskets, etc.)	unit	1,000.0	1	1,000	0.5%
• Regenerative Blower	unit	350.0	1	350	0.2%
• Telephone Dialer	unit	350.0	1	350	0.2%
• Lab Equipment	unit	4,000.0	1	4,000	2.2%
• Misc. Equipment	unit	1,500.0	1	1,500	0.8%
Subtotal				**16,634**	**9.1%**
Labor					
• Building, Electrical and Plumbing				20,000	11.0%
• Equipment Set-Up				20,000	11.0%
Subtotal				**40,000**	**21.9%**
Total				**182,521**	**100.0%**

Table 2. Detailed equipment list.

Equipment	Unit	Price($)/Unit	# of Units	*Total*($)
Broodstock System (Includes fish)				
• Tanks, 8-ft Fiberglass (1000 gals.)	tank	1,200.0	4	4,800
• Female Broodstock (4 lbs. each)	fish	50.0	20	1,000
• Male Broodstock (1 lb. each)	fish	8.0	32	256
• Pump (2-hp)	unit	565.0	4	2,260
• Foam Fractionator	unit	1,495.0	4	5,980
• Bubble Bead Filter	unit	1,340.0	4	5,360
• Filter Sump	unit	94.0	4	376
• Regenerative Blower	unit	350.0	1	350
• Heat Pump (¾-hp)	unit	2,200.0	4	8,800
• UV System (200 watts)	unit	2,100.0	4	8,400
Subtotal				**37,582**
Larvae System				
• Tanks, 4-ft Fiberglass (300 gals.)	unit	600.0	4	2,400
• Pump (2-hp)	unit	565.0	1	565
• Foam Fractionator	unit	1,495.0	1	1,495
• Bubble Bead Filter	unit	1,340.0	1	1,340
• Filter Sump	unit	94.0	1	94
• Regenerative Blower	unit	350.0	1	350
• Heat Pump (¾-hp)	unit	2,200.0	1	2,200
• UV System (200 watts)	unit	2,100.0	1	2,100
• Incubator	unit	200.0	1	200
• Feeder Controller	per system	260.0	1	260
• Feeders	per tank	150.0	4	600
Subtotal				**11,604**
Algae Station				
• Lights, Fittings, Valves, Tubing	unit	500.0	1.00	500.00
• Flasks and Miscelaneous	unit	150.0	1.00	150.00
• Cylinders (18" dia., 5" high)	unit	134.0	8.00	1,072.00
• Carboys (5-gallon containers)	unit	16.0	8.00	128.00
Subtotal				**1,850**
Rotifer Station				
• Tanks, 180-Liter Polyethelene	unit	200.0	4	800
• Wood Frame, Fittings, Valves, Tubing	unit	250.0	1	250
Subtotal				**1,050**
Nursery				
• Tanks, 8-ft Fiberglass (1000 gals.)	tank	1,200.0	2	2,400
• Pump (2-hp)	unit	565.0	1	565
• Foam Fractionater	unit	1,495.0	1	1,495
• Bubble Bead Filter	unit	1,340.0	1	1,340
• Filter Sump	unit	94.0	1	94
• Regenerative Blower	unit	350.0	1	350
• Heat Pump (¾-hp)	unit	2,200.0	1	2,200
• UV System (200 watts)	unit	2,100.0	1	2,100
Subtotal				**10,544**
Total				**62,630**

Table 3a. Operating costs (assumes 6 spawns per year, 20% survival): 20,000 eggs/tank x 4 tanks x 6 spawns/yr. x 0.2 = 96,000 fingerlings.

Items	Unit	Price/Unit($)	#Unit	*Total($)*	% of Total	Per Fish
Receipts (assume 6 spawns, 24 total tanks/year)						
• Flounder Fingerlings	fish	2.00	96,000	**192,000**		
Variable Costs						
• Female Broodstock (4 lbs. each)	each	50.00	4	200	0.16%	$0.00
• Male Broodstock (1 lb. each)	each	8.00	6	48	0.04%	$0.00
• Feed						
- Broodstock: Frozen Squid, Dry Feed	pound	1.00	2,044	2,044	1.65%	$0.02
- Rotifers: Algae Paste	1 liter	80.00	9	720	0.58%	$0.01
- Artemia	can	25.00	24	600	0.48%	$0.01
- Larval Feed	per tank	25.00	16	400	0.32%	$0.00
- Nursery Feed	per tank	187.50	16	3,000	2.42%	$0.03
• Initial Algae Disks	disk	3.00	15	45	0.04%	$0.00
• Initial Rotifers	quart	1.00	15	15	0.01%	$0.00
• Chemicals						
- Formalin	treatment	25.00	8	200	0.16%	$0.00
- Hormones	treatment	5.00	80	400	0.32%	$0.00
- Sea Salt	pallet	4.00	1,386	5,544	4.47%	$0.06
• Electrical Usage						
- Pumps and Filters	month	1,150.00	12	13,800	11.13%	$0.14
- Building Heat and AC	month	200.00	12	2,400	1.94%	$0.03
• Oxygen	100 cu ft	0.30	900	270	0.22%	$0.00
• Repair/Maint. of Equip.	month	150.00	12	1,800	1.45%	$0.02
• Managerial Labor	month	3,000.00	12	36,000	29.03%	$0.38
• Part-time/Weekend Labor	month	1500.00	12	18,000	14.52%	$0.19
• Office Overhead	month	100.00	12	1,200	0.97%	$0.01
• Interest on Above Operating Funds	dollar			1,520	1.23%	$0.02
• Marketing Cost	dollar			3,000	2.42%	$0.03
Subtotal, Variable Costs				**91,206**	**73.56%**	**$0.95**
Fixed Costs[1]						
• Payment on Land and Const. Debt	dollar			14,701	11.86%	$0.15
• Payment on Equip. Debt	dollar			11,285	9.10%	$0.12
• Property Taxes and Insurance	dollar			5,000	4.03%	$0.05
• Oxygen Tank Rental	month	150.00	12	1,800	1.45%	$0.02
Subtotal, Fixed Costs				**32,787**	**26.44%**	**$0.34**
Total Costs[2]				**123,993**		**$1.29**

[1]Excludes annual depreciation, estimated at $7,107. [2]Includes a total cost of financing at 7% over 10 years.

Table 3b. Returns summary.

Returns to Owner's Management, Labor, and Capital	Per Fish	Farm
Returns Above Variable Costs	$1.05	$100,794
Returns Above Total Costs	$0.71	$68,007
Breakeven Price Per Pound Above Variable Costs	$0.95	—
Breakeven Price Per Pound Above All Costs	$1.29	—

Table 4. Sensitivity analysis: total cost ($) per fish based on changes in number of spawns per year and survival. $1.29 is the baseline assumption for the budgets.

Spawns Per Year	Survival						
	5%	10%	15%	20%	25%	30%	35%
1	31.00	15.50	10.33	7.75	6.20	5.17	4.43
2	15.50	7.75	5.17	3.87	3.10	2.58	2.21
3	10.33	5.17	3.44	2.58	2.07	1.72	1.48
4	7.75	3.87	2.58	1.94	1.55	1.29	1.11
5	6.20	3.10	2.07	1.55	1.24	1.03	0.89
6	5.17	2.58	1.72	**1.29**	1.03	0.86	0.74
7	4.43	2.21	1.48	1.11	0.89	0.74	0.63
8	3.87	1.94	1.29	0.97	0.77	0.65	0.55
9	3.44	1.72	1.15	0.86	0.69	0.57	0.49

In hatcheries, optimum efficiency is achieved by maximizing survival of fish and spawning broodfish during the entire year. *Table 4* gives the cost of production per fish based on changes in survival and the number of spawns per year. The baseline assumption of 20 percent survival is considered "good" for the hatchery, yet 25 percent or even 30 percent could be achievable as the operator gains experience. It is unlikely that survival would exceed 35 percent.

The number of spawns per year could be increased, with an estimated maximum of nine spawns for this size facility. The number of larvae and nursery tanks become the limiting factor.

Flounder eggs could also be a source of revenue, with sales of about $0.01 per egg. A hatchery can have an excess of several million eggs per year at a potential income of $10,000 per million sold.

Summary

Successful operation of a flounder hatchery requires a great deal of knowledge and experience. Estimated returns to the owner/operator are significant: the $68,007 in returns to owner's management is in addition to the manager's labor cost budgeted at $36,000 per year.

The production of flounder fingerlings is now technically feasible, and sales of fingerling fish or eggs can be made to the scientific community for research. However, the sale of a large number of fingerlings will require demand from flounder growout facilities.

The TRS water filtration systems emphasize water conservation and rely on the use of synthetic sea salt to maintain optimum salinity for each life stage. The broodstock systems at UNC-W are semi-closed (about 20 percent/day exchange) seawater systems with some recirculation to maintain constant temperature.

Flow-through water systems are optimum to maintain water quality for hatcheries, but they require that the hatchery be located near a source of abundant, clean salt water with at least 33 ppt salinity and must comply with effluent discharge requirements.

Hatcheries with water reuse systems can be located inland, but must allocate a significant portion of their operating budget to the purchase of synthetic sea salt.

The combined use of a water reuse system with a source of saline groundwater likely will result in the most economical combination of low-cost land and abundant, high-quality water.

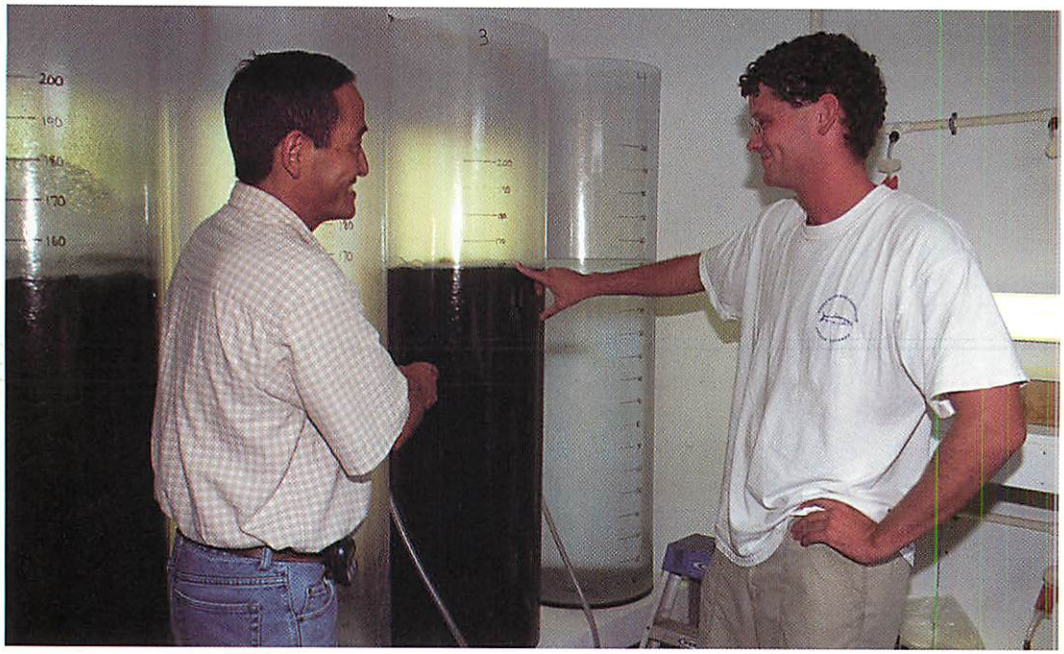


Figure 14. Wade O. Watanabe and graduate student check algae tank system — an important part of a hatchery equipment investment.

Appendix A: Rotifer Culture

Rotifers are small (50- to 150-microns long), soft-bodied metazoans. Their slow swimming motion allows them to be easily captured and eaten by fish larvae. Unfortunately, rotifers are mostly devoid of nutrition and must be enriched with commercially available supplements. The following section describes the daily routine needed to culture rotifers and the procedures used to enrich them just before they are fed to the flounder larvae.

Four poly-tanks (200 L) are used to culture rotifers, with an additional reserve tank of heated water for exchange. Salt water at 20 to 25 ppt is made using chlorinated/dechlorinated freshwater and synthetic sea salt. Tank volumes are maintained at 90 L, but smaller volumes can be used to start new batches. Submersed aquarium heaters are used to maintain culture temperatures at 26 to 28° C. Supplemental light is provided (12-hour light cycle) and air is supplied to each tank. Each tank also has a mesh screen to prevent build up of contaminants in the culture tank. (*See Figure 15.*) Tanks are covered with plexiglass covers to prevent contamination.

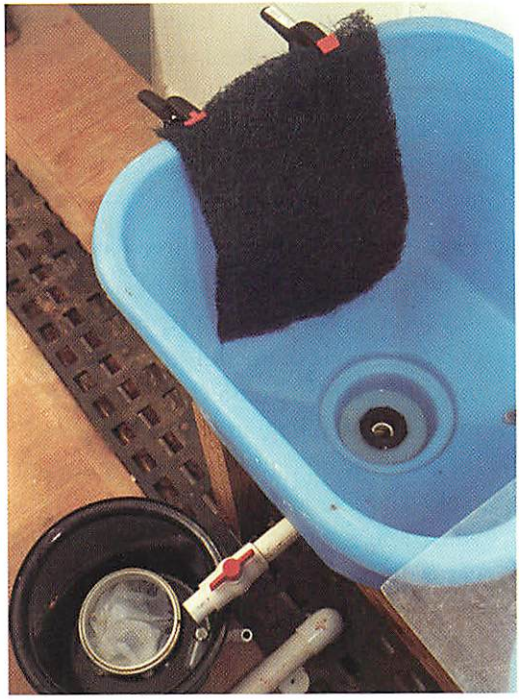


Figure 15. Rotifer tank with a mesh screen on the side and a drainage system filter bag inside a 5-gallon bucket.

The Daily Routine:

1. The temperature and salinity are recorded for each tank. Rotifers are sampled using a 1-mL glass pipette with 0.1 mL divisions. A small rubber bulb will help to suction water into the pipette. Sample the rotifer tanks with the pipette and count the rotifers in the pipette using a small hand-held magnifying loupe. These magnifying loupes can be purchased at most photographic supply stores. Our loupe has small grooves cut into the base to hold the pipette steady while the rotifers are counted. (*See Figure 17.*) Use this same pipette to count the rotifers in the larviculture tanks to determine how many rotifers the fish have been eating. (*See Figure 16.*) Take 3 to 4 samples from different areas of the tank and use the average of those numbers to calculate feeding rates. (*See Table 5.*)
2. Screens are used in each tank to trap large particles. Screens can be made

from air conditioning filters, weighted at the bottom with lead weights, and suspended vertically along one side of the tank. Each screen is removed daily, rinsed and soaked overnight in fresh water to kill protozoans.

3. Each day (for a total of three days) approximately 15 to 30 L of water — depending on water quality and rotifer condition — is drained from each culture tank. Rotifers are collected in a 55-micron screen bag and either removed for enrichment, or replaced in the culture tank. The inside of the tank above the water line is wiped out with a sponge. Be careful not to knock any loose material into the tank. Remove foam with a dip net. Preheated seawater is added to bring the total volume to 90 L.
4. Approximately 15 to 20 mL of *Nannochloropsis* paste is added daily to each tank — 7.5 mL in the morning and 7.5 mL in the afternoon. When a new culture is started, approximately 30 mL is added. The paste may be blended in 1 L of seawater. Populations increase when feed allotments are split into mutiple feedings throughout the day.
5. After three days of culture, the rotifers are harvested by draining the tank completely. Rotifers are collected in 55-micron mesh screen and rinsed with clean seawater. The tank is scrubbed and refilled to 90 L with salt water and heated to 26 to 28° C. Add 15 mL of the old culture water to the new culture. Rotifers are carefully transferred back to the culture tank and 30 mL of *Nannochloropsis* paste is added.

Rotifer Contaminants

Rotifer populations can decline due to the presence of ciliated protozoans. One procedure used to remove the ciliates from the rotifer population is to filter the water with a 50-micron screen and flush the rotifers with water to dislodge the ciliates. Another procedure involves bleaching the entire tank of rotifers. This is a more precise method that should kill most of the ciliates. To do so, the water level in the tank should be 90 L. First remove the mesh screens and then add 9 mL of bleach directly into the tank. After exactly 30 minutes, add 0.175 g of sodium thiosulfate dissolved in 100 mL of salt water. It is best to do this right before a full water exchange for the rotifers.

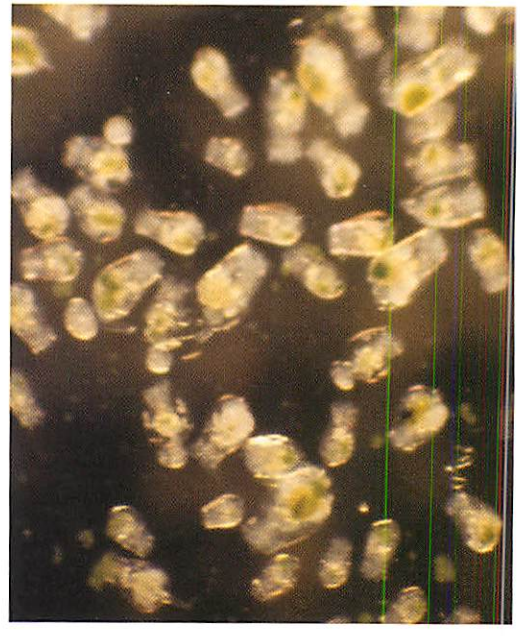


Figure 16. Rotifers as seen under a microscope. Several rotifers have eggs attached.

Rotifer Enrichment

1. Sample rotifer stock tank with a 1 mL pipette diagonally from top center to bottom outside corner. Count the rotifers with a magnifying loupe. (*See Figure 17.*)
2. Calculate the number of rotifers per mL and record on rotifer stock tank data sheet.
3. Based on number of rotifers needed per tank, calculate volume of rotifer tank water needed. (*See Table 5.*) ***Note:*** **Values from table are on a per tank basis.** Multiply this number by the number of tanks to be fed.
4. Filter the rotifer tank water through a 55-micron screen and rinse with clean seawater.
5. Add rinsed rotifers to enrichment container. Use 1 L of water per 1,000 liter of the larviculture tank to be fed.

Enrichment Procedure

Commercially available supplements are used to boost the nutritional value of rotifers. We use a product (Algamac 2000®, from Aquafauna BioMarine, Hawthorne, CA, USA) that is high in DHA (docosahexaenoic acid) concentrations and is easy to use. Other high-DHA products are available and are equally effective for enriching rotifers.

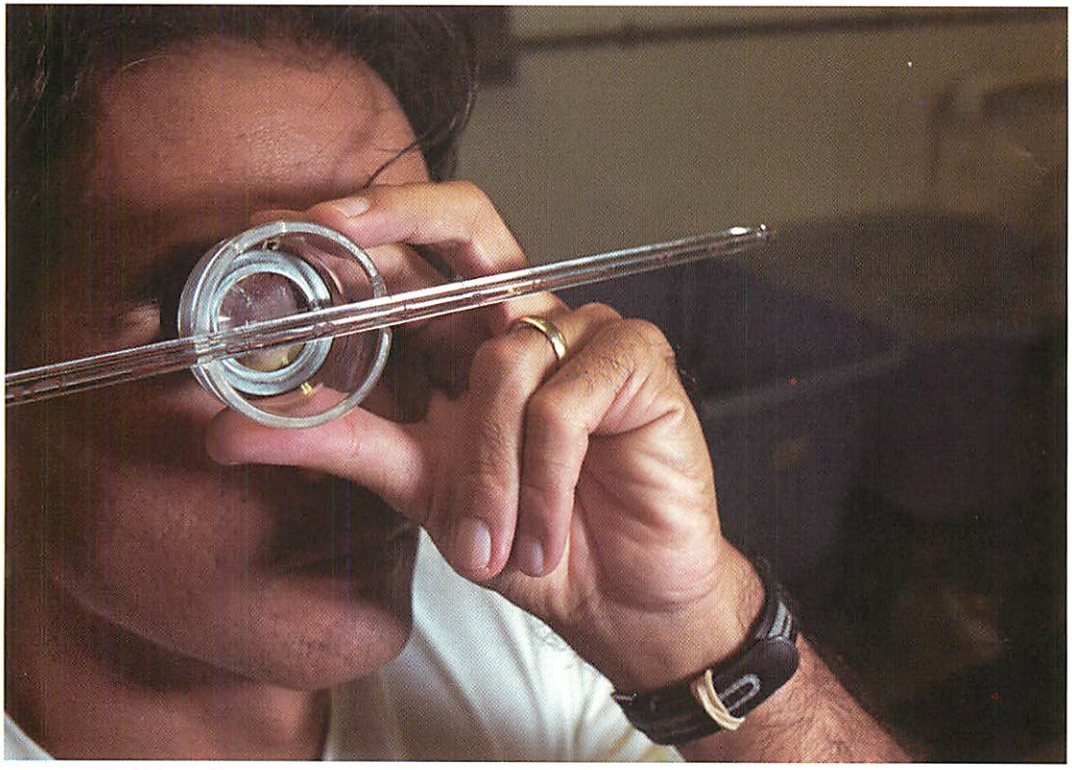


Figure 17. Counting rotifers with a magnifying loupe.

6. Add 1 L of clean water to regular kitchen blender.
7. Add 10 g of Algamac 2000 (about one teaspoon) per 100 million rotifers (multiply number of rotifers/L in step 2 by number of liters in enrichment container). "Grind" in blender for 3 minutes.
8. Add mixture to rotifers in enrichment tank.
9. Let rotifers enrich for 2 hours.

Harvest

10. Leave air on for uniform mixing.
11. Filter one liter per tank through a 55-micron screen and rinse well with clean seawater before feeding to fish tank.

Rotifer Stocking Table

Table 5 shows the number of liters to harvest from the stock rotifer tank to maintain rotifer densities in a 1,000-L larviculture tank. To use this table, first count the number of rotifers in the larviculture tank to determine how many rotifers are needed to maintain desired density. Find that number in the first column on the left. Next, count the number of rotifers in the rotifer-culture tanks, then find that number along the top row of the table. Move vertically down the column until you reach the row that corresponds to the number needed. That number is the volume of liters needed to harvest from the stock rotifer tank and to add into the larviculture tank.

Note: **Check to be certain rotifers are alive before they are fed to the fish.** Occasionally, rotifers die during enrichment. Feeding dead rotifers to fish can kill fish within a few hours.

Table 5: Volume (L) of rotifers from stock tank needed to feed larviculture tanks.

Rotifers/mL needed	Rotifer count (No./mL) in stock tank						
	200	400	500	600	700	800	1000
2	10	5.0	4	3.3	2.8	2.5	2
4	20	10.0	8	6.6	5.7	5.0	4
6	30	15.0	12	10.0	8.5	7.5	6
8	40	20.0	16	13.2	11.4	10.0	8
10	50	25.0	20	16.6	14.2	12.5	10

Appendix B: *Artemia* Hatching and Enrichment

Artemia are small (250 to 500 microns in length) shrimp that are widely available and easy to hatch. Commonly known to aquarium hobbyists as "sea monkeys" or "brine shrimp," many people are familiar with the dried cysts that are sold in aquarium supply stores. Larger quantities can be purchased through most aquaculture supply companies. The dried cysts, which are basically encapsulated eggs, must be incubated and hatched before they can be fed to flounder larvae. Similar to rotifers, newly hatched nauplii are nutritionally incomplete so they must be enriched. (*See Figure 18.*)

1. Add 1.8 g/L of cysts to seawater. Use vigorous aeration and heat lamps until hatch is complete (approximately 24 hours). ***Note:*** **Make sure air is entering at lowest point of container to prevent any cyst settlement.**
2. After hatch is complete, turn off air and let unhatched cysts settle to bottom. Drain out the bottom water with unhatched cysts. Most *Artemia* should still be up in water column attracted to lights from above.

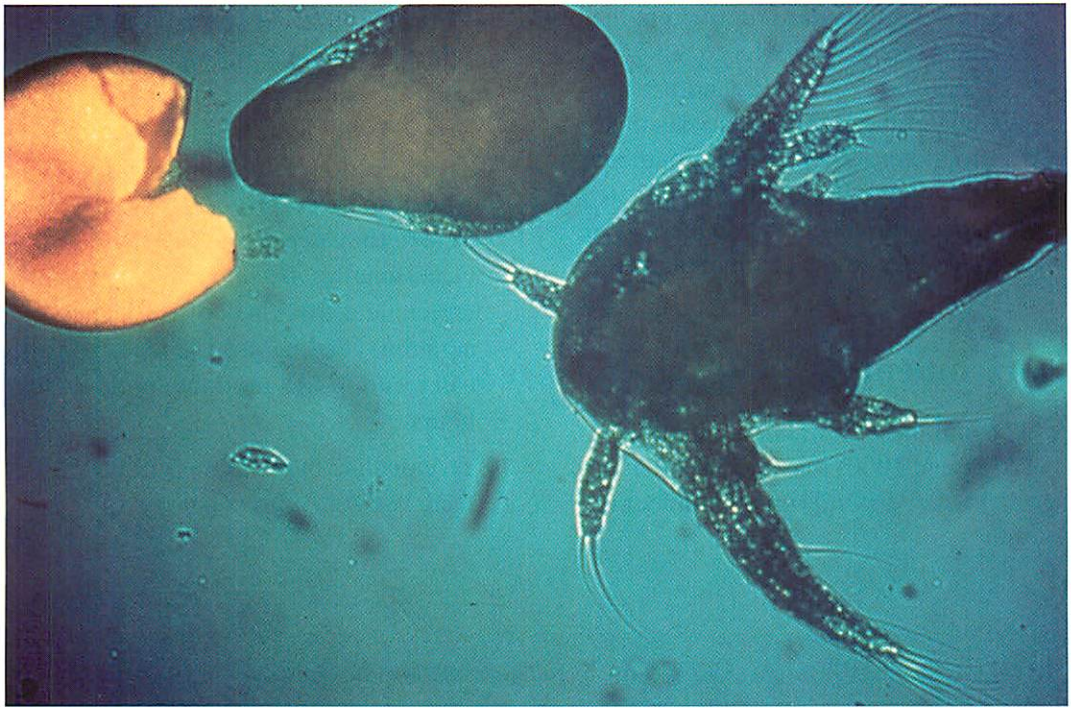


Figure 18. **Artemia** ***egg emerging from cyst (above) and recently hatched nauplius at right.***

3. Leave air off and move light to bottom of cone. Live *Artemia* will move to the light. After 5 to 15 minutes, drain concentrated *Artemia* from cone through a 105-micron filter (68-micron for smaller species). ***Note*: Stop draining before floating cysts are harvested.** Rinse collected *Artemia* with seawater and place in another bucket with moderate aeration to keep them alive while you complete the next steps.

Counting

4. The counting procedure for *Artemia* is similar to the rotifer counting method described in the previous section. Use the same type of 1-mL pipette and magnifying loupe as described in the previous section on rotifers. (*See Figure 17.*) Take 3 to 4 samples and use the average of these samples to calculate feeding rates. Often *Artemia* numbers are too high to be counted by pipette. To reduce the concentration of *Artemia*, add seawater to the original bucket.

Enrichment

5. The amount of enrichment material (Algamac 2000) that will be added to the bucket depends on the number of *Artemia*. Therefore, an acceptable count is important to avoid excessive use of enrichment material, which can suffocate *Artemia*.
6. For every 100,000 to 500,000 *Artemia*, add 10 g of Algamac 2000 to 1 L of seawater in blender. Mix on "grind" for 3 minutes and add mixture to *Artemia* in enrichment container. Be sure to wait at least 4 to 6 hours after hatching to do the enrichment.
7. Using vigorous aeration, enrich for 2 hours, using light when enriching with algae.

Harvest

8. Leave air on for uniform mixing.
9. Filter 1 L per tank through a 105-micron screen and rinse collected *Artemia* well with clean seawater before feeding to fish tank. ***Note:* Check to be certain *Artemia* are alive before they are fed to the fish.** Occasionally, *Artemia* can die during enrichment from lack of sufficient aeration. Feeding dead *Artemia* to fish can result in fish mortality within a few hours.
10. To calculate the volume (in liters) to feed, use the formula below:

100 ÷ number *Artemia* counted/mL = number liters to be fed per 100,000 *Artemia*

Appendix C: References

General

- Daniels, H.V. 2000. *Species profile: Southern Flounder*. Southern Regional Aquaculture Center Publication No. 726. Texas A&M University, College Station, Texas, USA.

Algae Culture

- Tamaru, C.S., W.J. FitzGerald, Jr., and V. Sato. 1993. *Hatchery Manual for the Artificial Propagation of Striped Mullet (*Mugil cephalus L.*)*. C. Carlstrom-Trick (ed.) Guam Aquaculture Development and Training Center. Department of Commerce, Tamuning, Guam.
- Wyban, J.A. and J.N. Sweeney. 1991. *The Oceanic Institute Shrimp Manual: Intensive Shrimp Production Technology*. The Oceanic Institute, Honolulu, HI, USA.

Plankton Culture

- Hoff, R.H. and T.W. Snell. 1993. *Plankton Culture Manual, 3rd edition*. Florida Aqua Farms, Inc., Dade City, Florida, USA.

Appendix D: Credits

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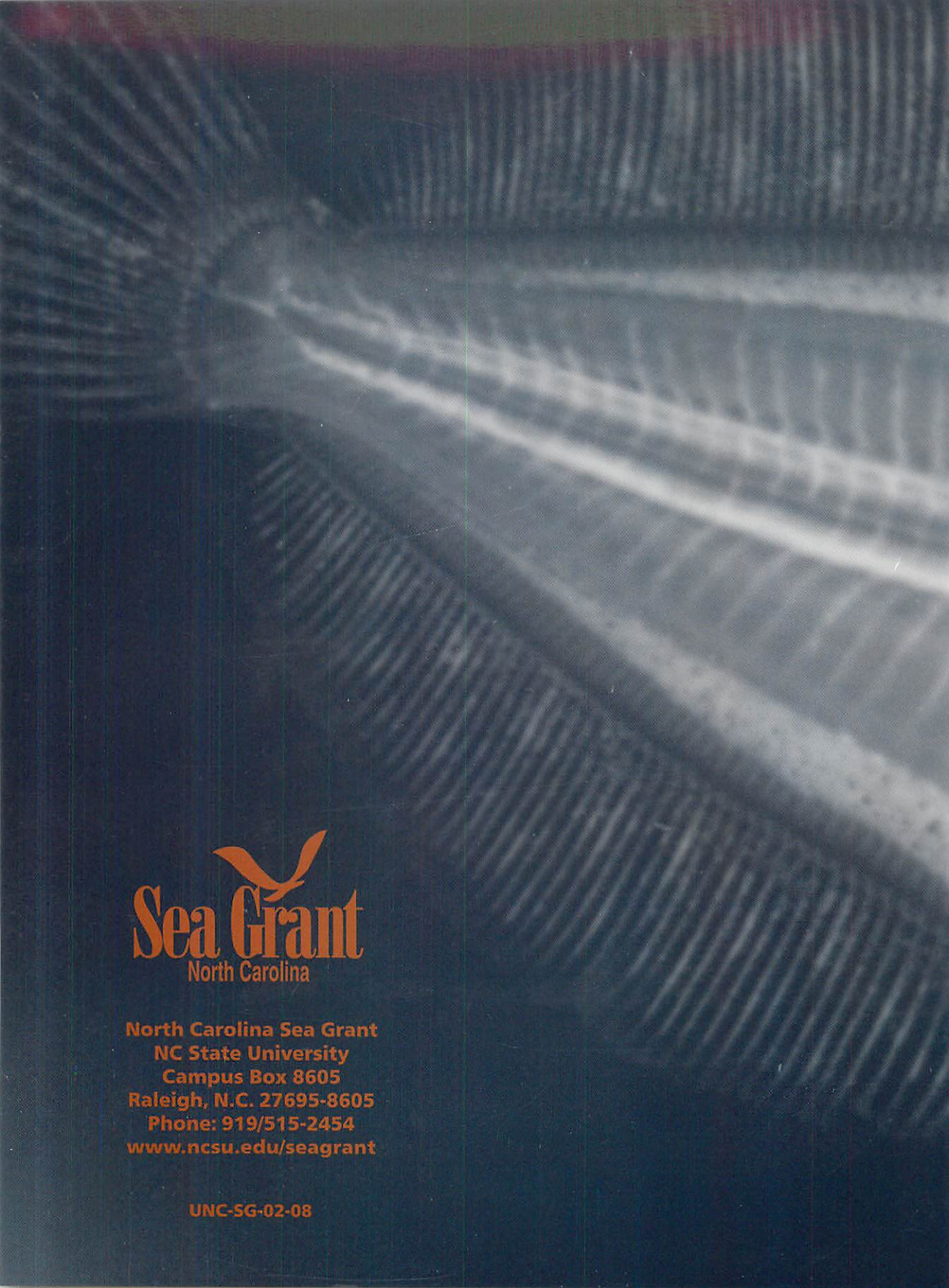

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