Pacific Threadfin,
*Polydactylus sexfilis* (Moi),
Hatchery Manual

2nd Edition

By

Chatham K. Callan, Charles W. Laidley,
Anthony C. Ostrowski & Augustin Molnar

Center for Tropical and Subtropical Aquaculture
Publication Number 159
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Photograph of wild-caught juvenile Pacific threadfin juveniles being transferred to OI facilities for use as future broodstock.
Preface

Pacific threadfin, *Polydactylus sexfilis*, known in Hawaii by its Polynesian name “moi,” has emerged as a premier aquaculture candidate for Hawaii and the Pacific. These fast growing, near shore inhabitants are among the most amenable marine finfish species to raise in controlled environments. This species is adaptable to a variety of culture conditions, with high survival rates and efficient feed utilization on practical, pelleted feeds. Pacific threadfin also exhibit exceptional market characteristics essential for commercial success. This fish has an excellent flesh quality, and is in demand in Asian and Hawaiian markets. Pacific threadfin are also sought in nontraditional markets such as the U.S. mainland and Europe.

Commercial scale production of Pacific threadfin is technically feasible, but as with any cultured species, economic viability relies on efficient operations and skilled personnel. The hatchery is the base of the production operation, providing healthy high-quality seed on a reliable and consistent basis. This ensures that growout operations are not compromised and have the best chance to attain economic success. Emphasis must be placed on appropriate facility design and trained personnel.

This hatchery manual provides a detailed step-by-step methodology for commercial scale production of threadfin through the hatchery phase, based on techniques developed over the past 20 years at the Oceanic Institute. It is based largely on the original Pacific Threadfin Hatchery Manual (Ostrowski & Molnar, 1998) but contains many revised protocols and updated, compiled data from the time since that manual was released. It is presented in the same concise, technical format intended for use by practicing fish culturists and farmers and is designed for quick reference to troubleshoot problems when they arise. It is not intended as a review of biology or culture techniques.

Following the procedures included, the Pacific threadfin culturist should be able to duplicate the successes that have been accomplished at OI.

This manual is divided into six chapters, Chapter 1 is the introduction. It presents a brief overview of Pacific threadfin, how interest in modern day aquaculture evolved and the intent of the manual. A brief ecological treatise is also given, including some systematic, differential distribution and reproductive observations from the wild and edible qualities of the species.

Chapter 2 covers components essential for the establishment of a long-term holding and breeding program for Pacific threadfin. Proven methods for capture, transport, and quarantine of animals from the wild are outlined. Facilities design, water quality parameters, and general care, selection and stocking procedures are described. Techniques for maturation, spawning, and egg collection are presented.
Chapter 3 describes the techniques for producing live feeds required for marine larvae during the early portions of hatchery rearing. In this chapter, the methods for production of rotifers and *Artemia* are discussed. Additionally, the utilization of commercially available algal paste products as a replacement for live algae in rotifer production and enrichment are covered.

Chapter 4 describes the larval rearing stage, from egg stocking through metamorphosis and harvest. Facility descriptions are given as are techniques to estimate egg stocking and hatch rates. Larval feeding behavior and the introduction of live feeds, including background algae, rotifers, and *Artemia* is described. It is assumed that the reader is familiar with general methods of live feed mass culture (discussed in Chapter 3). In addition, a detailed description on the use of practical, pelleted feeds in the hatchery is described. This chapter also includes all pertinent water quality characteristics essential to Pacific threadfin larval rearing. Chapter 4 ends with methods to harvest fish and estimate survival as a means to determine the success of the run.

Chapter 5 describes the Pacific threadfin nursery. Facilities design and optimum stocking and feeding rates for rapid growth is presented. The chapter ends with a description of harvest and transport methods.

Chapter 6 presents diseases and pathologies encountered in the Pacific threadfin hatchery. It describes diagnostic and treatment methods for both infectious and noninfectious diseases. Also described are conditions which are not necessarily health-threatening, but are important in overall production strategies. Emphasized is implementation of preventative measures, the best way in which to maintain the health of animals.

References and an appendix listing standardized data sheets are provided at the end of the manual. These data sheets can be used in conjunction with each chapter to customize hatchery operations at individual facilities.

It is understood that the technologies presented in this manual are not the only ones that can be used for Pacific threadfin, but are proven, reliable techniques for consistent production essential for a commercial operation. This manual has been modified and improved throughout its development to the present date. Publication at this time represents a point in the evolution of Pacific threadfin knowledge and aquaculture. Future improvements in culture techniques are imminent as commercial production expands. It is hoped that this manual will provide a model upon which modifications and adaptations can be implemented to optimize hatchery production at a particular site or facility.

The views expressed in this publication are strictly those of the authors and do not reflect the views of the Center for Tropical and Subtropical Aquaculture or the U.S. Department of Agriculture or any staff of those agencies.
Photograph of Oceanic Institute - Waimanalo, Hawaii from above
The goal of successful culture – healthy market-size moi, ready for harvest.
Chapter 1 – Overview of Pacific Threadfin Culture

The Pacific threadfin is a member of the tropical and subtropical Polynemid family whose Hawaiian name “moi” means “fish of the kings” and is rapidly becoming a premier aquaculture species in Hawaii and throughout the Indo-Pacific. It is highly prized for its excellent flesh quality, fast growth, and adaptability to conditions of captive culture.

It was formerly harvested commercially but rapid declines in nearshore populations over the last 30 to 40 years have led to dramatic declines in commercial catches (Tamaru, 1997). The high popularity for both sport and food has led to considerable efforts to culture this fish for aquaculture (Ostrowski and Molnar, 1998) and stock enhancement (Friedlander and Ziemann, 2003; Ziemann, 2004) efforts. With the advent of commercial offshore cages, pressures on increasing egg supplies and elevating fingerling production capacity and efficiency are immense.

Efforts to culture this animal began in the early seventies when local researchers and state of Hawaii agencies became concerned with the rapid decline in natural stocks, and began looking to aquaculture as a tool to provide fingerlings for stock replenishment (Rao, 1977). However, it
was soon realized that moi aquaculture could provide a potential new industry for the islands that was both socially and environmentally appropriate to the region.

Figure 1.2. Moi were historically reared in nearshore fishponds (top photo) throughout the Pacific Islands. New open ocean cage technologies (bottom photo) provide a sustainable alternative to wild-fishery harvest.
Biology

The basic biology of the Pacific threadfin was originally documented in unpublished thesis work by Lowell (1971) and Kanayama (1973). They described the species as bottom-feeding, living in the near-shore environment and utilizing primarily fish and crustaceans to meet its dietary needs. Subsequent work by May (1979a,b) demonstrated that the species could be successfully spawned in captivity and that small numbers of larvae could be reared to juvenile stages. However, substantial improvements in hatchery technology would be required to support requirements for stock enhancement or farming. Further evaluations of potential feeds, the culture environment, and tolerance limits for salinity and dissolved oxygen were published by Szyper et al. (1991).

The family Polynemidae is distributed throughout the tropical Atlantic Ocean and Indo-Pacific areas of the world. There are numerous species in the Indo-Pacific, but only one species is found in Hawaiian waters, Polydactylus sexfilis. The body of P. sexfilis is deep and compressed like other members in the family. The head is moderately conic in shape. The eyes are large and covered with a well developed adipose eyelid, similar those of mullets. The pectoral fins have six filaments. The Latin name sexfilis is derived from this anatomical feature. The body color is a dull silver dorsally, shading to white on the belly. Young Pacific threadfin, two to four inches in length, have distinctively different color patterns. Three broad vertical bands of dark brown are located above the lateral line. The area between these bands is light gray, as is the rest of the body.

Ecology

Adults typically inhabit turbid waters and are usually found in large schools in sandy holes along rocky shores and high energy surf zones. Spawning occurs inshore and eggs hatch offshore. Larvae are pelagic but after metamorphosis enter nearshore habitats, including surf zones, reef and stream entrances. Juveniles and adults are benthic feeders, preferring largely penaeid and caridean shrimps. They feed throughout the day.

Reproduction

Spawning behavior has been observed in the wild from June to September. Eggs are small and average 0.75mm in diameter with a large oil globule. The Pacific threadfin is a protandrous hermaphrodite. It matures first as a male at a fork length of about 20 to 29cm, and then transforms into a female at 30 to 40cm following a hermaphroditic stage.
Market Popularity

Found in many high-end restaurants, Pacific threadfin is highly regarded for its flesh quality and commands premium prices in wholesale and retail markets. Pacific threadfin is a tender, moist, white-fleshed fish typically served grilled, steamed, poached, baked, or smoked. Pacific threadfin are marketed in Hawaii, the continental U.S., Asia and Europe through seafood distributors located in Hawaii. This fish is sold whole or whole-gutted in three sizes: 8-12oz, 1-1.5 lb, and 1.5-2 lb (in limited quantities). This popular and versatile fish has a shelf-life of 14 days, and can be served in many different ways.

Culture Milestones

Researchers at the Oceanic Institute began working on Pacific threadfin in the mid 1990’s under initial financial support from the Center for Tropical and Subtropical Aquaculture (CTSA). In less than one decade, the Institute was successful in taking the existing fledgling research and converting it into a commercially viable technology that has the potential to diversify the Hawaiian economy and create valuable new economic opportunities in the islands (Ostrowski et al., 1996; Ostrowski and Molnar, 1998). Some of the specific milestones over this period include:

1. The establishment of broodstock populations generating a year-round (although seasonal) supply of viable eggs.

2. Development of live feeds and larviculture protocols for large-scale captive production of threadfin postlarvae.

3. Establishment of low-water, high-flow nursery methods to improve survival and reduce cannibalism during nursery phases of operation.


5. On-site farmer training program (sponsored through the DLNR) to ensure hands-on training in support of technology transfer.

6. Production and distribution of approximately two million eggs and 200,000 fingerlings per year to assist initial commercial startups on Oahu, Maui, Molokai, and Hawaii.

7. Initial testing and demonstration of submersible offshore cage systems for large-scale growout of moi to market size (joint Sea Grant funded collaborative effort between OI, UH and Cates International, Inc.; Ostrowski and Helsley, 2003).
8. Startup of commercial demonstration hatchery facility in November 2003, producing nearly two million moi fingerlings to date for offshore and onshore growout in Hawaii.

9. Development of the first genetically-select line of Pacific threadfin with 25% increase in growth performance compared to non-selected controls (Laidley and Liu, 2005).

The development of captive culture technology for generation of fingerlings in combination with the successful demonstration of commercial scale growout in submersible cages, led to the June 2001 startup of the first commercial offshore cage culture in the United States, Cates International, Inc. The emergence of offshore cage culture technology has also created an unexpected demand for large numbers of hatchery reared moi fingerlings to meet stocking requirements of 100,000 to 200,000 fingerlings per 2600m$^3$ cage.
Figure 1.3. Photograph of divers tending to an off-shore cage of Pacific threadfin fingerlings.
Chapter 2 - Broodstock

Figure 2.1. Photograph of Pacific threadfin broodstock in holding tank.

Biology of Broodfish

Pacific threadfin are protandrous hermaphrodites, maturing as males at age 5-7 months and changing to females as early as age 1.5 years. Individual fish can possess both sperm and developing eggs simultaneously as they change sex. This species is not sexually dimorphic. Pacific threadfin spawn naturally in captivity approximately once a month for 3-6 consecutive days. Although this species will spawn year-round, peak spawning occurs in the summer months (April-October).

Acquisition

Due to heavy fishing pressure, adult moi are typically hard to collect. However, juveniles and young adult males are commonly found in sandy-bottom near shore waters and are captured by surrounding with a seine net (0.5” eye). There may be restrictions on the number, size, acceptable equipment, and/or season for collecting Pacific threadfin. Prior to collection, be
certain that all local regulations & requirements are followed. Alternatively, Pacific threadfin can be raised from eggs produced in captivity. Domesticated stocks can be selected for desirable characteristics, such as more rapid growth (Laidley and Liu, 2005), however the inclusion of these stocks in offshore growout environments is cautioned.

**Transport**

**Handling**

Moi are sensitive to handling, with repeated handling often leading to abrasions and infections. Therefore, gentle handling is key to a successful transport. Examples of gentle handling are to minimize the use of nets, keep the fish in water as long as possible and have no drastic changes in salinity or temperature. The following are used in transport of moi:

- **Nets**
  - Nylon nets (0.5 cm mesh) are suitable for quick transfers between tanks. Use smooth and soft nets to help prevent injuries.

- **Bags**
  - Plastic bags (preferably opaque) are better for slightly longer transfers. Place up to four fish in a bag with enough water to cover their opercula.

- **Buckets**
  - Use 5-gallon buckets to transfer juvenile fish captured by near shore seining. Provide aeration if fish are not immediately transferred to a larger tank.

- **Tanks**
  - Transport fish long distances in round, plastic tanks. Tanks should have covers that prevent fish from jumping out, but allow ventilation.

**Transport Conditions**

Water in the transport container should be the same salinity and temperature as the capture site. Avoid an instantaneous change in temperature of more than 2°C. Ice can be used in hot weather to maintain the temperature. Water conditioner such as *Stress Coat™* can be added to provide an artificial slime coat that reduces the chance of infection. Provide oxygen with a compressed oxygen bottle, regulator, and diffuser. The oxygen level should be kept above 5 mg/L.
Figure 2.2. Photographs of transporting Pacific threadfin juveniles to OI holding facilities.
Quarantine

Newly acquired fish should be quarantined from existing stocks to prevent the possible introduction of diseases or parasites. The following protocol has been used at OI to successfully quarantine new stocks.

When brought onsite, fish are held in quarantine tanks (separate from established stocks) to minimize the possible introduction of pathogens. These areas use dedicated equipment that is not used elsewhere and personnel must adhere to strict biosecurity protocols (use of hand wash, foot baths, and equipment sterilization). Fish are maintained and carefully observed for any unusual behavior (lethargy, flashing, gasping) which may indicate possible disease. However, it should be considered likely that fish could transfer pathogens without demonstrating any external symptoms. Therefore, all staff working with or near quarantined animals must be aware of the potential for disease transmission and minimize any potential transfer through proper adherence to quarantine protocols. These include the following:

1) All original transport water must be treated (Chlorinated 200ppm, Ozonated or other) prior to disposal. **No transport water is to be dumped elsewhere on-site.**

2) Posting of appropriate signage to inform all staff of dedicated use of quarantine tanks/systems.

3) Use of labeled, dedicated equipment for these areas (nets, scrubbers, buckets etc).

4) Use of preventive barriers such as hand wash stations, footbaths and disinfection areas for equipment.

5) Following working in these areas all equipment must be rinsed with freshwater and sterilized using a chlorine solution (200ppm).

6) If staff have come in contact with the quarantine area they must rinse/shower and change clothes if necessary prior to working in any non-quarantine areas.

Although it is possible for newly acquired fish to transfer any number of pathogens or disease, our primary pathogen of concern is the marine parasite *Cryptocaryon irritans*. *Cryptocaryon irritans* is an insidious and ubiquitous external parasite that is commonly encountered in newly acquired fish, and in rare occurrences, in established collections. This parasite has a 21-28 day life cycle, alternating between a cyst, free-swimming, and attached forms. Therefore it is necessary to maintain fish in quarantine for at least four weeks to ensure no transfer of parasites to established stocks.
Typically all incoming fish receive prophylactic treatments of either hydrogen peroxide dips or hyposalinity exposure. Hyposalinity treatment is the preferred method, due to the extended life cycle of the targeted parasite. However, in some cases this method may be impractical (large numbers of large animals, or if the species is particularly stenohaline).

Quarantine Treatment Protocols

A) Hyposalinity Treatment

- Salinity is gradually decreased from 32ppt to 11ppt at a rate of 3-5ppt per day.
- Once the salinity reaches **11ppt**, the fish are held following normal husbandry protocols and carefully observed for **four weeks**.
- After this period the salinity is slowly brought back up to 32 ppt (approximately 2-4ppt per day).

B) Hydrogen peroxide (HP) treatment

- This begins by lowering the tank volume to 1/3 of its normal operating volume. Once the water has been shut off (aeration remains ON) and is no longer leaving the tank (overflowing standpipe), HP is added directly to the tank at a concentration of 100 ppm.
- The fish are bathed in the HP solution for 30 minutes. During this 30 minute treatment period, the fish should be checked on every 10-15 minutes.
- Following the treatment period, the water is flushed from the tank as quickly as possible using maximum water flow. The tank is flushed for at least **two hours**, ideally into an isolated dispersal system.
- Following flushing, the tank is brought back up to normal volume. The effluent may then be re-directed to communal dispersal system.
- The treatment is repeated three times a week for four weeks.
Figure 2.3. Photograph of 25m³ broodstock holding tank.

**Tank Configuration**

**Dimensions**

A round tank 6 m in diameter and 1 m deep with a water level of 80 cm is recommended (Fig. 2-3). Seawater enters through a submerged inflow manifold to create a circular current. Debris will accumulate in the center of the tank and be removed via the bottom of the center standpipe as a result of this current.

**Standpipes**

A pipe located in the center of the tank should nearly reach the water surface and have holes on the bottom, collecting water from the top and bottom. Set the water level with a standpipe outside the tank, where an egg collection system can be installed. Secure the outside standpipe with glue. Typically, a double standpipe system is used to prevent the water from draining if
either standpipe is knocked over. Tanks should be kept clean by removing any debris from the tank bottom to reduce the incidence of disease outbreaks.

Aeration

The aeration system should be sufficient to maintain dissolved oxygen levels above 5 mg/L. Increase oxygen levels by placing five air stones around the perimeter of the tank. Additionally, pinching the water inflow pipes to create a spray at the surface will increase gas exchange.

Shading

Cover broodstock tanks with a double layer of 80% shade-cloth or a roof (Fig. 2-3). Shading helps to control algal growth. This will assist in keeping the tank clean for longer intervals and also limits the growth of biofilm, which can harbor bacteria and parasites.

Egg Collection

Eggs are collected from the effluent with a 0.5 mm mesh net (dimensions 43 cm³, volume 80 L) attached to a PVC frame. The net is weighed down with a PVC ring (33 cm diameter x 5 cm height). This frame is seated in a separate tank, through which the tank effluent can flow, leaving the majority of the mesh submerged. At OI, we have modified our tanks to accommodate permanent egg collector boxes for this purpose (Fig. 2-4). Alternatively, standard 55 gallon barrels can be modified to house the egg collector nets (Fig. 2-5).

In the later set-up, the height of the pipe leading to the barrel determines the water level in the tank. The barrel has its own outside standpipe, so eggs remain in enough water to avoid being damaged. The height of the barrel should not be less than 54 cm, to provide enough room for the net to fully expand and not be sucked into the drain. In all cases, the more gentle the flow into the egg collector the better. Avoid large drops (>6") in water height and minimize turbulence within the net as much as possible.
Figure 2.4. Photographs of new (left) and old (right) style egg collecting tanks.

Figure 2.5. Photographs of egg collector net and net in place under tank effluent.
**Water Quality**

**Water Source**

Seawater from onshore wells or directly from the sea should be filtered before use to reduce contamination.

**Water Exchange and Loading Rate**

Water flow should be sufficient enough to produce six turnovers daily at a loading rate of 0.24 kg fish/Lpm of seawater. This level is sufficient to keep the fish healthy, tanks clean, and allow egg collection with little turbulence.

**Salinity**

Pacific threadfin tolerate a wide range of salinities. For example, a five minute freshwater dip directly from and back to seawater is a treatment used against parasites. Pacific threadfin can survive for extended periods in brackish water (10-20 ppt). However, salinity between 33 and 36 ppt most closely approximates their natural habitat and enables the eggs to remain buoyant.

**Temperature**

Pacific threadfin have spawned in temperatures ranging from 24-30°C. At OI, the water temperature remains at 26-27°C year-round, which has facilitated continuous spawning in this species.

**Dissolved oxygen**

The aeration system and water flow should be sufficient to maintain dissolved oxygen (DO) levels above 5 mg/L. Pacific threadfin can tolerate DO levels to 4 mg/L with no apparent ill effect, but broodstock should not be subjected to any unnecessary stress. Monitor DO levels if water quality is poor or loading rate excessive.

**Additional Factors or Variables**

Ammonia, nitrate, and nitrite levels do not need to be monitored if the previously mentioned recommendations are followed and the water source is of good quality.
Feed Regimen

Schedule

Pacific threadfin are fed once daily to satiation. It is better to underfeed than overfeed, as decaying feed fouls the water quality and makes disease outbreaks more likely.

Diet

The nutritional requirements of broodstock Pacific threadfin have not been thoroughly researched. Broodstock have been fed raw and pelleted diets. Whole (chopped) squid, shrimp, and smelt are fed on alternate days. However, raw food can be a source of contamination, and great care must be taken in the handling and processing of these feeds. Prepared diets offer additional biosecurity, but typically don’t support optimum spawning performance. OI recently developed a modified broodstock diet for moi consisting of raw shrimp (10%), squid (49%), smelt (40%) and a vitamin mix (1%). These ingredients are blended together and then pushed into sausage casing. After being frozen, the “sausages” can then be cut to the desired size for feeding (Fig. 2-6). This method allows for the delivery of additional vitamins or other nutrients that could be lacking in raw/frozen feeds. Additionally, Moore-Clark produces a 55% protein pelleted diet that Pacific threadfin will consume, but long-term effects on health and reproduction have not been examined.

Amount

On a daily basis, broodstock eat approximately 3-6% and 0.6-0.9% of their body weight on raw and pelleted diets, respectively. The calculated dry matter intake of raw food (0.75-1.5%), which contains approximately 75% water, is slightly higher than the dry matter intake of pelleted food.
Cleaning

Maintaining a clean tank promotes population health, and reduces the occurrence of parasite outbreaks. Methods include scrubbing, siphoning, or (only when necessary) transferring the fish to clean tanks. Tank walls, bottoms, pipes, airlines, egg collectors, etc., should be scrubbed with a scouring pad once every two weeks. During the cleaning, fish are not removed from the tank. The procedure is outlined as follows:

- Drain tanks to 50% of normal volume (40 cm) and clean.
- Following cleaning, increase water flow to remove debris quickly. Stop scrubbing if the water becomes turbid and wait for water to clear.
- Rinse all equipment used with freshwater and allow to dry.
- Siphon any debris remaining in the tank after scrubbing and flushing.
Figure 2.7. Photographs of evaluation procedures of Pacific threadfin broodstock in holding tank. Clockwise (from top left) corralling fish prior to handling, getting weights, anesthetizing fish and implanting PIT tag, capturing fish in plastic bag, transporting fish in bag with water, expressing gametes for sex ID.
Examination Protocol

Capture

Prior to capture, the water in the tank should be lowered to approximately 1/3 normal volume. Pacific threadfin are then corralled with a barrier (Fig. 2-7). The barrier consists of interlocked square (32” x 32”) panels made with PVC pipe and covered with mesh (1/4” eye) netting. Holes are drilled in the frame, so it will sink in the water. Capture fish from the barrier by hand or with a plastic bag and transfer them quickly to the anesthesia tank.

Anesthesia

A 300-L tank is recommended for examining Pacific threadfin. The anesthesia tank can be set up directly inside the broodstock tank. Fill tank with 150-L seawater and place an airstone in the tank bottom. Pacific threadfin can be anesthetized with 90 ppm of tricaine (MS-222™). Add Stress Coat™ to the water to reduce the risk of infection from handling, stress, or injuries.

Safety of use and disposal of MS 222

1) Wear protective clothing, gloves and goggles when handling the MS-222 powder.

2) If possible, work inside a fume hood to prepare a concentrated stock solution by mixing an appropriate amount of MS-222 powder in a small volume of water.

3) Dilute the stock solution further as required.

4) Wear gloves and use a utensil to stir until all powder is dissolved.

5) Wear gloves to handle animals exposed to MS-222 if particularly sensitive to this chemical.

6) Dispose of MS-222 wastes by flushing down the drain to a sanitary sewer with an excess of water.

7) If in a remote location where a sewer may not be readily available, further dilute the solution with water and dump wastes on land in a location away from water.

8) Do not discard MS-222 directly into surface water, storm water conveyances, or catch basins.
Figure 2.8. Photographs of Pacific threadfin broodstock evaluation and equipment. From top left (clockwise), measuring fish length, evaluation of gills and check for goiter, PIT tag implanter, PIT tag reader, coded PIT tags for fish ID, implanting the PIT tag in anesthetized fish.
Condition Appraisal

After 1-2 minutes in anesthesia, fish lose equilibrium and can be easily handled. The following characteristics are investigated (Fig. 2-8):

- Examine fish for physical abnormalities, such as blindness, scoliosis, missing opercula, lesions, or growths.

- Look for macroscopic parasites attached to gills, eyes, or skin. With a spatula, scrape gill filaments and skin of five fish from each tank.
  - Place scrapings on a slide, add a drop of seawater, smear with a cover slip, and examine at 100 X with a compound microscope.

- Measure weight and fork-length at least annually to assess condition, as well as monitor loading and feeding rates.

- Fish can be tagged at this time with PIT tags for subsequent identification. Tags are implanted using a syringe applicator in the dorsal musculature.
  - Tags should be read using a PIT tag reader after implanting to ensure they are in place.

- Sex determination can be made by gently pressing on the abdomen to express sperm/eggs, or by canulation.

- The condition factor index (CFI) is a measure of the health of the individual based on the correlation between weight and length.
  - CFI equals 100 * wt ÷ (lth³), where wt is the weight of the fish in grams and lth is the fork length in centimeters.
  - Ideal broodstock candidates have a CFI above 2.10. CFI typically decreases during the spawning season.

Spawning

Reproductive Season

Captive Pacific threadfin have spawned naturally in every month of the year, with the highest frequency from April through October. The longer the fish are held in captivity, the broader the natural spawning season becomes.
Spawning Frequency & Lunar Periodicity

Pacific threadfin typically spawn for 3-6 consecutive days. Most fish spawn predictably near the third lunar quarter, 8-10 days post full moon (Fig. 2-9). Other groups have been unpredictable, spawning in any lunar quarter, with intervals between spawning series ranging from one week to two months.

![Graph showing spawning frequency and lunar periodicity](image)

**Figure 2.9.** Pacific threadfin spawning typically peaks 8-10 days after the full moon.

Production Numbers

Group spawning produces an average of 70,000 eggs per kg of female (min. 100; max. 400,000). A tank stocked with 30 fish (20 females, averaging 1.5 kg) can produce 78 million fertilized eggs per season, at an average of 1.5 million fertilized eggs each spawn (Fig. 2-10).
Spawn Time

Pacific threadfin spawn during the night between 6 p.m. - 12 a.m. This remains constant and does not appear to be affected by locating the tank indoors or outdoors. By dawn, most fertilized eggs have reached the embryo stage.

Figure 2.10. Total egg production from three tanks (~100 fish total) of moi broodstock over 2009-2010.
Egg Evaluation

Egg Biology

Spawned eggs are 0.75-0.80mm in diameter and have a single oil droplet. Viable eggs are transparent and buoyant in salinities greater than 33 ppt. At 26°C, larvae hatch about 19 hours after spawning or around 3 to 5p.m. Examine egg development microscopically at magnifications of 40 – 100 X. Spawning and hatch times can be estimated from the development series in Figure 2-12.

Figure 2.11. Photomicrograph of Pacific threadfin eggs shortly after being collected on the morning following spawning.
Figure 2.12 (a) Photomicrographs of Pacific threadfin eggs undergoing embryonic development.
Figure 2.12(b) Photomicrographs of Pacific threadfin eggs undergoing embryonic development.
Preliminary Examination

Microscopically examine a subsample of eggs the morning following a spawn. Spawning is a group event; many females and males may spawn simultaneously. A low fertilization rate (percentage of eggs fertilized) may not reflect the quality of the fertilized eggs. Instead, appearance and uniformity of egg development are the criteria used to decide if the eggs should be collected.

Transfer from collector

Fill a 5-gallon bucket with 3-L of seawater. The net can be raised to concentrate the eggs while allowing them to remain submerged. Using a container with smooth edges, skim concentrated eggs from the water into the bucket. The remaining eggs can be transferred by inverting the net and gently rinsing into the bucket.

Quantifying

Step 1: Total count:

Fill the bucket containing eggs to 15 L with seawater. Place an air stone in the bucket to gently mix the eggs. Remove five 10 mL samples (or samples of at least 100 eggs), place on a screen and count (Fig 4-10). Estimate the density in units of eggs per milliliter. The density equals the number of eggs counted, divided by five (number of samples), and divided by ten (sample size in milliliters).

Step 2: Separation of viable eggs

Raise the salinity in 2 ppt increments until the eggs float or the salinity reaches 36 ppt. One gram of salt added to a liter of water will raise the salinity 1 ppt. Dissolve salt in a small amount of seawater, add to the bucket, and mix well. Remove the air stone and wait for the viable eggs to float to the surface. Dead and unfertilized eggs will sink at this salinity. Skim viable eggs from water surface into another 5-gallon bucket containing 3-L seawater. Repeat the counting procedure in Step 1.

Qualifying

Examine a subsample microscopically to measure the fertilization rate (Fig. 2-13). Count the number of fertilized eggs in a 100-egg sample. If the fertilization rate is less than 70%, remove the air stone to see if dead eggs sink and can be removed by siphoning. Otherwise, consider discarding the spawn as the dead eggs may foul the water quality in the larval tank.
Microscope egg assessment

Place (six) 1ml samples of eggs in a multi-well counting tray. Using a hand counter, tally the number of unfertilized, fertilized but not viable (eggs have died in development), and viable eggs.

**Total # of eggs** = (the total count ÷ total sample volume) X volume of container holding the eggs

**Viability Rate (%)** = # of viable eggs in count ÷ total eggs counted in sample

**Fertilization Rate (%)** = (# of fertile eggs in count + # of viable eggs in count) ÷ total eggs counted in sample

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**Figure 2.13.** Evaluation of Pacific threadfin eggs utilizing a dissecting microscope is essential in determination of egg quality.
Figure 2.14. Using a multi-well counting tray will aid in examination of egg quality and allows for multiple samples to be processed in one observation.
Figure 2.15. Evaluation of Pacific threadfin eggs utilizing a computer paired with a microscope can allow for very precise measurements of egg characteristics.
Chapter 3 - Production of live feeds

Figure 3.1. Photomicrograph of Rotifer (left) and Artemia nauplii (right).

Overview

The consistent production of live feeds can be one of the most challenging aspects of marine fish hatchery operations. Most marine fish species require live prey at first feeding, as they transition from endogenous yolk reserves to exogenous feeding. Marine rotifers (Brachionus plicatilis & Brachionus rotundiformis) and brine shrimp nauplii (Artemia) are commonly used at first feeding to substitute for the larvae’s natural prey items. Though significant advances have been made with regard to the culture and nutritional manipulation of these live food items (benefiting larval growth and survival), and some formulated alternatives are commercially available, a suitable complete replacement for live feeds in the hatchery has yet to be established.

Rotifers

Rotifers are the first food item consumed by larval Pacific threadfin. Rotifers are widely distributed, eurohaline, and relatively simple to culture, making them the generally adopted first feed for many cultured marine fish species. Rotifers vary in size depending on strain and culture conditions. Adult sizes range from 123 to 315 µm (lorica length). The mean weight of an individual is 3 µg. The s-type (Brachionus rotundiformis) is commonly used in Hawaii. The
advantages of this specific strain are its short doubling time (24 hours), its wide tolerance ranges for temperature, salinity, and dissolved oxygen, and its small size (100 to 200 µm lorica length).

Currently we employ two methods for the mass culture of rotifers, a standard batch-culture method and a continuous production method. The batch-culture method has the advantage that cultures are maintained in multiple vessels, at various degrees of culture age, providing a back-up should one or more of the cultures crash. This method also involves the least amount of equipment. The continuous culture method has the advantage of a smaller footprint and can achieve higher rotifer densities with less labor. However, this method is more complex to set up initially, requires more equipment and closer observation for peak performance.

**Batch-culture method**

The batch culture method utilized at OI is similar to methods described for other marine hatchery operations. Culture vessels ranging from 200L to 1,000L are typically utilized as individual culture tanks, with the size and number of tanks in use being dependant on the total rotifer needs of the hatchery. Under normal operating conditions, following the protocol described within, we typically achieve rotifer densities of 1,000-1,300/ml at the conclusion of a 3-day cycle (Fig. 3-2).
Figure 3.2. Diagram of typical 3-day batch culture method for rotifer production.
Set-up

Once the appropriate size and quantity of culture tanks are identified, the batch culture process begins by preparing the culture vessels. Ideally, the rotifer production area should be separated from other parts of the hatchery to reduce contamination of the cultures and maintain hatchery hygiene. This area (Fig. 3-3) should have a supply of aeration, seawater and freshwater, a floor drain for disposal of culture water, and a sink for cleaning and rinsing equipment. Prior to initiating a culture, tanks and associated equipment should be cleaned and dried followed by good freshwater rinsing just prior to starting up.

Each tank should be marked with graduations so that the approximate volume can be determined at various water heights within the tank. Tanks should be fitted with a bottom valve for ease of draining and this valve can be subsequently connected to a hose for harvesting the rotifers. We utilize quick disconnect fittings for this purpose.

Figure 3.3. Rotifer production labs at OI. Quick disconnect fittings are used for easily moving hoses from one production tank to another and for draining rotifers into the harvest bag.
Figure 3.4. Flock mats are used to trap debris in rotifer production tanks. These mats are made with course material that needs to be weighted down to prevent floating in the production tanks.

The harvest net should be made from mesh that is 20-50µm in size. It should be made with enough surface area that rotifers can be harvested in reasonably large batches (50-100million at a time), without clogging the net. We typically utilize a net that is approximately 0.3m square X 0.3m deep (Fig. 3-3).

The culture tanks should be arranged so that they are a sufficient height off the ground to accommodate gravity draining of their contents into a harvest container that will collect the rotifers. The tanks will also need clearance above for access to the cultures, water quality measurements, and maintenance of the tanks and associated equipment.

Stocking

Prior to inoculation, the culture tank should be filled with a combination of seawater and freshwater to obtain a salinity of 20ppt. A submersible heater can be used (if necessary) to maintain the water temp at 28-30C. Airstones should be placed near the bottom of the culture vessels, and tested to ensure they provide enough air for vigorous circulation and DO levels of >5 mg/L.
To help reduce overall bacteria levels, and prevent ciliate contamination, the culture water should be chlorinated prior to stocking. We typically add 1ml of 12% Cl/L of culture water (~100ppm) and allow to stand for 30 minutes (no aeration). After the chlorination period, sodium thiosulphate can be added (.1g/ml of Cl added) and aeration restored to neutralize the chlorine. After 30 minutes, the residual chlorine level can be checked using readily available swimming pool test strips to ensure all the chlorine has been eliminated from the water.

Rotifer cultures are inoculated (Day 0) to achieve an initial density of 200 rotifers per ml of culture water. Rotifers can be counted by using a 1ml graduated, glass pipette and magnifying loop (10x). Normally three to four counts of rotifers occupying 0.1 ml volume of the pipette are averaged, and then multiplied to get an accurate estimation of the total population size. Algae paste is then added to provide food for this new population, and a flock-mat (Fig. 3-4) is suspended in the center of the tank to catch detritus.

**Feeding**

Rotifer cultures can be maintained on a number of commercially available products. We utilize a combination of Nanochloropsis (Nano) and Isochrysis (Iso) pastes by Reed Mariculture for growth and short-term enrichment of our rotifers (Fig. 3-5). Our feeding protocol is as follows: On day 0 (inoculation) the new culture is fed 0.6ml Iso + 1.25ml Nano per 1 million rotifers in culture. This feeding is repeated at the end of the day, for two total feedings per day. On days 1 & 2, cultures are fed 0.5ml Iso +1.0ml Nano per 1 million rotifers in culture, twice daily. On day 3, the culture is harvested (generally first thing in the morning) and therefore this culture is not fed.

Since the new culture is always started with the same number of rotifers, and the cultures tend to reproduce in a predictable way (nearly doubling each 24 hours), the quantity of food offered at each feeding on any given culture day tends to remain stable, and can therefore be used in planning for supply procurement.

*Figure 3.5. Commercially available algae pastes have largely replaced the need for live algae in the production of rotifers.*
Daily Maintenance

Rotifer cultures need to be monitored at least twice daily. Typically the cultures are counted in the morning to get an estimate of population size, determine daily feed requirements and estimate the harvest volume necessary for the larval feed requirements. During counts, the technician should also make observations of rotifer health including % of individuals with eggs (Fig. 3-6), speed of movement, and presence of dead rotifers and ciliates, as these can all be indicative of the overall health of the population. Temperature, salinity, and DO should all be measured in the morning and at the last feeding (late afternoon).

![Figure 3.6. Photomicrographs of rotifers under dark (left) and light (right) field views. Note the visible eggs trailing off the back of healthy female rotifers.](image)

Harvest

On day 3, the oldest cultures will be harvested, and used to feed the larval fish and to restart new cultures (Day 0). The procedure is as follows.

1) Count the culture to be harvested and calculate the volume needed to start your new culture. Turn off (unplug) heaters that are being utilized in the tank. Fill your harvest container with new, flowing seawater and place the harvest net inside.

2) Drain the first few liters of culture water into the waste-drain, as it likely contains settled food, dead rotifers, and other debris you don’t want mixed in with the collected rotifers. Then, position the drain hose to empty beneath the surface of the water, inside the harvest net.
3) Slowly begin draining the culture tank into the harvest net, being careful not to go too quickly as high velocity will quickly damage the collected rotifers. Periodically massage the collection bag (Fig. 3-7) from the outside, to free any rotifers that may be accumulating on the bag. Aeration should be used within the harvest container, along with the flowing water to help maintain DO levels at high (>6 mg/L) levels.

4) Once the volume that is necessary for the starting culture has been drained, temporarily stop the process by closing the drain valve. The harvest net can then be carefully lifted from the water and the rotifers transferred directly to their next culture tank or to a holding container while the culture tank is being prepared. If the latter applies, be sure to utilize plenty of aeration to keep the DO of the holding container above 5mg/L.

5) The remaining volume of culture can now be harvested and used for feeding of larval fish. Excess volume can be discarded, or utilized to bolster populations in other cultures if performance is lower than expected (based on previous counts).

Figure 3.7. Photograph of flock mat (left) after several days of use. The mat will need to be cleaned thoroughly and allowed to dry in the sun prior to re-use. The harvest bag (right) should be rinsed with clean seawater during harvest to keep the rotifers from getting stuck to the bag.
Tank cleaning

After the tank is emptied, it will need to be thoroughly cleaned prior to re-use. A degreasing agent such as “Simple Green” works well when applied with freshwater via a spray bottle to help break down and remove oil residue from the algae paste. All components of the tank (air lines, heaters, airstones, flock mats) should be cleaned with this, scrubbed free of any excess, and then rinsed with freshwater.

Flock mats will accumulate considerable amounts of algae and detritus and take several minutes to rinse clean using a high pressure hose. Once clean, these can be hung in the sunshine for 1-2 days for further drying out. Airstones should be replaced with each new culture and can be soaked in a (200ppm) chlorine solution for 24-48h to get thoroughly clean.

Once the culture tank and associated equipment is clean, it is best if it can be left to dry for 24h prior to being utilized as a new culture tank. Therefore, it is advisable to have a few extra culture tanks available so that they might be rotated through this “dry” period.

Enrichment

Harvested rotifers should be transferred to a clean tank at a density of \( \leq 1,500 \text{ per ml} \) (1,000/ml makes calculations easy) for enrichment prior to feeding to larval fish. Vigorous aeration should be utilized to keep DO >5 mg/L. There are a wide variety of commercial enrichment products available for this purpose; however we have found that the same algae paste used for rotifer rearing works exceptionally well as enrichment.

Add 0.5ml/million rotifers each of Iso and Nanno paste (total of 1ml paste/million rotifers). Allow rotifers to remain in this enrichment for at least two hours prior to feeding. After two hours, re-count the rotifer population and determine the amount of volume required to harvest to satisfy the morning’s first larval feed requirements.

Harvest of the enrichment tank utilizes the same procedure as harvesting the culture tank. Condensed rotifers can then be fed (volumetrically) to the rearing tank. The remaining rotifers can continue to remain in the enrichment for the duration of the day, and will be utilized for the later feeding. This allows a portion of the rotifers to get a longer enrichment period, increasing the amount of important highly unsaturated fatty acids (HUFA) to be delivered to the larvae. The enrichment tank should be cleaned daily as described for the culture tanks.
Continuous culture system

In order to intensify rotifer production, and reduce daily labor requirements, a higher density, continuous rotifer production systems can be utilized. There are commercial systems available for purchase (Aquatic Ecosystems Inc, Apopka, Florida), however we have developed a simplified, flow-through design that has proven efficient and reliable.

![Continuous culture system](image)

**Figure 3.8.** Photo of continuous culture system for rotifers. The primary culture tank (white) houses the reproductive culture and continuously overflows into the smaller (black) tank, which serves to collect the rotifers. The smaller tank is harvested daily to feed the fish in the hatchery.

**Set up**

The main components of the continuous production system are the main culture tank, a collection tank for concentrating the harvested rotifers (Fig. 3-8), a source of feed, feed delivery pump, and source of new water (Fig. 3-9).

We utilize a 500L culture tank and 200L collection tank as the two main vessels. Aeration is supplied to both tanks to maintain DO > 5mg/L. Seawater (31ppt) is not diluted, as with the batch method, since 50% of the tank volume will be exchanged daily. The tank is filled with seawater
and a new culture of rotifers is added to a density of **2,000 per ml**. This allows for a standing crop of rotifers in the production tank to be ~ 1 Billion.

A flock mat should be utilized as with the batch culture method, and cleaned regularly (daily). A submersible heater can be used to maintain a temperature of 28-30°C.

Water flow into the production tank should be set to a rate = to ~50% of the water volume daily. The excess water and rotifers will leave the production tank at this rate, filling the collection tank slowly over 24h. In the morning, the collection tank can be harvested and enriched as described above.

![Figure 3.9](image1.png)

**Figure 3.9.** Important components of the continuous production system are fine control valves to regulate water flow, a peristaltic pump for dosing algae paste, graduated measuring stick for estimating volume, and an insulated container with ice for maintaining the algae paste feed.
Feeding

Rotifers should be cultured on a mixture of Iso and Nano algae pastes totaling 1ml per million rotifers per day (0.7ml Nano paste /million rotifers /day + 0.3ml Iso paste per million rotifers per day). Feed approximately 40% of the total feed amount [Iso paste (0.1ml/million) and Nano paste (0.3ml/ml)] directly to the production tank once daily. The remainder (60%) of the feed (Iso paste @0.2ml/million; Nano paste @ 0.4ml/million) should be diluted into several liters of seawater. This diluted solution will be dispensed to the rearing tank via a peristaltic pump, set to deliver the entire contents of the solution over a 24 h period.

Our 500L test system utilized ~1 liter of algae paste (700ml Nano, 300ml Iso) per day. 600ml of the algae paste was diluted into 4.5L of seawater. This solution was kept cool by placing it in a cooler with ice, and was mixed using aeration. We used a 14-rpm peristaltic pump (Mityflex from Aquatic Ecosystems Inc.) that utilized 1.6mm ID tubing to deliver ~3ml of feed solution per min. At this flow rate, the entire contents of the feed solution were dispensed in 24 hours.

Using the above 500L system, a 50% exchange rate, and a starting population of 2,000 rotifers/ml, we routinely harvested **200-300 million rotifers per day** from the 200L harvest tank.
It is likely that production could have been increased further with additional food, water exchange, and the use of oxygen. However, 200-300 million rotifers/day was enough to support ~8m³ of larval rearing tanks (producing ~80,000 fingerlings at day 25).

**Operation**

While this system does achieve labor savings by reducing the number of culture tanks in use, it needs to be monitored closely for peak performance. Daily checks should include: rotifer population counts in production tank and harvest tanks, monitoring of the water flow rate into the production tank, monitoring of the feed delivery rate, routine checks of water quality parameters (DO, Temp, NH₃), regular (daily) cleaning of the flock mat(s).

We have operated the system continuously for over ten days, however it is recommended that the production tank be completely harvested, cleaned and re-started every 5-6 days to maintain system hygiene.

**Common problems**

- Due to the compression of the nylon tubing during operation, the feed line will periodically pinch in the peristaltic pump. This slows the delivery of feed to the culture and can limit rotifer population growth.
- Slowing of water exchange rate due to water use elsewhere (if on shared system) or if water supply line gets air-locked.
- Low DO due to clogging airstones, or accumulation of feed on bottom of tank.
Culture of *Artemia*

*Artemia* (Fig. 3-11) brine shrimp inhabit harsh and diverse environments in temperate zones. They are tolerant to a wide range of salinity, temperature, and dissolved oxygen levels. Their life cycle includes a wintering egg stage called a cyst, which is a dormant blastula protected in a chitinous shell chorion. Cysts can germinate after more than 100 years. Application of *Artemia* in aquaculture utilizes this dormant stage. Incubated cysts hatch within 18-36 h. Incubation time varies by strain, temperature, salinity, and light intensity, while hatching size varies by strain. In general, *Artemia* hatch at about 600 µm in length and 180 µm in width as non-feeding instar I nauplii with yolk reserves. Within a few hours (6 h at 26°C) they molt into obligate filter feeding install II metanauplii at 700 µm in length and 200 µm in width, and feed on suspended particulate matter less than 50 µm.

**Figure 3.11.** *Artemia* cysts are widely available and have a long shelf-life if properly stored (left). The resulting newly hatched nauplii and later instar stages (right) are useful feeds for rearing marine fish larvae.
Hatching

High quality *Artemia* cysts will typically yield **200,000-300,000 nauplii per gram**. The steps utilized for hatching of *Artemia* cysts are as follows:

1) **Hydration:**

Cysts should be hydrated in freshwater, and disinfected prior to hatching. Cysts can be placed in freshwater (up to 100g/L) and aerated vigorously to be kept in suspension. The dehydrated cysts will change shape and become spherical once they have fully hydrated (~1hr). At this point, chlorine can be added directly to the vessel for cyst disinfection and partial de-capsulation. Our typical procedure is to empty one can of cysts (450g) into ~8L of freshwater, hydrate for one hr.

2) **Disinfection (Chlorination):**

For 450g of cysts in 8L of water, add 500ml of 12% Chlorine, treat for approximately 5-8 minutes (cysts will change color from brown to grey to tan). Once the majority of the cysts are a tan color (or eight minutes has elapsed), we stop the chlorination by rinsing the cysts through a 100 micron mesh bag. Sodium thiosulfate can also be used to immediately neutralize the chlorine. Note: this procedure does not completely de-capsulate the cysts, but does partially remove the outer shell layer and disinfects the cyst.

3) **Rinsing & Tank Set-up:**

After rinsing with freshwater for at least five minutes, the cysts can be transferred to a hatching tank (1g cysts/L volume of hatching tank). The salinity of the hatching tank should be 25-35ppt; temp =27-29°C; pH = 8.0-8.3.

4) **Hatching:**

Vigorous aeration should be used to keep the cysts suspended and DO > 5mg/L. Bright overhead lighting should be used to stimulate hatch. Cysts placed in the hatching tank in mid afternoon will be hatched by early the next morning.
Figure 3.12. *Artemia* production lab at OI. Various size tanks are utilized for hatching and enriching *Artemia* based on the quantity required in the hatchery.
Harvesting

Hatched nauplii can be estimated by taking a 1ml sample directly from the hatching tank and counting with both a glass pipette and a loop magnifier (10x). The nauplii can be harvested by draining the contents of the hatching vessel through a 100 micron bag to condense and collect the nauplii. The procedure utilized for harvesting is as follows (Fig. 3-13):

1) Prior to draining, the overhead light and aeration should be turned off, and the tank should be allowed to settle for about 15 minutes. The surface of the tank should be covered (to darken the tank) and a portable light can be used to illuminate a small area near the drain opening to attract the nauplii towards the drain.

2) A harvest container supplied with flowing seawater and aeration should hold the harvest net in position to collect the nauplii (similar to as described for the rotifers). Unhatched cysts may settle to the bottom, but all shells should float to the surface.

3) The contents of the tank should be slowly drained through the harvest net. Slow draining is the key to clean separation of nauplii from egg shells. Most (70-90%) of the nauplii will be collected in the first 20-30 minutes of harvest, as they will actively swim towards the drain (light). A small glass beaker can be utilized to visually inspect the water entering the collection net.

4) Once significant numbers of nauplii decrease and/or the presence of cysts or shells are detected, the harvest should be suspended. The harvested nauplii can then be transferred to a secondary holding container for estimation of number or for subsequent enrichment.

5) Collected nauplii should be rinsed in clean seawater for several minutes to assist in the flushing of bacteria accumulated in the water during hatching.
Figure 3.13. *Artemia* harvesting procedure. From top left (clockwise): separated cyst shells at surface, light to attract nauplii to bottom drain, slow flow rate to minimize cyst harvest, harvest net with collected nauplii, rinsing the harvested nauplii, separated and clean nauplii.
Enrichment

Artemia lack many of the essential fatty acids required by marine fish larvae, and therefore should be enriched prior to being used as a feed. There are many commercially available enrichment products available for use with Artemia, and selection by the culturist will likely depend on regional availability and cost.

Supply

Enrichment emulsions containing 30% DHA are recommended for Pacific threadfin larval rearing. OI has used the following commercial enrichment emulsions with successful results: Algamac 2000, 3000 and 3050; Selco and Super Selco from Aquafauna Bio-Marine, Inc., P.O. Box 5, Hawthorne, CA 90250 USA.

Shelf-life

If kept cold, products can be stored up to six months unopened. Once opened, possible contamination can decrease their shelf life to 2-3 months. Spoiled enrichment can lead to large percentages of dead nauplii or total “crashed” batches. Artemia that have experienced either total or partial crashes should not be fed to larvae.
Enrichment Procedure

*Artemia* metanauplii can be enriched up to a density of **100 per ml.** The tanks must be well aerated to maintain a DO level higher than 4 ppm. Approximately 3-5% daily mortality of *Artemia* is common after handling and enrichment. The procedure is as follows:

1) Newly hatched, separated, and rinsed nauplii are placed into enrichment tanks with clean seawater and vigorous aeration. Adding enrichment emulsion to the water will decrease dissolved oxygen levels, which must be maintained above 4 ppm. **Nauplii density should not exceed 100/ml.**

2) Enrichment emulsion is measured into a kitchen blender with water and mixed for five minutes. The diluted emulsion is then added directly to the *Artemia* enrichment tanks. Typically, the enrichment is not added until the nauplii are **older than 6h post hatch,** as they are not capable of feeding until that point.

3) The *Artemia* remain in the enrichment overnight and are harvested the next morning (~12-16h later).

4) If *Artemia* are being enriched for more than 16 h, the tank is drained through a 100 µm nytex mesh bag, and the *Artemia* are replaced with new seawater and fresh emulsion. All tanks are cleaned with “simple green” and freshwater between each use; tanks are rinsed with diluted bleach (10%) between uses.

5) Collected *Artemia* should be rinsed (again) in clean seawater for several minutes to assist in the flushing of bacteria and enrichment media that accumulated in the water during the enrichment process.
Figure 3.14. Routine microscopic examination of live feeds is essential in determining their health and suitability as food for larval fish.
Figure 3.15. Successful production of live feeds will lead to full and robust larvae in the hatchery. Pictured here are moi larvae at five days post hatch.
Chapter 4 - Larval Rearing

Figure 4.1. A technician carefully siphons the bottom of a larval rearing tank. This stage of the hatchery operations is very labor intensive.

Overview

Larval rearing is the most labor intensive stage of the Pacific threadfin hatchery operations (Fig. 4-1). As with the production of other marine species, this stage of hatchery operations requires the simultaneous culture of live feed organisms used for feeding the larvae. Many of the basic techniques for rearing marine finfish are utilized in the culture of moi, however the timing of feed introduction, feeding rates, and specific management practices may vary from species to
species. Pacific threadfin eggs are hatched in the larval rearing tank where the larvae are reared for approximately 25 days and weaned onto dry feeds prior to harvest. Utilizing the techniques outlined in this chapter, survival rates of 20-30% through this period are consistently achieved.

**Larval Biology**

The larval stage is defined as hatching to metamorphosis (Bond, 1979). Two larval stages are recognized: prolarva, which retains a yolk sac, and postlarva, which has absorbed the yolk sac, but is still unlike the juvenile stage. During the larval stages, many ontogenetic changes occur (Fig.4-2). Some changes relate directly to adult form and development, while others are specialized and are presumed to have functional significance for planktonic existence.

Pacific threadfin larvae hatch (Day O) undifferentiated with a large yolk and single oil droplet. Functional eyes and mouth develop by D3, when larvae initiate feeding. A coiled mid-gut is evident at this time. Swim bladder inflation occurs on D9 and notochord flexion is evident by D14-15. It is also at this time that a full complement of digestive enzymes is present in larvae and fins begin to develop. By D21-D25 metamorphosis is complete and larvae have a fully developed digestive system. By D25 fish have formed scales and a lateral line and exhibit a silver or gray body pigmentation with vertical bands.

![Figure 4.2: Diagram of moi larval development from yolk-sac larva (hatch) to fully metamorphosed juvenile at day 25.](image-url)

**Figure 4.2.** Diagram of moi larval development from yolk-sac larva (hatch) to fully metamorphosed juvenile at day 25.
Facility

The size of a hatchery and types of material used to supply it can vary widely based upon the larval production plan and live feeds (algae, rotifers, and *Artemia*) production capabilities. Normally, hatcheries raise their own live feeds which, historically, required significantly more space than needed to contain the larval rearing section alone (Fig. 4-3). However, through the use of improved live feeds production technologies, and commercially available live algae replacement products, hatcheries can significantly reduce the space and labor requirements dedicated to these areas. Therefore, choice of hatchery design is dependent upon the level of control desired, risk assessment, and the availability of resources.
Tank Design

The primary unit in the hatchery is the larval rearing tank (Fig. 4-4). Circular larval rearing tanks are recommended for Pacific threadfin. At OI a range of tank sizes have been tested and proven successful in rearing moi. Comparable survival and growth rates have been obtained utilizing 1.2m diameter, 1m³ and 2.4m diameter, 5m³ tanks, where in both cases the depth is 1m. We utilize tanks made of fiberglass and the interior is painted with a black epoxy (bottom may be painted a lighter shade of grey to facilitate viewing of larvae). Interior walls should be smooth, and the bottom normally has a slight slope toward the center drain to which a center standpipe can be fitted.

Each rearing tank is provided with an inflow of fresh and sea water, air inlet to supply up to six airstones, illumination and tank drain. In our facilities, the tank and system plumbing is made from poly vinyl chloride (PVC). However, high density polyethylene (HPDE) is also widely used in hatchery facilities. Water supply and drain pipes need to be sized to adequately deliver and drain the water volume necessary (in the case of a 5m³ tank, the inflow pipe is 1” in diameter with a 2” drain). Water flows out of the tank through the center standpipe, and the height of the water within the tank is regulated by an external standpipe.

The interior center standpipe should be easily removable, and provide a large surface area (top to bottom) of 250 µm nytex screen to prevent the eggs and larvae from being flushed out. Cutting out large portions of PVC pipe and replacing them with this mesh can accomplish this. When the larvae grow, center standpipes with increasing screen sizes can be easily changed to accommodate dry food and increased flow rates.

Figure 4.4. Larval rearing tank in operation. Notice the placement of the surface skimmer (side of tank) and airstones in the center of the tank.
Figure 4.5. Photographs of the upgraded flow-through water treatment system commissioned to study the microbial ecology in the marine finfish hatchery. The top left panel shows the vacuum degassing column, which effectively uses the weight of the water column to create a vacuum leading to degassing of the supersaturated well water in the sump tank (lower left panel). Following degassing, the water is filtered through pressurized crushed glass filters (upper right panel), followed by bag filtration (5μm and 1 μm) and UV sterilization (lower right panel) prior to supply of larval rearing tanks.
**Figure 4.6.** Newly commissioned recirculating water treatment system (RAS) for study of microbial ecology in the marine finfish hatchery. The left panel provides a schematic of system design and the right panel is a photograph of the actual water treatment system located outside of the marine finfish research hatchery at OI.

**System Configuration**

Bacteria in the rearing systems have profound effects on the organisms being cultured. It was determined that the original system design of the OI research hatchery was not adequate to prevent the occurrence of bacteria blooms that were later associated with hatchery crashes. Therefore, two new systems were installed including a much improved degassed water treatment system (FTS) and a recirculating water treatment system (RAS) as an alternative approach to stabilizing the microbial environment in the larval hatchery.

The improved water treatment system (modeled after successful upgrades in the OI production hatchery) included a vacuum degassing column (designed by Akvator, Norway), crushed glass replacing sand in the pressurized sand filtration unit (based on reports that bacterial retention is lower on glass after backwashing than on traditional sand), and a series of bag filters (5µm and 1 µm), followed by UV sterilization (Fig. 4-5). The sequential order of these components was determined to be critical, as each filtration component can become a source of bacteria, rather than a means of removing it. The system was also designed to minimize bacterial retention by removing all possible side loops, tees, and other areas that might facilitate non-flowing water,
which we have shown aids microbial proliferation. The system was outfitted with sampling ports after each stage of the treatment process to facilitate water/microbial sampling for system study.

A recirculating water treatment system was also commissioned to examine the use of recirculating aquaculture system (RAS) technologies to supply water for larval rearing (Fig. 4-6). The commissioned system takes larval rearing tank water effluent and use a 60µm screen drum filter and protein skimmer to remove live feeds and other particulates, a kaldness™ bead biofilter for removal of toxic nitrogenous wastes, 5µm and 1µm pressurized bag filters for final particulate removal, heat pump to control temperature and UV sterilizer unit to reduce bacteria and other pathogens from re-entering the larval rearing tank system. A side loop vacuum degassing tower is used to lower total gas pressure.

![Changes in Bacterial Levels Due to Water Treatment (Log Scale)](image)

**Figure 4.7.** Overall changes in bacteria levels at various points in the OI marine hatchery filtration systems as a result of improved systems’ design and management.
Tank & System Preparation

Prior to the initiation of larval rearing runs, the entire water delivery system (from source to rearing tank) should be cleaned & disinfected. At OI, we recently began routine dry-down periods (30 days) followed by chlorination of system components prior to start-up.

**Step 1:** Fill the water delivery system and all hatchery piping with chlorinated (200ppm) seawater and allow the system to sit for 24 hours. In the case of recirculation systems, the water delivery can be set to continuously loop this chlorinated water throughout the system for this period, with the biofilter bypassed.

**Step 2:** After 24h has passed, water flow can be initiated to flush the system of chlorinated water. Chlorine levels should be tested after several hours and neutralized with sodium thiosulfate if necessary. Prior to use, the larval rearing tank and all supplies should be kept clean and dry.

**Step 3:** The tank should be thoroughly rinsed with fresh water before it is filled. The central stand pipe is initially fitted with a 250µm screen. Submersible water heaters are utilized when necessary to keep the water temperature 26-27°C. After filling, aeration is provided through a central air ring placed around the center standpipe. Four additional air stones are placed evenly around the tank.

Sea water should be flushed through the system at a rate of 2-3X/day along with aeration for at least ten hours prior to stocking eggs. Air supply is regulated through plastic air valves.

*Figure 4.8.* Photographs of 4m³ rearing tank used for larval rearing. The tank walls are black, while the bottom is painted a lighter shade of grey to facilitate seeing the larvae. A screened mesh standpipe (left) provides ample surface area to reduce water velocity of tank effluent which is drained through an external standpipe (right) to maintain water level.
Salinity Adjustment

Moi eggs will sink, requiring more aeration to stay in suspension and to hatch unless the incoming water is \( \geq 35 \text{ppt} \). Increased aeration tends to cause more variable hatch results, and often leads to lower survival in the early larval rearing period. If necessary, the salinity and incubation protocol can be modified to adjust for areas with lower ambient salinity. Just prior to stocking, the water flow can be turned off, and salinity adjusted to 35ppt (using coarse salt). Once 35ppt has been obtained, the aeration levels can be reduced to just enough to keep the eggs in gentle motion.

Egg Stocking and Incubation

Egg rinsing procedure

Prior to stocking, eggs should be rinsed for 2-3 hours to remove unwanted debris and to reduce over-all bacteria levels associated with the eggs.

A 200L cylindrical fiberglass tank is used for rinsing, counting eggs, and monitoring development prior to stocking into the larval rearing tanks. The setup is basically the same as for the larval rearing tank described above.

During rinsing, water exchange should be a minimum of 100% exchange per hour. Aeration should be adjusted to ensure all eggs are evenly suspended in the water column. Eggs are gently transferred into this tank utilizing a plastic pitcher. Embryonic development at this time is usually late gastrula to early embryo (approximately 8.5 hours post-spawn at 26°C)

Condition is determined by taking a small number of eggs and viewing them under a compound microscope. Determine the percentage of eggs with clear embryo development from a minimum of 100 eggs.
Figure 4.9. Eggs are rinsed in a clean tank with high water flow (100% tank volume per hour) and high aeration to keep the eggs in suspension and to remove debris.

Figure 4.10. Photographs of technicians utilizing the screen counting method of estimating the number of eggs in a given volume of water.

Separating and Counting Eggs

Egg density in the 200L tank is determined volumetrically. The water to the 200L tank is turned off (aeration remains on), and eggs are allowed to mix uniformly. A 60 ml sample is taken by pipette and spread evenly on to a sieve and counted. The sieve is constructed of 6” PVC pipe (~4” high) and covered on one end with a 150µm mesh screen (Fig. 4-10).

This procedure is repeated four more times for accuracy. Egg density in the 200L tank is calculated from the equation:

\[
\text{eggs per ml} = \frac{\text{average of five counts}}{60 \text{ ml}}
\]
Stocking Density

The 5,000L (5m³) larval rearing tank has a working volume of 4,000L. The recommended initial stocking density of eggs with normal embryo development is 40 eggs per L. The eggs are removed from the rinsing tank using a graduated beaker. The appropriate volume is removed from the 200L tank and placed gently into the larval rearing tank using the following equation:

Volume needed from 200L tank =
\[
\text{Total number of eggs needed in rearing tank} \div \text{Density of eggs in 200L tank}
\]

Incubation

Eggs are incubated in the larval rearing tank with continuous very gentle aeration (center airstones only) and a daily water exchange of 400% (if the supply water is 35ppt). The eggs must be kept suspended in the water column, but not moved around too vigorously as the fragile larvae will be damaged upon hatch. If the salinity is below 35 ppt, the eggs should be incubated in static (no water turnover) conditions, with adjusted salinity (as described above) and gentle aeration. During incubation, a small sample of eggs should be examined under the microscope to determine the stage of development and estimate hatching time.

Hatching

Pacific threadfin hatch approximately 19 hours after spawning at 26-27°C. The time of hatching is inversely proportional to the incubation temperature. Newly hatched larvae are more sensitive to environmental factors than eggs, which are protected by shell and chorion. Between larval days 1-3, the larvae show stereotypic motor functions, which are not directional.

Air driven surface skimmers (Fig. 4-11) are utilized from hatching onward to rid the surface of its oily film produced by the hatching embryos and subsequent feed additions. Once 50% of the eggs have hatched, water supply can be re-initiated (in the case it was suspended due to low salinity) and set to turn the tanks over 400% daily. This will slowly return the water quality to ambient conditions, while allowing enough time for the larvae to fully hatch. One day after hatching, the dead unhatched eggs are siphoned from the bottom of the tank to waste.
Vertical Sample

The number of larvae in the rearing tank is determined 16-18 hours after hatching. This value helps to estimate the hatching percentage, initial larval density, and percent survival at the end of the larval rearing trial. The number of larvae is estimated by the vertical sampling technique (Fig. 4-12). Airstones are not removed during sampling because they help to keep the larvae evenly distributed in the tank. A 4.5-ft. long, 2” diameter PVC tube equipped with a ball valve is used to sample larvae. With the valve open, insert the tube into the water obliquely, starting at the tank wall and ending at the tank bottom near the standpipe. Then, close the valve and slowly
withdraw the tube from the water. Just before the tip is removed from the water, place a hand over the open end to prevent water from spilling out. Slowly empty the contents from the tube are into a 3,000mL graduated plastic beaker. Repeat until five random samples are taken from the tank.

Record the volume of the water in the beakers. Then count the larvae by carefully pouring the contents of the beaker into the mesh sieve used for estimating egg number (above). After all the water & larvae have been transferred, a paper towel can be used to dry off the mesh (from the underside), revealing the larvae on the screen.

The following formula is used in the estimation of the larval density of the samples:

\[
\text{Larvae/L} = \frac{\text{Number of larvae counted}}{\text{volume of water in sample (L)}}
\]

To estimate the density of larvae in the tank, the average of the five samples is taken. To determine the total number of larvae in the larval rearing tank, multiply this average density by the working volume of the rearing tank.

\[
\text{Total number of larvae} = \text{avg density five samples (larvae/L)} \times \text{volume of tank (L)}
\]

To obtain the hatching percentage, the total number of larvae is divided by the total number of eggs stocked.

\[
\%\text{ Hatch} = \left(\frac{\text{Total number of larvae per L}}{\text{Number of fertilized eggs stocked}}\right) \times 100
\]

After the vertical sample is completed, only the center airstones remains to provide gentle aeration and mixing.

Figure 4.13. Day 2 moi larva ready to begin exogenous feeding. Note the presence of very little yolk, fully pigmented eyes, and open mouth.
Feeding Larvae

Feeding Behavior

At 2-3 days post-hatch, the larvae become positively phototactic and rheotactic and directional movement begins. These behaviors assist their diurnal positioning at the surface of the larval tank and allow larvae to thoroughly search for food in the water column. When the mouth fully opens (D3 post-hatch), larvae are able to ingest live food from the surrounding water. Hunting and capture behavior has several phases: locating prey, orienting toward prey, aiming, attack, seizing, ingesting, and using visual (prey movement) and tactile cues (prey which is too solid is regurgitated). During this period from endogenous to exogenous feeding, high mortalities can occur if the proper feed and environmental conditions are not presented.

Another important feeding behavior to note is cannibalism. With moi, this behavior is normally observed at approximately D 18-19 posthatch, when the larvae are about 10.0 mm. At this stage there is usually a bimodal size distribution and larger fish tend to prey on the smaller fish. This behavior (and associated mortalities) can be greatly reduced with proper feeding and tank management.

Live Food

One of the primary challenges in rearing Pacific threadfin larvae is the maintenance of an adequate supply of live food (Algae, rotifers and Artemia). It is crucial that food items be produced in sufficient quantities for the different larval stages. Feed organisms must be of appropriate size, motile, palatable, digestible and of high nutritional quality. In addition, they must be amendable to mass production under controlled conditions.

Live Microalgae

Microalgae form the base of the larval rearing food chain and use in rearing tanks improves larval rearing survival (over clear water). Algae provide nutrition for rotifers that are not consumed immediately by larvae. Live algae may help stabilize water quality by adding oxygen, removing nitrogen, and potentially acting as bactericides. Algae also provide contrast within the rearing tank, which improves sight recognition by larvae and enhances their ability to catch prey.

Nannochloropsis oculata has been used in the OI hatchery, although other green algae may be used. N. oculata is small (2 µ), eurohaline, and reproduces rapidly. It can be grown at high densities, but due to advances in commercially available algae paste products, live algae are no longer necessary for moi production.
Figure 4.14. Live microalgae is the base of the larval rearing food chain, but has been largely replaced with commercial algae paste products.

Figure 4.15. Application of algae paste products to the larval tank needs to be done gently. Algae paste should be first diluted in seawater and added slowly to the tank over multiple areas.
Algae Paste

Due to the labor demands of live microalgae production, concentrated algae paste products can provide considerable cost and labor savings to the hatchery operation. Moi are a species that has proven amenable to the replacement of live algae with paste, and we currently employ a methodology that no longer uses live algae in any portion of our moi hatchery operations. Our current protocol utilizes a concentrated *Nannochloropsis oculata* product (Nanno 3600) from Reed Mariculture Inc., which contains 68 billion cells/ml. The product is shipped frozen and has a long shelf life. The substitution of this product for live algae has not negatively affected growth or survival over the past ten years of testing.

Use of Algae in Larval Tanks

*N. oculata* (live or paste) is introduced into the larval rearing tanks on the second day post-hatch. Approximately 300,000 cells/ml are introduced on D2 post-hatch. The density of algae is adjusted so the water column is turbid enough so that the bottom is just out of sight. The precise amount of cells/ml may vary depending on tank depth, color and illumination but should typically range from 200,000-500,000 cells/ml. When live algae are utilized, they are added prior to adding rotifers in the larval rearing tank due to the volume of water added.

The number of liters of live algae to add to the larval rearing tank is determined by the following equation:

\[
\text{Algae to be added (e)} = \frac{\text{Desired algal density (cell/ml) x tank vol. (ml)}}{\text{In-tank algal density (cells/ml) x 1,000}}
\]

Paste algae addition would be calculated in a similar way, but would likely involve much less water volume to be added due to the highly concentrated stock supply. Prior to addition, the volume to be added should be diluted in clean seawater so that it can be slowly applied to the rearing water and evenly distributed throughout the tank.

It is very important to prevent unwanted debris from entering the larval rearing tank when adding algae. A 20µm nytex screen should be utilized to filter out any particulate matter.

Feeding Regimen – Rotifers

Rotifers are presented in rearing tanks on D2 post-hatch (in the late afternoon), one day prior to initiation of feeding. On D3 post-hatch and each day thereafter, rotifers are added to the rearing tank two times per day (early morning and late afternoon) to an optimum density of 20 rotifers per ml. Rotifers are harvested, counted, and then enriched before being transferred to the larval rearing tanks. Rotifers are added at an initial density of 20/ml (a total of 80 million per 4m3
tank). The volume of rotifers from the harvest to be added to the larval tank is estimated using the following formula:

\[
\text{Volume of rotifers to be added} = \frac{\text{Total number of rotifers needed}}{\text{Rotifer density in holding vessel}}
\]

**Figure 4.16.** Rotifers can be easily counted using a 1ml pipette and 8X magnifier.

**Density Determination**

Rotifer density in tanks is estimated by taking five 1-ml samples from a tank with a pipette, and counting the number of rotifers with a magnifying eyepiece (10X). To increase the density of rotifers up to 20/ml, the following equation is used:

- Average number of rotifers/ml = \( \sum \) (count 1-5) ÷ 5
- Total number of rotifers to be added/ml =
  
  \( (20 \text{ rots/ml} - \text{avg. number of rotifers/ml counted in tank}) \times \text{tank volume} \)

- Volume of rotifers =
  
  \( \frac{\text{Total number of rotifers to be added}}{\text{rotifer density in harvest container}} \)
Feeding Regimen - *Artemia*

Pacific threadfin larvae are fed new-hatched *Artemia* metanauplii starting on larval D8 and a combination of new-hatched and enriched nauplii starting on D10 (Fig. 4-18). The density of metanauplii in the larval rearing tank is 0.20 per ml initially. This is increased gradually to 2.12 to 3.20 per ml, and 6.2 to 7.0 per ml when the larvae are D15 and D23 respectively. The amount of *Artemia* remaining in the larval rearing tank should be calculated daily before feeding and larval behavior should be observed. Normally, enriched *Artemia* metanauplii are fed until larval D24.

Approximately 250-300 metanauplii can be consumed by a single D17 larvae.

Enriched *Artemia* metanauplii are fed to larvae in small amounts, five to six times per day, termed pulse feeding. Pulse feeding ensures that larvae are consuming *Artemia* replete in essential nutritional components since metanauplii rapidly excrete the enrichment media. Also, excess *Artemia* in the rearing tank compete with the larvae for oxygen and produce toxic...
metabolites. *Artemia* densities should be adjusted accordingly. *Artemia* are pulsed when densities fall below 75% of the daily targeted amount.

![Figure 4.18](image)

**Figure 4.18.** Enriching *Artemia* is essential for the health and survival of the fish larvae.

**Dry Feed**

Dry, practical feeds are introduced into the Pacific threadfin rearing tanks on larval D8 with the introduction of *Artemia*. A slow transition to dry food yields a better survival. Weaning too early or too quickly is often accompanied by disease, cannibalism, and other defects which affect the survival rate. Physical and chemical water quality parameters must be closely monitored when feeding dry food.

**Diet Types**

A high quality commercial salmon larval diet can be used for successfully weaning and feeding Pacific threadfin, (i.e., Nutra Marin, Perla Marine, or Nutra Starter, Skretting/Bio-Oregon). Moore-Clark Co. Nutra Starter Salmon size 0 and 1 (300-700 µm and 600-1200 µm) have been utilized for many years with excellent results. Recently, we have started utilizing Otohime© (Japan) diets as the first dry diets to be introduced. These diets come in a range of sizes...
(A1=250µm, B1=250-350µm, B2=360-650µm, C1=580-840µm) and have greatly accelerated the weaning onto later dry diets.

**Figure 4.19.** Photographs of commonly used dry diets in the moi hatchery. Larvae are initially offered a very fine (250µm) “powder” diet. As the fish grow they are transitioned onto larger pellets.

**Figure 4.20.** Automatic belt-feeders can be utilized to dispense dry feeds to the larval rearing tanks. These feeders are powered by a spring clock that retracts the belt towards the front of the feeder, dumping any contents on the belt into the tank. These feeders allow the dry diets to be continuously distributed to the fish over the entire day.
Feeding Regimen – Dry Diets

Careful application of dry feed is very important. The exact amounts and size to be given varies, depending upon the number of larvae in the tanks and their growth. At the end of each day the tank bottom should be examined for unconsumed dry feed. The dry feed is then adjusted up or down the following day.

On larval D8, only a small amount of feed (~ 1.0g/day) should be introduced into the tank. The feed is sprinkled over the water surface of the tank until the larvae are observed actively feeding on the particles. During this critical period in larval rearing, increased mortalities may occur. The amount of dry food is gradually increased each day depending on the demand of the larvae.

Feed is administered manually at the beginning of the weaning period. A small amount of feed is given at one hour intervals. This allows larvae time to consume most of the feed before it sinks. This also assists in maintaining good water quality.

When the larvae adapt to dry feed and consume most of the feed during the hourly feedings, a 12-hour belt feeder is installed (Fig. 4-20). This usually occurs on approximately D14. The belt feeder is attached to the rim of the larval rearing tank approximately 1-2 ft. beyond the water inflow pipe. The total daily amount is determined, divided by two, and half the amount is spread over the feeder belt to distribute feed evenly over a 12h period. The remaining portion can be added to the belt feeder at the end of the day to provide food overnight (the next 12h period).

When dry feed is introduced, it is important to change the standpipe screen from 500 to 1,000µm. This reduces the chance of the screen getting clogged by the extra feed and maintains the water circulation in the larval rearing tank.

Survival

Survival rates average consistently between 20-30% of hatched larvae. Mortalities occur on a daily basis and are often difficult to track. However, there are two periods of mortality very noticeable in the larval rearing tanks. The first period is between D6-D8, in which losses of about 5-10% are expected. These mortalities are due to starvation and/or poor feeding during the transition to exogenous food items. The second period of mortality is from approximately D12-D14, when larvae undergo several physiological and morphological changes. By D12 overall survival in the tank is approximately 50-70% of hatched larvae. Total mortality between D12-D14 is approximately 25% of the number of larvae remaining, or about 10-15% of hatched larvae. Mortality after this period is very small and most of the animals remaining in the tank will survive to harvest at D25.
Water Quality

Temperature

The recommended rearing water temperature range for Pacific threadfin is 26-28°C. At these temperatures, the larvae can be harvested 22 to 25 days post-hatch. If water temperature cannot be achieved under ambient conditions, submersible electric heaters should be utilized.

Lighting & Photoperiod

Natural daylight in the hatchery is supplemented with artificial illumination. A fluorescent lamp connected to a timer is attached above each larval rearing tank (Fig. 4-21). Each light has a diffuser to provide a light intensity of no more than 1,500 lux at the water surface. The young larvae and postlarvae are sensitive to light shock. It is advisable to maintain constant light conditions and avoid sudden changes. Moi can be raised on ambient photoperiod (12h L:12h D). However (as reported for other marine species), 24h light has recently been utilized with excellent results and may provide for additional feeding and improved survival and growth. The use of 24h light is now our standard protocol for the hatchery period.

Figure 4.21. Additional lights can be utilized to supplement ambient lighting conditions in the hatchery.
Aeration

Airstones are placed in the larval rearing tank for several reasons. Aeration provides essential oxygen and also promotes CO2 removal. The current generated by the airflow ensures that the live food is distributed uniformly and prevents thermal stratification.

The aeration will need to be monitored and routinely adjusted. Large bubbles achieve the highest degree of mixing but have a negative effect on the larvae. Small bubbles are best for diffusing gases into the liquid medium. The targeted aeration protocol is a medium sized bubble with gentle flow, just enough to keep the feed and larvae in motion.

Chemical Characteristics

A summary of the key chemical characteristics of water for rearing Pacific threadfin larvae is presented in Table 4.1

Table 4.1 Targeted water quality parameters for larval rearing of Pacific threadfin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>32 - 35ppt</td>
</tr>
<tr>
<td>Temperature</td>
<td>26 - 28°C</td>
</tr>
<tr>
<td>pH</td>
<td>7.8 - 8.3</td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>5 - 7mg/L</td>
</tr>
<tr>
<td>Ammonia</td>
<td>0.00 - 0.02ppm</td>
</tr>
</tbody>
</table>
System Operation

Water Exchange

Water exchange is very important in intensive larval rearing, since it provides a means to remove waste and stabilize water quality in the rearing tank.

Rotifers are the exclusive food for larvae during the early rearing cycle (D2-8 post-hatch). If water flow is too high during this stage, many rotifers and algae will be lost.

The recommended water exchange rate used at OI is shown in Figure 4-22.

When the water flow in the larval rearing tank reaches 300%, the standpipe screen should be changed to a larger mesh size (500 µm).

On day 20 post-hatch, the water exchange is increased to 500%. The standpipe screen should also be changed to 1,000 µm at this time. Dry feed amounts are steadily increasing during this period.

The water inflow rate to obtain a certain exchange rate is calculated by the following equation:

\[
\text{Flow rate/min} = \frac{(\text{tank vol (L)} \times \text{exchange rate (\%/D)})}{24 \times 60}
\]

Figure 4.22. Water exchange (% daily) in the rearing tank during the larval rearing of Pacific threadfin.
Figure 4.23. Water quality testing is an important aspect of all hatchery operations. Portable meters can be used to measure temperature, salinity, dissolved oxygen, and pH. A bench top colorimeter is used to measure ammonia, nitrite and nitrate.

Daily Routine

Tank Monitoring

Water quality is monitored to ensure optimum conditions exist in the larval rearing tanks. Measurements of water temperature, salinity, dissolved oxygen, and pH levels should be taken and recorded twice daily, in the morning and afternoon. Live feed densities should also be monitored to determine the amount of rotifers or Artemia to be fed. Rotifers are fed twice per day and Artemia are fed five times per day. Dry feed should be fed hourly by hand during the early weaning stage and excesses should determined.
Tank Maintenance

The flow rate is determined 1-2 times daily. Aeration is also checked twice per day. The central standpipe screen and ring siphons are checked and cleaned when necessary. The surface of the rearing tank is skimmed when necessary and the bottom is siphoned.

Surface Skimming

The surface is skimmed to remove the oily waste that often forms on the surface of rearing tanks. A clean surface provides unobstructed exchange between the air and water, and facilitates the ability of larvae to gulp air to inflate their swim bladders. An automatic surface skimmer is installed on day 0 and turned up when dry feed is introduced. This is to remove the oily film that leaches from the feed particles. The skimmer consists of a Styrofoam square (35x35cm) frame with one side open (Fig. 4-24). A ½” diameter PVC pipe (35 cm in length) is fixed to the open side. The pipe has 1mm holes along its length. One end of the pipe is closed and the other is connected to a compressed air circuit. The air tube is adjusted so it is blowing across the surface. This will concentrate the oily film into the skimmer's trap. The scum from the skimmer is removed with a beaker whenever necessary.

Figure 4.24. Surface skimmers will accumulate oils and other organic materials and need to be cleaned frequently. Paper towels can be utilized to soak up the accumulated material.
Bottom Siphoning

Beginning on larval D1, the tank is siphoned to remove the dead and unhatched eggs. Dead eggs and larvae appear as white spots on the bottom of the black tank. The white spots are carefully siphoned off the bottom of the tank into a bucket.

The siphons for bottom cleaning are divided into three categories: 3.5, 4.5, and 7.0mm. The siphons are constructed from a glass rod about 1 m in length, which is connected to a 2-3 m long plastic tubing. The tip of the glass rod has a short piece of tubing attached, which is cut at an angle. Normally, the contents of the tanks are siphoned into standard 20L buckets. Dead larvae are counted when siphoning is completed. If the number is low, they are counted by visual examination. If the number is large, it is counted volumetrically. The dead larvae are poured through a fine mesh hand net and rinsed. All larvae are placed into a 5-L graduated beaker and aerated vigorously. Five aliquot samples are taken with a 50 ml beaker or a 10 ml graduated pipette. The dead larvae are counted from each sample. The total number of dead larvae is calculated using the following equation:

\[
\text{Number of dead larvae} = \frac{\text{Avg. five counts} \times \text{volume of water in beaker}}{\text{Volume of sample}}
\]

Figure 4.25. Photographs of technician siphoning larval rearing tank and siphon attachment created for vacuuming the tank bottom.
Harvest and Transfer

Stress Test

A stress test is conducted on fry prior to transfer to the nursery. Stress is defined as the reaction of an organism to a physiological imbalance or a disturbance to the normal physical environment. Weak fish overreact to stress.

D22-24 post-hatch Pacific threadfin larvae should be subjected to a stress test to determine whether they are able to survive the harvest process.

D22 post-hatch, transfer buckets are filled with seawater to the 15-L level and aerated gently. Random samples of 100 fry are removed from the tank by hand net and held suspended in the air for 15 seconds (Fig. 4-24). Fry are placed into one of the buckets. Keep the collected fry in the buckets for one hour.

After one hour, the number of live and dead fish are counted and recorded. Survival rate is determined by dividing the number of live fish by the total number of fish collected in the sample and multiplied by 100 to give a percentage of survival:

% survival = (number of fish alive ÷ total number of fish collected) x 100

Repeat this estimation of survival three times. Average the survival percentage obtained from three buckets. If survival is 90% or above, the fry are considered harvestable. Harvest should be postponed if survival is less than 90%.
Once the fish have passed the stress test, the larval tanks are ready for harvest. Fish are gravity drained into a harvest cradle for transfer to nursery tanks and for estimation of growth and survival.

**Harvesting**

Under optimal conditions, D24 Pacific threadfin are approximately SL average 10-15 mm and 30-40 mg wet weight. At this age, metamorphosis is complete and the fish are ready to be harvested from larval rearing tanks to the nursery.

**Procedure**

Post-metamorphosed Pacific threadfin are extremely fragile and require special handling techniques.

The bottom of the larval rearing tank must be siphoned clean prior to harvesting. Prepare a 500L rectangular fiberglass tank with seawater, install a flow through system with screen, and place an airstone in the tank. Fill five 15L buckets halfway to the top with seawater and place an airstone in each. To begin harvest, lower the water volume by turning off the water source and drain the tank through the center standpipe mesh. The water level in the larval rearing tank should be
lowered to 0.5m. When the larval rearing tank is ⅓ the original volume, the fry are drained into a harvest cradle (Fig. 4-26).

The cradle is used to collect and concentrate fish (Fig. 4-27). It is constructed from a 33” long, 12” diameter PVC pipe. The center 29” is cut out leaving two 2” handles on either side. Each end is covered with PVC plate. One plate is cut 7” high; the other plate is cut 9” high. A PVC ring with 1mm meshed screen is glued inside each end. This prevents fish from being lost from the cradle.

Fry are concentrated into a small volume of water and are transferred from the cradle to buckets for visual estimation of numbers.

**Estimating Numbers Harvested**

Estimating the number of Pacific threadfin fry from the larval rearing tank consists of sampling a fixed number of fry (1,000) in a bucket and comparing it with other buckets. The numbers are then adjusted visually (Fig. 4-26).

Once the fish are in the harvest cradle, fill the 15-L plastic buckets with 10 L of seawater and count 1,000 fry into a single bucket with seawater and gentle aeration.

Excess water in the bucket is removed to bring the volume back to 10-L. This will be the **standard bucket**. Place two buckets with seawater on each side of the standard. Place fry with a plastic scoop (not net) into each bucket until the density appears to be same as the standard. When the number reaches 1,000, it is recorded on the data sheet and the fry are quickly transferred to the nursery tank. The total number of fish harvested is estimated by the number of buckets filled in this manner.
Figure 4.2. Once fish are harvested and condensed, they are transferred into buckets for visual estimation of number. Fish are transferred by scooping out of the cradle and into the buckets until the numbers visually match those in the counted “standard” bucket.
Final Growth and Survival Estimates

Growth

Randomly scoop a sample (minimum 100) of fry from the harvest cradle of the 5,000L fiberglass tank. Place these fry into a 15L bucket filled with seawater. Be sure to provide aeration to the bucket.

Place a plastic bowl on the top loading digital scale and tare the balance to zero. Fry can be collected from the bucket with a scoop net; all water must be drained from the net. The fry are then placed directly into the bowl on the scale and weighed. After weighing, all fry are placed back into the bucket with seawater. Anesthesia (MS-222, 40,000ppm) is then added at 2ml/L. Wait until the fry are completely immobilized.

Individual fry are then collected from the bucket and placed on a large glass slide. A minimum of fifty fish are measured with a caliper to determine standard length.

Calculate the average body weight and standard length per fry from the total sample and record. This value is important because it is used to determine the amount of dry feed for the first nursery phase.

Survival

Survival rate is one of the most important procedures during the harvest process. To obtain a survival percentage of the larval rearing tank, divide the number of fry harvested by the number of larvae hatching and multiply by 100.

Utilizing the methods described in this chapter, survival of Pacific threadfin through the hatchery phase (Day 25) at our facilities has averaged 30% (Table 4.2).
Table 4.2: Mean performance of 4m$^3$ tanks at the OI CAAMB Hatchery over the 2009-2010 production period.

<table>
<thead>
<tr>
<th>Run #</th>
<th>Larvae/L</th>
<th>Hatch (%)</th>
<th>No. Harvest</th>
<th>Fish/L</th>
<th>Survival (%)</th>
<th>Wt (g)</th>
<th>SL (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30.4</td>
<td>67.4</td>
<td>27,917</td>
<td>7.0</td>
<td>23.3</td>
<td>0.06</td>
<td>10.7</td>
</tr>
<tr>
<td>2</td>
<td>20.4</td>
<td>48.1</td>
<td>23,667</td>
<td>5.9</td>
<td>29.3</td>
<td>0.08</td>
<td>12.2</td>
</tr>
<tr>
<td>3</td>
<td>22.6</td>
<td>56.5</td>
<td>38,000</td>
<td>9.5</td>
<td>41.9</td>
<td>0.07</td>
<td>12.3</td>
</tr>
<tr>
<td>4</td>
<td>18.7</td>
<td>46.7</td>
<td>24,750</td>
<td>6.8</td>
<td>36.0</td>
<td>0.08</td>
<td>11.8</td>
</tr>
<tr>
<td>5</td>
<td>22.8</td>
<td>57.0</td>
<td>19,750</td>
<td>4.9</td>
<td>22.2</td>
<td>0.08</td>
<td>13.3</td>
</tr>
<tr>
<td>Avg</td>
<td>23.0</td>
<td>55.1</td>
<td>26,817</td>
<td>6.8</td>
<td>30.5</td>
<td>0.08</td>
<td>12.0</td>
</tr>
</tbody>
</table>
Figure 4.28. Photograph of D25 moi larvae in buckets, ready to be transferred to nursery tanks.
Chapter 5 – Nursery

Figure 5.1. The proper set-up of the nursery tank to establish the correct amount of water turn-over and current speed is essential to reducing cannibalism.

Overview

The transition period from hatchery to grow-out was once a major bottleneck in the production of moi fingerlings, as cannibalistic behavior could impart severe losses to fish at this stage. To mitigate these losses, a two-stage nursery system was utilized (Ostrowski & Molnar, 1998) that facilitated rearing large numbers of fish through the highly cannibalistic phase and into grow-out. However, as hatchery technology has intensified production, and fingerling demand has increased, there arose a need for more intensive nursery technologies to satisfy a growing off-shore production industry.

One of the largest changes to the previous moi rearing protocols was the establishment of a high-density, single-phase nursery tank. Moi larvae were transferred from hatchery (one time) into 20m³ nursery tanks for the entire 30-day nursery period. Other changes during this stage
included the use of multiple water delivery manifolds, liquid oxygen, and continuous (24h/day) feeding. The resulting survival, growth and total number of fingerlings delivered throughout five production runs are summarized in Table 5-1. Approximately 310,000 fish were produced during the hatchery portion of each production run, resulting in the use of four 20m$^3$ nursery tanks running with approximately 80,000 juveniles each. The average survival through this portion of the cycle was quite high at nearly 87%, although mean survival did decrease as the fish were maintained to larger sizes towards the latter portion of the year. Despite a couple mortality events during runs # 2 & 4 (due to power failures), successful execution of the following nursery protocols allowed for approximately 1 million fingerlings to be delivered to off-shore cages over that period.

Table 5.1: Nursery stage performance of moi fingerlings generated from a collaborative project between OI and Hukilau Foods Inc. at the CAAMB Hatchery. The fish were transferred to off-shore cages during the 2009-2010 production period.

<table>
<thead>
<tr>
<th>Run</th>
<th>Stock</th>
<th>Harvest</th>
<th>Survival (%)</th>
<th>Avg Wt (g)</th>
<th>FCR</th>
<th>Harvest Day</th>
<th># to Cages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>320,000</td>
<td>298,049</td>
<td>93.1</td>
<td>4.2</td>
<td>1.45</td>
<td>52</td>
<td>240,000</td>
</tr>
<tr>
<td>2</td>
<td>429,000</td>
<td>386,000</td>
<td>90.0</td>
<td>4.6</td>
<td>NA</td>
<td>50-64</td>
<td>200,000</td>
</tr>
<tr>
<td>3</td>
<td>312,000</td>
<td>266,430</td>
<td>85.4</td>
<td>7.2</td>
<td>NA</td>
<td>55-76</td>
<td>225,880</td>
</tr>
<tr>
<td>4</td>
<td>267,000</td>
<td>209,790</td>
<td>78.6</td>
<td>7.9</td>
<td>1.82</td>
<td>58-60</td>
<td>135,000</td>
</tr>
<tr>
<td>5</td>
<td>222,000</td>
<td>200,000</td>
<td>90.0</td>
<td>6.4</td>
<td>1.61</td>
<td>50</td>
<td>200,000</td>
</tr>
<tr>
<td>Avg</td>
<td>310,000</td>
<td>290,067</td>
<td>86.8</td>
<td>6.0</td>
<td>1.62</td>
<td>Total</td>
<td>1,000,880</td>
</tr>
</tbody>
</table>

Analysis of water use and feed consumption during the nursery stages indicated very consistent application of nursery water flow and feed over multiple runs (Fig. 5-2). The resulting average total feed used for production of 290,000 (6.0g) fingerlings equaled 2,400kg, with FCR values ranging from 1.45 to 1.82. FCR was sensitive to overall survival, and the highest FCR was recorded in October, largely due to a mortality event (loss of 68,000 fingerlings) just days prior to transport. Likewise, in June, low DO caused the loss of approximately 42,000 fingerlings,
highlighting the fact that this high density nursery protocol is very sensitive to mechanical interruptions, and further back-up protocols should be considered. Typical water use for each 20m$^3$ tank ranged from 100lpm to approximately 500lpm. Feed consumption in each tank ranged from 0.01kg/day to approximately 35kg/day. Mean water usage for all four tanks during the nursery period averaged 1,323lpm with a minimum of 468lpm, and a maximum daily use of 1,965lpm.

Figure 5.2. Mean daily water flow (top panel) and mean daily feed consumption (bottom panel) over three production runs for each 20m$^3$ moi nursery tank containing approx 80,000 fingerlings during the nursery stage of production cycle.
Facilities

System Design

Tanks

Two sizes of nursery tanks have been utilized at OI (5m$^3$ and 20m$^3$). The tank is typically constructed of fiberglass and painted with black epoxy (Fig. 5-3). The 5m$^3$ tank has a working volume of 2,000L with a water depth of approximately 38cm at the tank wall and 58 cm at the bottom of the center drain. The larger diameter, 20m$^3$, round fiberglass tanks have a 4” center drain. The setup of the system is similar to that for the smaller tank, except that tanks are operated at a volume of 6-7m$^3$. If the nursery tanks are located indoors, ambient light should be supplemented with artificial light. Outdoor tanks should be covered with a double layer of 80% shade cloth to help minimize algal growth.

Figure 5.3. Photograph of empty nursery tank set-up. A horizontal spray bar with adjustable spray-angle is utilized to control water velocity (shown in operation in Fig. 5-1).
Two standpipes are utilized to control the water depth (Fig. 5-4). An external standpipe will serve as the primary means to regulate water depth in the tank. A mesh covered inner standpipe is utilized in the central drain of the tank to flush feces and uneaten feed from the nursery tank. The bottom 2-3” of this pipe is perforated with many 2-4 mm holes (or cut-out in large sections) and covered with a 1.5 mm screen to prevent loss of fish. As the fish grow, this mesh can be changed to larger sizes, to allow for voiding of larger feed sizes and accommodate increased water flow. The inner standpipe height should be adjusted so that the water level is slightly above the top. This allows for the majority of the water containing all tank debris to drain from the bottom of the inner pipe. A screen placed over the top of the pipe will prevent loss of fish and act as back-up drainage in case the lower holes of the inner standpipe become clogged with debris.

Pacific threadfin juveniles will orient to the bottom of tanks. Therefore, tanks with deep water promote uneven distribution of feed particles to individuals, which can result in size disparities and increased incidence of cannibalism. Shallow water designs provide increased contact between fish and feed particles. This design also allows for feed to be more evenly distributed among all individuals, which will minimize size disparities as they grow.
Unidirectional current is created in the tank by using spraybars (Fig. 5-3). In smaller tanks, a PVC pipe is extended across the diameter of the tank (approximately 3-4” above the water surface) and coupled to a water source. The spray bar is assembled with threaded couplings so that the angle of the spray can be adjusted. A series of $\frac{1}{2}$” holes drilled along the sides of the pipe allow for the water to be injected at high velocity. The angle of the spray hitting the surface determines the speed of the water current. In larger tanks, a combination of vertical and horizontal spray-manifolds is utilized to create the current. When operating at high densities, oxygen can be injected into these manifolds to increase the DO.

**System Operation**

The two most important aspects of design and operation of the rearing tanks during the nursery phase are the correct application of feed and water current. The tanks are designed to provide a steady current across the entire bottom that can be increased as the fish grow. Also, feed needs to be evenly distributed across the tank at each application at nearly continuous intervals.

*Figure 5.5.* By utilizing the correct water velocity, fish will remain evenly distributed in the nursery tank, and cannibalism will be reduced. This technique can be applied to numerous species, including the amberjack (*Seriola rivuliana*) pictured here.
Current Speed

In a steady current, juvenile threadfin are positively rheotactic and naturally position themselves with larger individuals ahead of smaller. This orientation limits head-to-tail attacks of larger fish on smaller, reducing cannibalism. Current speeds should measure between 12-21 cm/s⁻¹. This corresponded to roughly 10-13 fish lengths/sec at stocking, and 4-5 fish lengths/sec near the end of the phase. This current should be adjusted so that the fish have to actively swim against it, but are not being overwhelmed by it, and drifting in the same direction. Occurrences of cannibalism tend to decrease after D40, and as the fish grow, the current is primarily used to provide an easy means to remove feces.

Figure 5.6. Fish are carefully transferred by bucket (not nets) to the nursery tank. The resulting flow should create an evenly distributed population of fish within the tank.
Stocking

The recommended initial stocking density for D25 moi is 5-8 fish/L, when the fish are approximately 40mg and 15mm in length. The loading rate at stocking should be 0.400 kg/15 lpm (0.027 kg/lpm).

Beyond D40, it is recommended that the density be adjusted, if necessary, to equal one fish/L (1g/L), with loading rates = 0.2 kg/lpm (flow rate = 30 lpm). This density can be increased, but additional water flow and supplemental oxygen will be required. If unmodified, final harvest density (at day 60) will be approximately 10 kg/m³ at a final loading rate of 1-1.2 kg/lpm (flow rate = 50 lpm).

Prior to stocking fish, nursery tanks should be washed, scrubbed, and rinsed with fresh water, then allowed to dry. The tank is then filled with seawater to the working volume one day prior to stocking.

Nursery tanks are stocked immediately following the harvest from the hatchery; density is determined by the length and weight measurements determined at harvest. Ideally, this process is done as quickly as possible to minimize stress on the animals.

Initially, water flow and aeration levels should be minimized when transferring the fish into the nursery tanks. Care should be taken to not expose the fish to nets or rough handling at this stage. Normally, the fish are transferred directly from the buckets they are visually estimated in (Fig. 5-6). The bucket should be slowly immersed in the nursery tank so that the fish can swim out.

After approximately one hour, the tank should be inspected and the water and aeration levels can be re-adjusted back to recommended levels. Some post-transport mortality (3-6%) can be expected in the first 24h.

Feeding Regimen

The correct application of feed during the Nursery Phase is key to maximizing growth and survival of fish. Feed should be offered within a few hours of stocking, in small amounts, frequently through the day. Effort should be made to distribute the feed evenly over the water surface to allow all fish simultaneous access to the food. Fish should be hand fed approximately hourly until dusk for a total feed amount equal to 10-15% of the fish mass stocked.

After the first day, feed is distributed to fish on a continuous basis using automatic feeders. At OI, a 12-h belt feeder is installed on to the rim of the tank, and placed slightly off center from the spray bar to facilitate even feed distribution. In larger tanks, or at higher density, suspended vibrating feeders can also be used to deliver the feed to ensure good distribution. The initial feeding rate should be 20% of the total wet weight of the stocked juveniles. This feed amount is
increased 10-15% per day; calculated by multiplying the previous day’s feeding amount by 1.15. Daily feed amounts are divided in half and each spread across the feeder belt at approximately 0800 h and then re-set again at 1600 h. This ensures fish will have food available 24h/day. Additionally, at the beginning and end of the day, fish should be hand fed a small portion of the ration to gauge feeding response.

If the feed is being applied correctly, it is common to observe a small amount of feed remaining on the bottom, centered around the standpipe. Feed should be decreased if the water is cloudy, a low DO level is present, or a large quantity of feed covers the bottom of the tank.

Figure 5.7. Photographs of feed bags for BioVita Fry by Bio-Oregon (left) and Marine Grower by Skretting (right). The Bio-Oregon feed is used in the early stages of nursery and the Marine Grower feed is used at the end of the nursery stage and throughout the remainder of the grow-out cycle.

Diet

Moi can be grown on high quality commercial marine fish diets (Fig. 5-7). The OI hatchery uses Otohime Diets (Reed Mariculture Inc. CA, USA) for weaning (A1 from Day 8-20; B1 from Day 16-25) and BioVita Starter (Bio-Oregon, Longview WA, USA) for nursery stages. Both diets are readily available in the USA. The sizes of the feed particles used are #0 (0.3-0.7mm) used from Day 25-35, #1 (0.6-1.2mm) used from Day 30-45, and #2 (1.1-1.9mm) used from Day 40-45. The exact time or phase to introduce a larger feed particle depends on the growth rate of
juveniles. Larger feed particles should be introduced slowly by mixing with smaller particles for a few days. Behavior should be observed to ensure fish accept the larger particles. Fish will normally accept a 2.0-2.5mm pellet by Day 45. The grow-out diet (3.0-6.0mm, used from Day 60 onward) currently being used widely in Hawaii is the Marine Grower diet from Skretting Inc. (Canada).

Growth

At 26°C, juveniles will normally grow from 0.05g to 1g and 15mm to 40mm from D26 to D40. However, growth rates are largely temperature and food dependant and can vary widely when conditions are not optimal. Instantaneous weight gain is approximately 20% body weight daily, during that period. As fish get larger (approximately 1g at D40), this growth rate decreases to approximately 10-12% per day (Fig. 5-8). Moi will normally reach approximately 11g by D60.

Figure 5.8. Moi reared under optimal conditions will have uniform growth, although some variation is always present. This photo of 100 fish collected at day 50 provides a good example of what the average population should look like.
Figure 5.9. Grading of fish causes additional stress and should be avoided if possible.

Grading

If the outlined system design and operation practices are followed, large intra-cohort growth differences will be minimized. This will greatly reduce the frequency and effects of cannibalism. However, if large differences in growth are observed during the initial stages of the grow-out period, grading of fish may be required. Juvenile Pacific threadfin can be effectively size sorted using a 5/64 or 6/64 inch stainless steel grader (Fig. 5-9). It should be emphasized that grading of fish does cause stress and can increase mortality and, therefore, should only be performed when other methods to reduce size differences have been exhausted.

Survival

Following the techniques and procedures outlined here, survival during this phase (Day 26-60) typically averages between 80-90%. Normally, some mortality is observed just after transfer into the nursery tank. However, this mortality should taper off quickly, with very little observed mortality from D28 forward.

Factors such as poor water quality, wrong feed size or inadequate feed distribution may increase daily mortality beyond 5%. Therefore, close attention must be paid to feed consumption, size distribution of fish and daily observed mortalities. Keep in mind cannibalism can also account for unseen mortalities, and can comprise a significant portion of total mortality.
Water Quality

Water quality parameters (Temp, Salinity, DO, pH) should be checked at least twice daily (0800 and 1600 h). Since this stage of production utilizes large amounts of dry feed, with fish at high density, DO becomes one of the most important parameters to monitor (Table 5.2). It is recommended that DO be monitored continuously via a submerged probe that can be coupled with an audible alarm.

**Table 5.2: Optimum physicochemical parameters of the nursery water**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Optimum Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>25-27</td>
</tr>
<tr>
<td>Dissolved Oxygen (mg/L)</td>
<td>5.0-6.8 mg/L (minimum 3.5 g/L)</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>32-34</td>
</tr>
<tr>
<td>pH</td>
<td>7.7-8.3 (minimum 7.5, maximum 8.5)</td>
</tr>
<tr>
<td>NH₃ (mg/L)</td>
<td>0</td>
</tr>
<tr>
<td>NO₂ (mg/L)</td>
<td>0</td>
</tr>
</tbody>
</table>

**Dissolved Oxygen**

Optimum DO level in the nursery tank ranges between 5.0-6.0 mg/L. DO levels below 3 mg/L will kill fish. Water flow or aeration/oxygen should be increased when DO falls below 5.0 mg/L. Oxygen consumption at this stage has been estimated at $0.177 \pm 0.035 \text{ mg O}_2/\text{g/h}$.

**Temperature**

Water temperature has a very large effect on growth of fish. Growth rates at 22-24°C are nearly half that at 26-27°C. Rearing moj at temperatures warmer than 27°C has not been investigated, but may provide for faster growth, shortening the nursery phase even further. At OI, our water temperature is very constant 26-27°C, and therefore all growth data provided are from fish reared in this temperature range.

**Water Exchange**

Good water exchange will ensure high DO levels and maintain water quality parameter at optimum conditions. Optimal water flow rates will vary depending on the size of tank and
number of fish stocked. At OI, we utilize 20m³ tanks stocked with ~80,000-100,000 fingerlings. Initial water flow rates are ~100L/min at day 25 and increase to ~500L/min at day 50 (Fig. 5-2).

**Daily Maintenance**

**Observation**

It is very important to observe the behavior of juveniles and the tank environment in the morning, before starting daily activities. Juveniles should be swimming against the current, almost belabored, and should be distributed across the middle two-thirds of the tank bottom away from the tank sidewalls and center drain. This indicates an appropriate current speed. Bunching near the center drain pipe or swimming with the current indicates that the current speed is too high. Fish milling about all areas of the tank indicate that the current speed is too low. Also, any chasing or cannibalism noted along with a lack of any feed residue is an indication that feed amounts are too low. Excessive feed buildup (i.e., more uneaten feed than feces is observed in the tank) is an indication of overfeeding. Remedial actions should be quickly taken.

**Cleaning**

The nursery tanks require daily cleaning to maintain optimal hygiene and fish health (Fig. 5-10). A simple siphon can be constructed using a 3 ft. section of ½” rigid PVC pipe and a flexible hose. The siphon can be utilized to remove any dead fish, excess feed, or feces. The number of dead fry should be estimated and subtracted from the total number stocked in the tank. After siphoning, a soft scrub pad should also be utilized to remove biofilm accumulation on the tank bottom and sides. This pad can be mounted on the end of a long pole so that the technician can clean the tank from the outside. Air lines and airstones will also require daily cleaning.
Figure 5.10. Tank cleaning is very important to maintain water quality and reduce the total bacteria load in the water.
Harvest, Packing and Transport

When the Nursery Phase and growout tanks or ponds are located at a different site from the hatchery, the fry will need to be transported. Packing and transport method will depend on the distance travelled. Fish can be safely transported with minimum mortality to distances that take up to ten hours. Preparation for transport takes place 1-2 days before the shipping date. Juveniles are not fed 24 hours before shipping. This ensures that their guts are empty upon shipping to avoid accumulation of feces in the transport system, which will deteriorate water quality. Information about the destination needs to be collected prior to shipping. The most important information is the location’s water quality and temperature. In-tank water temperatures prior to shipping should be similar to that of the destination site.

CTSA sponsored a small-scale two-year project to develop a practical test system for study of fingerling transport in effort to identify current safety limits for safe transport for the Pacific threadfin (moi), and identification of the critical factors affecting fingerling transport survival (Liu & Laidley, 2008).

Data from that project demonstrated a strong density-dependent effect of fish handling and transport on short-term fingerling survival. When moved under “standard” procedures typical of the trade, researchers began seeing significant fingerling mortality at densities greater than 10g fish per L (10kg/m³) (Fig. 5-11)

![Figure 5.11](image)

**Figure 5.11.** Transport stress-related mortality of 4g moi fingerlings during simulated transport at densities ranging from 10 to 50 g/L.
Survival at higher densities was effectively increased by exchanging transport water after handling fish to remove released scales and mucous, and by lowering transport water salinity to 25ppt.

Moi fingerlings also demonstrated a significant size-dependent sensitivity to transport stressors. Larger fingerlings exhibited much higher survival rates than at the smaller sizes at which fingerlings are normally shipped to offshore cages. However, the benefits of increasing tolerance to handling and transport stressors must be balanced by the need to move larger fish biomass, which counteracts many of the benefits associated with improved survival rates.

**Short Distance (Land)**

Juveniles are transported on land in a double-wall plastic transport tank (Fig. 5-12). Fill the transport tank with water of the same salinity as in the nursery. Aerate the water with pure oxygen. Collect the juveniles from the nursery tank with a pai-pai net and place them into the transport tank. Determine the average weight per larvae (using the same method described in the harvest section). Normal juvenile stocking density is approximately 7 juveniles per L. Higher densities can be used for shorter transports. Higher stocking densities can also be achieved by cooling the transport water. When fish reach the destination, the temperature should be checked in the transport tank as well as the destination. If temperature differences are greater than 5°C, the juveniles should be acclimated to the same temperature by placing water from the destination tank into the transport tank. Wait 15 minutes and check the transport tank. When the water reaches the same temperature as the destination tank, release the juveniles. If the temperature difference is 1-2°C, the juveniles can be transferred directly into the destination tank or pond. Lack of oxygen is undoubtedly the main reason for massive mortality during the transportation time. For this reason, it is very important to adjust the stocking densities, water temperature, volume, and aeration. Oxygen levels should never fall below 5.0 mg/L in the transport tank.

**Long Distance (Air)**

A different technique using smaller fish (D30, approximately 25mm) is used when air transportation is required and over long periods of time (3-10 hours). The preparation for air transport is more elaborate. Styrofoam coolers and cardboard boxes (30x36x7-1 cm) are used. Each cooler accommodates two plastic double bags (Fig. 5-13).

To prepare the bags for stocking, place a double plastic bag into a 15L plastic bucket. Add 8-9 L of water to the shipping bag. A subsample is taken to calculate the average weight of the juveniles. Typically, fish weigh 0.1g at D30. A density of 50-70g biomass can be placed into each bag, or approximately 500-700 juveniles. A total 1,000-1,500 fish are shipped in each box.
Fish are caught with hand nets, weighed, and placed into the bucket with the shipping bags. Air remaining in the inner bag is removed by holding the inner bag near the top and squeezing. The bag should be inflated with pure oxygen to a degree that it resists moderate finger pressure. The bag is then twisted and folded over and fastened securely with an elastic band or zip tie. The inner bag should be lightly squeezed to make sure there are no leaks. The outer bag is squeezed and sealed in the same manner.

A second bag of fish is prepared in the same manner. Both bags are placed on their sides so that the twisted sides are facing each other and the corners of the bag are against the corners of the Styrofoam cooler. This configuration allows maximum swimming area for the fish and reduces the chance of juveniles becoming trapped in a pinched corner. The lid of the Styrofoam box is placed on the cooler.

The cooler is then placed into a cardboard box and sealed with strapping tape. The cardboard box should be kept in the shade and protected from getting wet. The box can be covered with a plastic tarp until shipped.

Figure 5.12. Transport tanks supplied with pure oxygen are used for transporting fingerlings short distances over land.
Figure 5.13. Styrofoam coolers in cardboard boxes are used to ship fingerlings longer distances by air.
Chapter 6 – Health Maintenance

Figure 6.1. Moi gill tissue sample showing parasite infection.

Disease Prevention and Control

Prevention is the best defense against disease. Disease prevention methods for broodstock include using aseptic techniques, maintaining good water quality and a clean tank, proper stocking densities, and providing quality feed. For larvae and juveniles, hygiene is most important and must be incorporated into the daily activities of the hatchery. Cross contamination from live food cultures and from larval rearing tanks must be avoided. *Artemia* nauplii should be rinsed with sterilized or fresh tap water before being introduced into the larval rearing tank. At the end of a production cycle, all tanks should be cleaned thoroughly and rinsed with freshwater. After each larval rearing season the hatchery is shut down and cleaned. All pipes are pressure cleaned and all units are washed and disinfected with chlorine, then rinsed with fresh water and allowed to dry.
Aseptic Techniques

Avoid contamination from one source to another. It is important to clean all equipment (nets, barrier, thermometer, etc.) after use and before placing into another tank. Rinsing tanks with freshwater and allowing them to dry (when possible) between hatchery runs is a good practice. Humans can also be a source of contamination. Technicians should clean only one tank a day to avoid potential contaminant transmission to unaffected tanks. In addition, daily tasks with the highest risk level should be performed last. For example, feeding should be conducted before tank cleaning, and tank cleaning before necropsy.

Water Quality

Maintain optimal conditions by providing sufficient water exchange and aeration. Provide a stable environment without sudden, drastic changes in temperature and salinity. Clean tanks regularly.

Observation

Observe fish daily for any unusual behavior. Common signs of disease include: loss of appetite, lethargy, irregular swimming patterns, and flashing (i.e., fish scrape their sides along the bottom of the tank).

Examination

Screen fish periodically for diseases or parasites. Anesthetize and examine fish for parasite or irregularities (swelling, discoloration, fin deterioration, bleeding, etc.). Examine scrapings of gill and skin tissue under a compound microscope (Fig. 6-1).

Isolation

An infected fish or tank of fish should be isolated to prevent the disease from spreading. Clean and/or disinfect all personnel and equipment that comes into contact with diseased fish.
Rapid Response

Even under optimal conditions, disease and parasite outbreaks occur. Diseases can often exist at low levels, waiting for the right opportunity or environment to multiply. Early detection and prompt treatment are important methods for controlling diseases. Hesitating even one day could result in the loss of an entire tank of fish. Once a disease is detected and identified, treatment should begin immediately. A quick response should decrease the number of mortalities.

Specific Pathogens and Conditions

Broodstock

At the Oceanic Institute, catastrophic Pacific threadfin mortalities have been caused by only two diseases, *Amyloodinium ocellatum* and *Cryptocaryon irritans*. Placing fish in freshwater for five minutes and then transferring to a clean tank is an effective treatment. Some fish have more than one disease, requiring different treatments. However, simultaneous treatments will often result in mortalities. Conduct treatments one at a time in order of urgency and allow for an interim recovery period.

Oodinium

*Amyloodinium ocellatum* is a common marine parasitic dinoflagellate that is round, oval, or pear-shaped. The trophont, ranging from 0.04 to 0.1mm, is found on the gill and skin of Pacific threadfin. The trophont appears light-brown to yellowish in color. Once mature, trophonts fall off hosts and within minutes form cysts. These cysts reproduce and release free-swimming flagellated dinospores. The dinospores remain alive 12-14h, during which time they can attach to another host.

**Diagnosis:** Clinical signs include respiratory distress (flared gills), flashing, loss of appetite, and lethargy. If any of the above conditions are noticed, examine gill and skin tissue under a microscope.

**Treatment:** Place the infected fish in a freshwater bath for five minutes, followed by transfer to a clean tank. Repeat three times, once every three days. Following treatment, examine gills and skin to determine if the treatment was successful. For severe outbreaks, increase water flow to 12 turnovers daily.
Cryptocaryon

*Cryptocaryon irritans*, also known as saltwater “ich” or “whitespot disease”, is found on the gills and skin of Pacific threadfin. This protozoan is round or oval and ranges in size from 0.03 mm to 1.0 mm. The trophont is surrounded by cilia and has a c-shaped macronucleus. Once mature, trophonts fall off the hosts, attach to a substrate, and form a cyst. Cysts reproduce, producing hundreds of free-swimming ciliates. Ciliates die if they do not attach to a host within 24 hours.

**Diagnosis:** Typically, a patch of white spots can be seen near the dorsal fin of Pacific threadfin. Behavioral observations include flashing, loss of appetite, lethargy, and respiratory distress. Microscopically examine gills and skin, particularly any white patches.

**Treatment:** Again, the most effective treatment is a five minute freshwater dip followed by transfer to a clean tank. Repeat 3 times, once every three days. Increase turnover rates for severe outbreaks.

**Other Symptoms**

Pacific threadfin have had other symptoms of disease, such as exophthalmia (pop-eye), blindness, fin rot, loss of balance, curved spine, and tumors (Fig. 6-2). There are many factors that can cause these symptoms, including bacterial and parasitic infections, poor water quality, nutritional deficiencies, collisions with tank walls, and heredity. However, these symptoms occur in such low numbers (1 out of 100) that it is easier to remove affected fish than to treat them.

*Figure 6.2.* Photograph of thyroid tumor (goiter) under moi gills.
Larvae and Juveniles

Marine fish larvae and postlarvae are particularly susceptible to protistan infections. These have not been experienced in large numbers in the Pacific threadfin hatchery at OI.

Protistan Infections

Primary routes of protistan infections into the larval rearing system are through egg transfer from the broodstock tank and egg collector, or through the live food. For this reason, broodstock management is very important. The Broodstock must be maintained under optimum environmental conditions. Daily observations of behavior and routine parasite checks must be performed. The bacteria protozoan or metazoan most frequently introduced from the broodstock tanks are Vibrio sp, Oodinium, Cryptocaryon, trematodes, and Argulus. During the nursery phase, juveniles are commonly infected by ciliated protozoans (Trichodina sp). This parasite attacks the gills. Infected individuals will display an erratic swimming behavior and flashing.

Diagnosis: Vulnerability to pathogens can be increased in larval rearing and nursery tanks by increased stress. Stress is increased by poor water quality, inadequate diet, over feeding, and improper or excessive handling. Careful observation of the feeding and swimming behaviors of larvae and juveniles will allow early diagnosis and treatment.

The first indication of a disease outbreak is an increase in daily mortalities. Feed consumption may be down in the tank. Unusual swimming behavior such as erratic swimming, flashing, or floating on the surface may be observed. When mortalities rise above 5-6% per day, this is an indication that a problem exists.

Treatment: Suspected individuals are removed immediately and isolated. The water exchange rate is increased to reduce bacterial or parasite densities. Since the United State Food and Drug Administration (FDA) has not approved any chemical for use with Pacific threadfin, a change in salinity is the only legal treatment for commercial hatcheries in the United States.
Deformities

The primary deformity noted in the Pacific threadfin hatchery is opercular malformations. Hatchery runs conducted at OI over the past have indicated that an average of 25% of harvested larvae exhibit some form of opercular deformity.

**Diagnosis:** The types of deformity noted range from wrinkled to missing or shortened opercula. Research has indicated that the gill deformities occur within the first two weeks of life and are independent of size at harvest. Unilateral and bilateral deformities occur. Approximately 9% of fish harvested have at least one missing opercula.

**Treatment:** Gill deformities have minimal effects on growth and survival under normal rearing conditions, but remedies for gill deformities are currently under investigation.
References


Appendix

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