

Determining Stocking Strategies and Culturing Winter Flounder (*Pseudopleuronectes americanus*) for Enhancement Programs



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Preface

This research project seeks to initiate restocking of winter flounder. Restocking is the “release of cultured juveniles into wild populations to restore severely depleted spawning biomass to a level

where it can once again provide regular, substantial yields” (Bell et al. 2008). Winter flounder enhancement research has never been proposed (or carried out) at this level in terms of numbers of fish stocked and regional participation involving fishing communities, scientists and aquaculture industry partners. Joining forces with the municipalities on Martha’s Vineyard, Mass. and the town of East Hampton, N.Y., all of which have a long and rich winter flounder fishery history, as well as nearby hatcheries capable of producing juvenile fish, is a logical next step in winter flounder enhancement research.

The purpose of this manual is to guide project participants with both field survey techniques and aquaculture training in conjunction with a training workshop held Nov. 10-11, 2010 at the University of New Hampshire (UNH).

Chapter 1 provides an overview of the research project, including goals and a timeline, as well as a brief history of winter flounder enhancement research. Chapter 2 lays out the methods for the year-long ecosystem analyses in the town/state waters that will be the first step in this multi-year regional project to explore restocking winter flounder. The data collected in these ecosystem surveys will be used to provide guidelines for stocking strategies. Chapters 3, 4 and 5 are the bulk of this manual and focus on how to raise winter flounder and live feeds. While we describe winter flounder and live feed production at UNH, we acknowledge that there are other variations to these methods that can be used with success. Fish produced by the hatcheries in Year 2 of this project will be used for large-scale pilot releases; stocking methodology as well as pre- and post-release sampling are discussed in Chapter 6. Chapter 7 describes how the project will be evaluated.

We remind users that this is a research project, and as such there is always some degree of uncertainty. Our hope is that this project indicates winter flounder populations can be aided through restocking, and that this manual will be of use to others who want to expedite the recovery of winter flounder.



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Chapter 1: Project Overview

The purpose of this research project is to initiate a regional winter flounder restocking effort following the “responsible approach” guidelines in two locations (Martha’s Vineyard, Mass., and East Hampton, N.Y.). This project is a regional collaborative effort that includes fishermen, scientists, the aquaculture industry, and fisheries managers who will engage in research to find ways to protect and enhance the winter flounder and its fishery.

Biomass of winter flounder, *Pseudopleuronectes americanus*, a target species of both recreational and commercial fisheries, is at an all-time low, and due to new unprecedented regulations, the largest of the three stocks is closed to all fishing activities in federal waters. Winter flounder population recovery could be expedited by enhancement (Waters 1996; Le Francois et al. 2002), and experimental restocking studies have been conducted since 1996 by scientists at the University of New Hampshire (UNH). The goal of past projects has not been to initiate large-scale releases. Instead, the “responsible approach” (Blankenship & Leber 1995; Lorenzen et al. 2010) has been applied to develop the processes needed to successfully enhance winter flounder by answering key questions in the event that large-scale restocking efforts occur. These experiments have shown that:

- 1) Winter flounder can be successfully cultured, tagged, conditioned and released into the wild, and
- 2) Released winter flounder then “behave” much like wild fish in that there are similarities in habitat utilization, movements, home range, growth and diet.

Unfortunately due to the lack of adequate space at UNH for large-scale, on-shore aquaculture, rearing the magnitude of hatchery fish to fully test the efficacy of restocking winter flounder has not been possible.

The possibility that restocking winter flounder could help the diminished wild stocks has elicited interest from several New England communities. Of these, officials and fishermen from both Martha’s Vineyard, Mass. and East Hampton, N.Y. have sought collaboration with UNH. Because both towns: a) have shown both enthusiasm and good organization; b) have historically high winter flounder populations, high quality winter flounder nursery areas, and existing wild spawning winter flounder stock; and c) have nearby aquaculture facilities capable of producing enough juvenile winter flounder for releases, UNH is working with these two communities to scientifically test, on a large scale, winter flounder enhancement strategies.

Rationale for Stocking Winter Flounder

Winter flounder is a commercially and recreationally important demersal flatfish found along the northwestern Atlantic coast, ranging from Georgia, USA, to Labrador, Canada, but is most abundant from Nova Scotia to New Jersey (Perlmutter 1947; Scott and Scott 1988). It is a long-lived flatfish and can reach a maximum age of 15 years and a maximum length of 58 cm (Fields 1988). Winter flounder is a hardy, eurythermal, euryhaline and freeze-resistant flatfish; it is prized for its thick, white fillets, which have a high market price compared to many New England flatfish. The species is typically exploited in coastal locations, although offshore areas, particularly Georges Bank and Nantucket Shoals, support important winter flounder fisheries as well. U.S. commercial and recreational fisheries for winter flounder are managed in federal waters under the New England Fishery Management Council's Northeast Multispecies Fishery Management Plan (FMP) and in state waters under the Atlantic States Marine Fisheries Commission's Fishery Management Plan for Inshore Stocks of Winter Flounder. In the U.S., winter flounder

stocks are classified as the Gulf of Maine (GOM), Georges Bank (GB) and Southern New England/Mid-Atlantic (SNE/MA) stocks. As with most groundfish species, catches have declined precipitously in recent years. For example, total U.S. commercial landings of all three stocks in 1981 were about 17,575 mt. Since then, catches have declined dramatically to 2,657 mt in 2007, the lowest landing of the 45-year time series (NEFSC 2008).

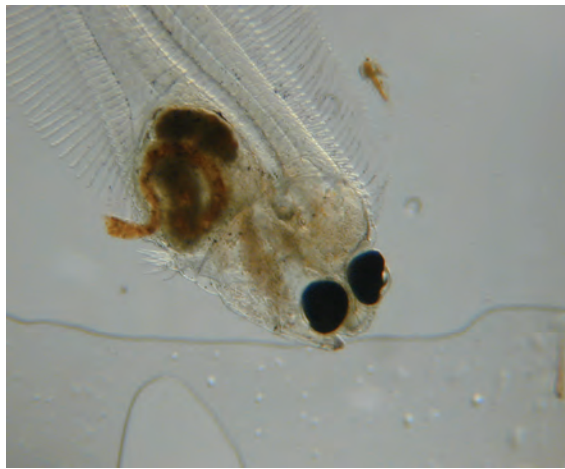
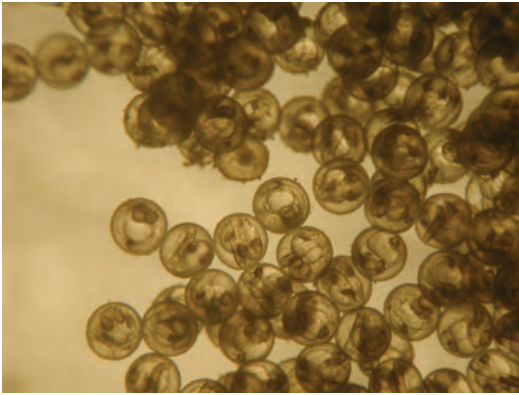
The goal of the current U.S. management program for winter flounder is to reduce fishing mortality to levels that will allow stocks to rebuild above minimum biomass thresholds and then remain at or near target biomass levels.

Despite strict regulations, winter flounder are

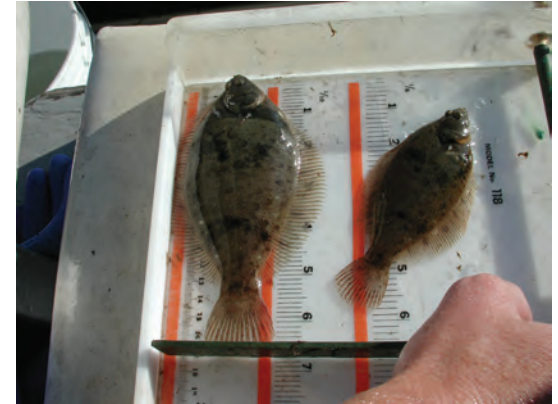
not rebounding. The most recent Groundfish Assessment Review Meeting (GARM III) report indicates that the SNE/MA stock is overfished and overfishing is occurring (NEFSC 2008). Spawning stock biomass (SSB) for this stock is very low; in 2007 SSB was estimated to be only 3,368 mt – about 9% of the SSB that would result in maximum sustainable yield (SSB_{MSY}). Partly because of this, the 2006 year class is the smallest year class on record (3.6 million). Even if fishing mortality is reduced to 0 during 2009-2014, scientists project that there is only a 1% chance that the stock can be rebuilt to SSB_{MSY} by 2014. In an unprecedented move, NOAA closed the largest of the three U.S. stocks (SNE/MA) to all fishing in federal waters in fall 2009 (NOAA 2009), and in New York there is at least one petition to close the inshore fishery as well (NY CCA 2008). While it is hoped that these more stringent fisheries regulations will allow winter flounder populations to rebuild to historic levels, recovery will not happen quickly. To jump-start the recovery of winter flounder stocks, using environmentally responsible enhancement techniques as an additional fishery management tool may be effective. In this, juvenile fish are stocked to overcome the high mortality associated with vulnerable early life history stages that prevent recruitment into the fishery. Many coastal communities are interested in pursuing this strategy and are looking to UNH for guidance.

Fishing boats in Montauk, N.Y.





Winter flounder eggs (left), juvenile eating Artemia (above) and adults being measured (right).



Culturing winter flounder for market and rearing juvenile fish for re-stocking are viable options for reducing fishing pressure and rebuilding this species (Waters 1996; Litvak 1999; Howell and Litvak 2000), and both have been the focus of research for well over 100 years. As early as 1890, wild broodstock were captured in fyke nets near Woods Hole, Mass., and spawned in government hatcheries (Bean 1890). Because winter flounder has been used as a model for laboratory experiments for 100+ years, much of the rearing protocol has been developed. Detailed winter flounder aquaculture techniques have been reviewed (Howell and Litvak 2000; Fairchild, 2010). Winter flounder have been cultured annually at UNH since 1996 where many aquaculture techniques have been refined (Fairchild and Howell 2001, 2004; Fairchild et al. 2007; Fairchild 2010). Despite operating from a small research facility (approx. 1,000 sq ft), Fairchild and colleagues consistently are able to produce winter flounder juveniles (approx. 20,000). At least 50,000 juvenile winter flounder (enough for large-scale experimental pilot releases) can be produced by relatively small hatcheries ($\geq 2,000$ sq ft), either in re-circulating or flow-through seawater systems.

Recent declines of many marine species have caused a renewed interest in marine fish stocking and enhancement, potentially useful tools to boost declining natural populations when used in conjunction with proper resource management. Winter flounder were among the species strongly suggested by a panel of fisheries and aquaculture experts in 1996 for further study (Waters 1996). As a result, the first winter flounder stock enhancement research project was initiated at UNH in 1999 and funded by N.H. Sea Grant (R/FMD-158, H. Howell PI). This research continues today as part of a larger marine stock enhancement mission, the Science Consortium for Ocean Replenishment (SCORE; <http://www.stockenhancement.org>). SCORE, funded by NOAA, is contributing financial and intellectual support towards this current regional winter flounder enhancement research project.

Project Goals

Year 1:

Train at least 12 regional project participants and conduct ecosystem analyses to determine appropriate stocking strategies for winter flounder in Martha's Vineyard, Mass. and East Hampton, N.Y. estuaries.

Specific Goals for Year 1:

1. Hold a training workshop for all project personnel, produce instructional materials.
2. Conduct ecosystem analyses in each region to determine appropriate stocking strategies for winter flounder by:
 - a. Evaluating the temporal and spatial abundance of wild winter flounder populations in each estuary.
 - b. Describing the size class distribution of winter flounder in each estuary.
 - c. Mapping the spatial use of the estuaries by different size classes of winter flounder.
 - d. Evaluating the temporal and spatial abundance of potential predators of juvenile winter flounder populations in each estuary.
 - e. Measuring the availability of potential prey of juvenile winter flounder in each estuary.
3. Make recommendations regarding winter flounder stockings based on ecosystem analyses.
 - a. Identify which season(s) and site(s) show promise for winter flounder stockings.
 - b. Based on the best stocking season/site combination, determine the most successful size-at-release for cultured winter flounder.
4. Prepare hatcheries for winter flounder aquaculture. Ensure hatcheries adopt responsible production techniques for raising fish fit for enhancement.

Year 2:

Conduct pilot-scale releases of 50,000 winter flounder in both Martha's Vineyard, Mass. and East Hampton, N.Y. estuaries and evaluate impact.

Specific Goals for Year 2:

1. Initiate production of winter flounder on Martha's Vineyard.
2. Produce and tag up to 50,000 juvenile winter flounder in each region for pilot-scale releases.
3. Test and compare two different release strategies in each region and evaluate the overall successfulness of the releases by:
 - a. Estimating the mortality (survival) of released fish.
 - b. Describing the diet transition in released fish.
 - c. Studying the movements of released fish.
4. Determine the impacts of the project through a final workshop.
5. Disseminate results to stakeholders.

Project Timeline

Timeline

Table 1.1 illustrates the project schedule. The timeline for Year 1 is straightforward. Since there are differences between regions (spawning time, for example) and the stocking strategy for Year 2 is not known yet (this will be determined based upon data collected in Year 1), there is some ambiguity in the timeline in Year 2. This will become clearer by the end of Year 1.

	2010												2011												2012											
	November	December	January	February	March	April	May	June	July	August	September	October	November	December	January	February	March	April	May	June	July	August	September	October	November											
Choose sampling stations and train personnel																																				
Bi-weekly sampling in estuaries																																				
Send data and benthic samples monthly to UNH																																				
Benthic sample analyses																																				
Data Analyses																																				
Prep Martha's Vineyard hatchery																																				
Start up rotifers																																				
Bring in broodstock																																				
Winter flounder culture, including live feed production																																				
Tag and release fish; pre- and post-stocking surveys																																				
Data Analyses																																				
Final workshop																																				

Responsible Approach

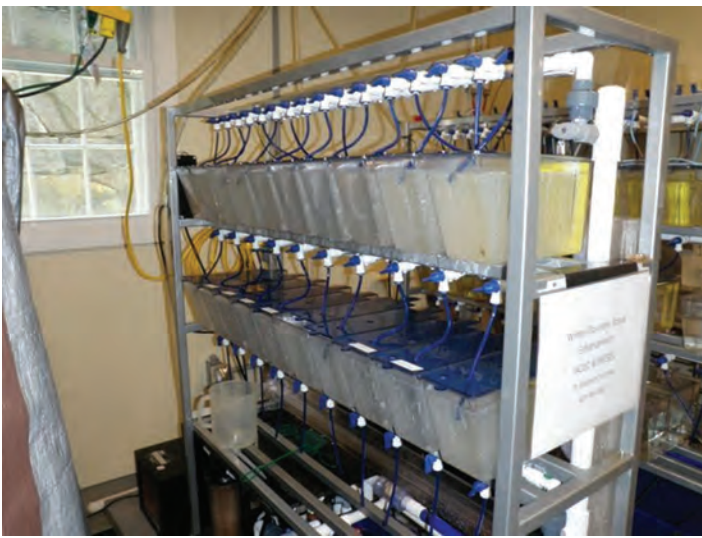
The central issues associated with developing and evaluating stock enhancement programs are described by Blankenship and Leber (1995) and Lorenzen et al. (2010), and are known collectively as the “responsible approach.” The responsible approach is based on multidisciplinary science to select appropriate stock enhancement candidate species and to rigorously evaluate pilot enhancement methods, strategies and programs. Included in responsible approach are three stages: 1) initial appraisal and goal setting; 2) research and technology development including pilot studies; and 3) operational implementation and adaptive management. Within these stages are a total of 15 elements that need to be addressed if enhancement is to be developed responsibly. The responsible approach has been incorporated into winter flounder enhancement research at UNH and is an integral part of this project. For further reading, both responsible approach papers (Blankenship and Leber 1995; Lorenzen et al. 2010) are included in the Appendix.

Lessons Learned at UNH in Winter Flounder Enhancement Studies



The goal of past projects has not been to initiate large-scale releases. Rather, our “responsible approach” has been to develop the process needed to successfully enhance winter flounder by answering key questions about optimal release strategies with small releases (<2,000 fish) in the event that large-scale stocking efforts occur. The studies we have done have been very successful. We have found that:

- cultured fish are more vulnerable to predators unless conditioned (Fairchild and Howell 2004);
- green crabs are a main predator of juvenile winter flounder and affect flounder distribution in N.H. (Fairchild and Howell 2000);
- released fish have similar diets to wild fish (Fairchild 1998);
- cultured fish begin feeding in the wild 12-18 hours post-release (Walsh Dissertation, in prep);
- cultured fish require two days to hone burial skills in sediment and can drastically change color in this period (Fairchild and Howell 2004);
- cultured fish have similar growth and survival to wild fish in-situ (Fairchild et al. 2005);
- many tags are effective for juvenile flounder, including visible elastomer and coded wire tags (Sulikowski et al. 2005), as well as acoustic transmitters (Fairchild et al. 2009) and t-bar tags (Fairchild, unpublished data);



*Learning
from the
past to
improve
the future.*

- fish can be transported at densities as high as 400% (fish bottom surface area to tank bottom area) as long as a two day recovery period is provided at the release site (Sulikowski et al. 2006);
- cultured fish can be analyzed histologically for sex ratio at 41mm TL (Fairchild et al. 2007), and sexual differentiation does not seem to be influenced by rearing temperature (Fairchild, unpublished data);
- many favorable release sites exist in N.H. waters (Fairchild et al. 2005);
- wild juvenile abundance is low at these sites (Fairchild et al. 2008b);
- cultured fish utilize similar habitats and have similar movements (<20m/d) as wild fish (Fairchild et al. 2009);
- acclimation cages aid the fish in adjusting to their new environment (Sulikowski et al. 2005; Sulikowski et al. 2006);
- benthic acclimation cages attract predatory green crabs (Fairchild et al. 2008a);
- caged fish maintain higher site fidelity than fish that were not caged (Fairchild et al. 2009);
- fish respond well to floating, in-situ acclimation cages (Fairchild, unpublished data);
- adults have spawning site fidelity (Fairchild, unpublished data);
- live hatchery feeds (e.g., white worms, burying marine amphipods or subadult stages of brine shrimp) provided during early juvenile stages (20-50mm), in lieu of formulated pellet feeds, increase growth and survival of flounder in the hatchery and decrease the post-release transitional feeding time in the wild (Walsh Dissertation, in prep); and,
- white worms are an ideal live hatchery feed because of rearing and harvesting ease, potential for en masse production, salt water tolerance, and a high nutritional profile (Walsh Dissertation, in prep).

Now that we understand so much about the biology, physiology, ecology and mechanics of stocking hatchery-reared winter flounder, we are ready to test these strategies through large experimental pilot-scale releases.

Chapter 2: Ecosystem Analyses

Rationale and Approach

For each region (East Hampton, N.Y. and Martha's Vineyard, Mass.), two estuaries will be studied for a 12-month period to determine the spatial and temporal distribution of the wild winter flounder population, potential predators and prey species, as well as other important parameters (water temperature, salinity, dissolved oxygen, substrate). These

Collecting data in the field.



estuaries were chosen, in part, because historically winter flounder were abundant in them, and they appear to have appropriate habitat for

juvenile winter flounder. In Mass., Lagoon and Menemsha Ponds will be surveyed. In N.Y., Napeague Harbor and Lake Montauk will be surveyed.

Sampling in each estuary will occur biweekly (twice monthly) for an entire year, weather permitting. Sampling will be conducted at several different stations spread out within each estuary. The stations will be selected by their physical parameters. They will be located in areas that are accessible at most tidal stages, have exposed sand bars or beaches at low tide for beach seine surveys, have few obstructions in the channels for trawling surveys, and will not impact other users. Although they will not be chosen randomly, there will be at least one

station in each major defining area of the estuary. All stations will be surveyed biweekly. Beam trawl and beach seine will be used on each sampling occasion to characterize the fish and macro-invertebrate community, including the wild winter flounder population. The catch from all sampling will be identified, counted and measured. To characterize the benthic community in the estuaries and, therefore, winter flounder food availability, benthic cores will be taken at each station.

Supplies Needed for Ecosystem Analyses

Motorized work skiff equipped with depth sounder and GPS
1-meter beam trawl with ¼" mesh net and tickler chains
Large bore sediment corer
Beach seine net (6 x 50 ft; delta ¼"; black) with 5-ft poles
Temperature data loggers (with necessary cables and software) and line, buoys and anchors
Measuring boards
Box sieves (as close to 1 mm mesh as possible) for washing benthic core samples
Calipers/measuring instruments
Waterproof paper, clipboard, binder, pencils, waterproof markers
Buffered formalin 10%
Dissolved oxygen meter
Salinity refractometer
Rose bengal stain
Water (Niskin) sampler
Widemouth jars for prey samples
Boots, waders
5-gallon buckets and plastic pitchers (1 qt size is good)
50-meter measuring device (can be a tape measure or 50 m length rope)

What to do at Sampling Sites

Each month you will sample biweekly (2 x month) each of the established sites.

Water quality sampling: Do this in conjunction with beam trawl sampling.

1. **Bottom water temperature:** A fixed submersible data logger (UTBI-001 temperature tidbit data logger, Onset Corp.) will record bottom temperature at each station hourly. Each data logger will be attached with cable ties to a line anchored by an 11-kg (25-lb) Danforth anchor. A surface buoy will mark the location. UNH staff will help with starting and deploying the data loggers. These will be downloaded periodically by UNH staff. The battery life of the data loggers is approximately five years.
2. **Collecting bottom water sample:** A sample of bottom water will be collected at each station with a water sampler (Niskin bottle). From this water sample, bottom salinity, temperature and dissolved oxygen will be measured and recorded on the Beam Trawl Data Sheet (only need to do once/sampling event).
 1. Open the valves and lower the water sampler to the bottom.
 2. Haul the sampler off the bottom ~1 foot.
 3. Send the messenger down to close the valves and bring the sampler to the surface.
 4. Open one side and pour the water into a pitcher or bucket.
 5. Immediately measure salinity, dissolved oxygen and temperature, and record on data sheet.

Measuring salinity:

1. Place a drop of water on the refractometer lense.
2. Close and lightly push down lid.
3. Look through ocular while pointing refractometer toward light.
4. Salinity is where the break/line is in the viewfinder.
5. Record number on data sheet.

Measuring dissolved oxygen:

1. Instructions for measuring dissolved oxygen with the YSI DO200 meter are included in the Appendix.

Beam Trawl Sampling: In areas deeper than 1.5 m, a 1-m beam trawl with a ¼" mesh liner will be used. Three replicate tows will be taken, each approximately 100 m long. The 1-m beam trawl is the international standard gear for juvenile flatfish studies. It has a low impact on the bottom and will not adversely affect the habitat. Beam trawl sampling should occur near low tide because fish will be concentrated in the channels.

1. Record date, location, tow number, tow coordinates and the time of low tide on the Beam Trawl Data Sheet (included in Appendix).
2. Cleat off bridle line to boat.
3. Fold end of net over on itself and using drawstring, secure with several half-hitches.
4. While boat is engaged at clutch speed, gently lower net over stern such that the trawl is deployed in the direction in which it will be towed. (Figure 2.1)
5. Feed the tow line out while maintaining tension to ensure that the trawl fishes correctly. You will generally want a scope of 3:1.
6. Tow against tide/current/wind (whichever is strongest) for 100 m (= 0.05 nm) at clutch speed (≤ 1 kt).
7. At end of tow, put engine in neutral and quickly haul in trawl.
8. Open codend and empty contents into a bucket or fish tote.
9. Identify, measure, count and record on data sheets all organisms caught, as well as tow depth (range and average), speed and the start and end times and GPS coordinates of the tow. Also record if cores were taken and how many.
 - a. For crabs, measure carapace width.
 - b. For lobsters, measure carapace length and record sex.
 - c. For finfish, measure total length.
 - d. For snails and sand shrimp, no measurements are necessary (just counts).
10. Release all captured organisms.
11. Repeat steps 1-10 for 2nd and 3rd tows.

*Figure 2.1:
The one-meter beam trawl deployed so that the net extends away from the stern of the boat.*



Beach Seine Sampling: In shallow water stations (<1.5 m) a 17 x 2 m beach seine will be used. Three replicate seines, each with an approximate swept area of 550 m², will be taken near low tide.



*Figure 2.2:
Walking the beach seine
parallel to shore.*

1. Record date, location, seine haul number and time of low tide on the Beach Seine Data Sheet (included in Appendix).
2. Measure out and mark on the shore a distance of 50 m. (We place the seine at the start mark and a bucket at the end mark.)
3. Stretch out net along beach and walk into water perpendicular to shore.
4. Two people (one person/pole) will walk the seine against the current. Make sure the purse/bag of the seine net is extended and the lead line is as close to the bottom as possible. It is helpful to have a third person in the water to untangle the net and make sure the bag is free floating.
5. Walking parallel to shore and keeping the lead line on the bottom, walk the seine against the current for 50 m. The inshore person should be about shin-to-knee deep, while the offshore person should be as deep as they can go. (Figure 2.2)
6. At the end of the tow it is important to keep the net moving so nothing escapes. Turn towards the shore and walk the seine completely onto the shore. Again, it is helpful to have a third person who helps gather and pull the bag up on the beach.
7. Once the seine net is on land, spread the net open and place contents into a bucket full of seawater. You may have to shake the net a few times in order to find all captured animals.
8. Identify, measure, count and record on data sheets all organisms caught, as well as the start and end times of the seine haul.
 - a. For crabs, measure carapace width.
 - b. For lobsters, measure carapace length and record sex.
 - c. For finfish, measure total length.
 - d. For snails and sand shrimp, no measurements are necessary (just counts).
9. Release all captured organisms.
10. Repeat steps 1-9 for 2nd and 3rd tows. You can either continue down the shore if enough shoreline exists (this is preferable) or repeat previous tow area.

Benthic Core Sampling: Multiple core samples will be taken at each site because benthic organisms are not evenly distributed and we want to get a good representation of what is present. Ten cores will be taken/site initially. After analysis at UNH, we will determine the minimum number of replicate cores that need to be collected. Take these benthic cores in conjunction with beam trawl sampling.

1. From the boat, in water <15 ft, hold onto end of coring handle and push coring tube firmly down into sediment.
2. Pry corer out of sediment, raise to surface and shake out contents into the plastic 0.77 mm sieve (Someone should hold out the sieve over the side of the boat.) (Figure 2.3)
3. Sieve the core sample by gently shaking the sieve in the water.



*Figure 2.3:
Benthic
core sam-
ple before
discharge
into the
sieve.*

4. Once the sediment has been washed away, use a pitcher of seawater to flush the contents into a wide-mouth container. Use the minimum amount of water necessary to wash the contents of the sieve into the pitcher to ensure that the formalin added later is not overly dilute. **Do not fill completely with water!**
5. Label container with date, location and sample number.
6. Repeat steps 1-5 until the correct number of core samples have been taken at the site.
7. As soon as you return to the office, top off the containers with 10% buffered formalin (preserves organisms) and a tiny pinch of Rose Bengal (stains all organisms pink, including your fingers). Caution: both formalin and Rose Bengal are carcinogenic, so please use care when handling them.
8. Alternatively, if weather conditions prevent comfortable processing of the cores in the field, they can be held in labeled Ziploc bags or other labeled containers for processing back on shore.
9. Each month, send core samples to UNH for analysis along with a completed Benthic Sample Inventory Sheet

(included in Appendix). At UNH, all organisms will be identified, counted and weighed. Numerical abundance and biomass will be calculated to determine the composition of the benthos.

What the Data Will Be Used For

Each month, data will be entered into spreadsheets and sent electronically to UNH. From data analyses, we will:

- Evaluate the temporal and spatial abundance of wild winter flounder populations in each estuary.
- Describe the size class distribution of winter flounder in each estuary.
- Map the spatial use of the estuaries by different size classes of winter flounder.
- Evaluate the temporal and spatial abundance of potential predators of juvenile winter flounder populations in each estuary.
- Know the availability of potential prey of juvenile winter flounder in each estuary.

Once the field work and analyses have been completed, enhancement strategies will be developed. Ecosystem analyses will guide the project participants in determining the best site in each region for pilot-scale stockings of winter flounder. These will be areas with appropriate conditions (temperature, salinity, dissolved oxygen and bottom type) for juvenile winter flounder. These areas also must be below carrying capacity; they must have excess resources

to support additional fish (i.e., food availability must exceed the needs of the wild winter flounder). The best season(s) and site(s) for releases will be the times and locations with low predator abundance yet high prey abundance. Optimal size-at-release for cultured winter flounder will be determined by the predator and prey composition at the proposed release site during the proposed release season. Survival in fish increases with size; smaller fish typically have lower survival than larger individuals due to the wide range of predators capable of eating them. However, hatchery costs increase with fish size due to the increase in space, feed and labor. The best size-at-release will be a compromise between these two conflicting demands. Knowing which predators are present (and what size flounder they can eat) will determine the most successful size-at-release for pilot-scale stockings. Based on the results of the ecosystem analyses, recommendations will be made for Year 2 of this project.



Chapter 3: Winter Flounder Aquaculture CML Style

These procedures were developed at the University of New Hampshire Coastal Marine Laboratory (CML). This facility is a flow-through hatchery/laboratory, with ambient-temperature water pumped from the mouth of the tidal Piscataqua River into header tanks on the second floor and then fed to the wet lab on the first floor. Organisms without strict requirements for the cleanest water are supplied through a simple gravity feed system. Organisms that need cleaner water are supplied by a pumped system, in which water is removed from the header tanks, pumped through a sand filter and an ultra-violet (UV) sterilizer (capable of 77 gpm at 30,000 $\mu\text{Ws}/\text{cm}^2$), and fed into a separate plumbing system. Because everything is flow through, there is no need to monitor and attempt to control nitrogenous wastes of any kind, nor to filter and treat wastewater. Adapting these procedures to a facility dependent on re-circulating systems will require significant changes, particularly with regards to water quality monitoring, stricter control of feed amounts, and treatment of effluent water.

System Requirements

Sanitation and Cleanliness

As with the live feeds, it's best to start with tanks that are as clean as possible. To that end, every part or piece of equipment that goes into the fish tanks should be sanitized using a bleach solution, an iodine solution or an acid bath. The tanks themselves should be treated similarly. At the CML, tanks are filled with freshwater, bleach is added (about 100 ml), and then they are allowed to sit for at least 24 hours. At the end of 24 hours, the tank is drained, rinsed very well, and the assembly and set-up is completed. Again, the sanitation treatment should be applied to anything that will eventually touch the water in the tank.

In a similar vein, it's best to get in the habit of working with clean hands around the fish tanks. Hatchery work, especially when setting up or taking down tanks, tends to be dirty, slimy stuff, and it's important to separate that from tanks of fragile larval fish. Get in the routine of washing hands or using hand sanitizer when working around the tanks (particularly after coming in from outside the hatchery) and try not to touch the water unless necessary. It sounds like a pain, but it's worth it if it can lower the chances of disease-related problems.

Broodstock Tanks

There's nothing particularly special about the required tanks for broodstock. They're really just holding tanks, so any quality tank that can be fed a steady supply of quality seawater will do just fine. If the space is available, it may be helpful to separate the males and females into two tanks, to make it easier to distribute them to the honeymoon tanks and also to prevent any spawning in the broodstock tanks (Figure 3.1).

Honeymoon Tanks

The honeymoon tanks are where the broodstock flounder will (hopefully) spawn, and where the eggs will incubate and hatch. Because there will only be 3-4 fish in the tank at a time, smaller tanks will work. At the CML, 0.9 m diameter circular fiberglass tanks are used (Figure 3.2). Circular tanks are preferred, but only because the favorable circulation patterns make it easier to keep the tank clean without constantly disturbing the fish. It's helpful to have as many tanks as possible for this step in the process. Not only does this give more opportunities for successful spawning, it also allows for moving egg masses into tanks untouched by wild fish, if desired.

Each tank should have some method of controlling the light and offering the fish some measure of isolation, as this will keep them calmer and hopefully help with a successful spawn. At the CML, each tank is covered with shade cloth (dark fiberglass window screen will work) secured around the tank with a length of shock cord (Figure 3.3). Each tank should also have some available method of directing fairly bright light onto the surface of the water. When the larvae hatch out they are strongly phototactic and will concentrate at the surface of a well-lit tank. This makes them much easier to collect in pitchers for the move into the larger grow-out tanks. At the CML standard clip-on lights are used for this role. Standard overhead lighting present in the facility will not be strong enough to sufficiently concentrate the larvae at the surface.

The honeymoon tanks occupy a unique place on the spectrum of water cleanliness. They will eventually hold larvae, therefore they should get the cleanest water possible (UV sterilized and filtered seawater). But they also hold wild-caught, adult fish, which will bring with them all the various bacteria and parasites that fish tend to pick up in the wild. The flow should be kept as high as practical without inducing violent circulation in the tank to ensure that the fish have enough oxygen. In circular tanks, set the flow up in a circular pattern to help concentrate debris at the center of the tank, where it can be removed via the standpipe or siphoning. As soon as some movement is seen in the eggs, the water flow will need to be reduced drastically to prevent damage to the newly hatched larvae. However, flow should remain high throughout the incubation period until that point. Too little water movement can encourage the buildup of fungus on the eggs, which will kill them.

Each tank should also have at least one airstone present to help with degassing and oxygenation. The size and number of airstones will depend on the size and configura-



*Figure 3.1 (left):
Adult winter flounder
in a broodstock hold-
ing tank.*

*Figure 3.2 (right):
Rack of 0.9 m-dia-
meter honeymoon tanks
at the CML.*



tion of the tanks being used. Supplemental oxygen is probably not necessary, assuming the water flow can be kept high. An oxygen diffuser can be added if desired, but at the CML it's never been necessary.

The honeymoon tanks can be drained by whatever method is preferred by the facility, as long as the drain has the ability to prevent the tiny, newly hatched larvae from escaping. Either external or internal standpipes can be made to work. The mesh size needed on the screen is approximately 200 μm , and care should be taken to ensure that there are no gaps that fish could get through and no obstructions that could trap them. At the CML, drainage of the honeymoon tanks is provided by internal standpipes equipped with "banjo" filters, so-called because of their resemblance to the instrument (Figure 3.4). These filters are constructed from easily attainable PVC parts and some small (200 μm) nylon mesh, available from aquaculture suppliers (Figure 3.5). Since the tanks at the CML have 2" drains, the standpipe starts with a piece of 2" PVC. On top of that is a 1 1/2" x 2" adapter to get to 1 1/2" pipe, then a 1 1/2" x 3/4" T-adapter, providing a 3/4" socket perpendicular to the standpipe. A piece of 3/4" pipe extends from this socket with a 90 degree fitting on the end. The banjo filter itself is constructed of a thin (1-2") slice of 3-4" PVC pipe covered on both sides with the fine mesh, attached with hot glue (Figure 3.6). A hole slightly larger than 3/4" is drilled into the side of the 3-4" sliver, and a short



Figure 3.3 (top left): Honeymoon tanks covered with shade cloth cover for isolation.

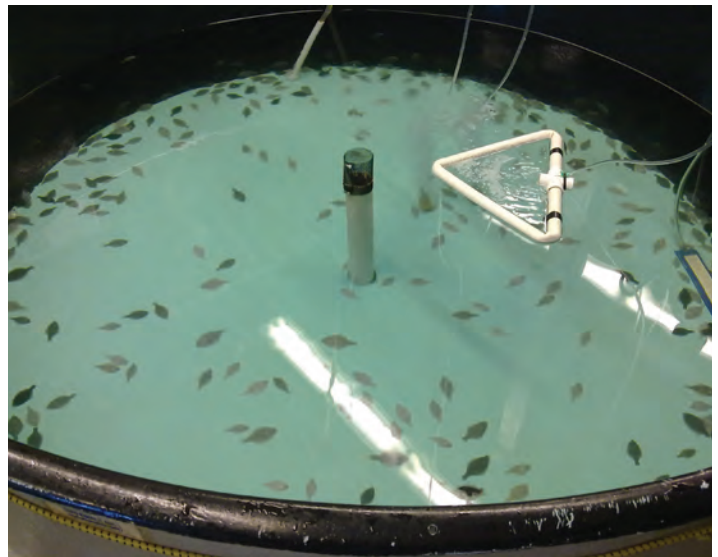
Figure 3.4 (top right): Honeymoon tank interior. Note the banjo filter setup.

Figure 3.5 (left): Close-up of banjo filter assembly.



Figure 3.6 (above): Close-up of the filtering area of the banjo filter.

Figure 3.7 (right): 1.8 m grow-out tank with 160 dph flounder. Note the water diffuser, oxygen diffuser, surface skimmer and standpipe screen.



piece of $\frac{3}{4}$ " pipe, perforated with approximately $\frac{1}{4}$ " holes, is inserted and pushed to the opposite side. The pipe is glued in place, and the filter portion is pushed into the 90 degree socket mentioned earlier. The top of the T-adaptor is open and if desired, a screen can be installed on it for safety if the banjo filter clogs. Obviously, any adaptation of this filter will work.

Grow-out Tanks

In many ways the initial set up of the grow-out tanks is just like having a larger version of the honeymoon tank. Again, the tank size, shape and configuration will vary based on the hatchery, but circular tanks are again preferred because of the reasons listed above. Within reason, the larger the tanks, the better, though again this will depend on the facility. Larger tanks can of course hold more fish, and feeding with live feed is much more convenient if there are fewer tanks to take care of. However, in some ways multiple good-sized tanks (i.e., a minimum of three tanks ranging in size from 1.5 to 3 m diameter) are preferable to one or two giant tanks, as they provide some margin of error should something go wrong with one of the tanks (water clogged, disease, etc.). At the CML, 1.8 m diameter circular fiberglass tanks with a central 2" drain are used.

At the CML, the grow-out tanks have been painted with a two-part epoxy aquaculture paint so that the tanks walls are black and the bottom is a light green color (Figure 3.7). This color combination was chosen because the dark walls improve the ability of the larvae to see their prey during the live feed stage and the light bottom allows the culturist to easily see how dense the tank is, as well as how dirty the bottom is getting, and if the fish have settled yet. The light bottom also encourages a lighter pigment on the fish. This makes it easier to see any stressed fish (since they generally darken in color) and can also help determine if a tank is too dense after the fish settle. An overly dense tank will have some fish that are substantially darker than others, having been forced to live on the dark

Figure 3.8 (right): Close-up of water diffuser.

Figure 3.9 (below): Water diffuser installed in tank. Note that 1/2" delivery hose fits snugly inside the diffuser.



sides instead of the light bottom. However, flounder have been successfully grown in tanks of many colors, so this scheme is by no means a requirement.

There is no strict requirement for light on the grow-out tanks. Obviously, there needs to be sufficient



light for the fish to see their food, but at the CML it seems that the standard (albeit fairly bright) overhead lighting is sufficient. If it's not easy to read a book at the surface of the tank, it probably needs more light. At the CML, fish are raised on an ambient photoperiod, though research continues on culturing fish with manipulated photoperiods as well.

The water delivered to the grow-out tanks should be as clean as possible, preferably UV treated and filtered in some way. It's best to deliver the flow through some sort of diffuser with multiple outlets on it in order to deliver the water to all levels of the tank. At the CML, the diffuser is simply a piece of 1/2" PVC with 1/4" holes down one side of it and an end cap (Figure 3.8). The 1/2" hose from the supply valve is inserted into the open end of the water

line (Figure 3.9). For the initial larval stage, there should be minimal current in the tank so diffusers with holes on one side can be turned so that they flow onto the wall of the tank, distributing the flow and preventing high current in the tank. Alternatively, a diffuser with holes on all sides could be used during the initial larval phase and swapped out for the previous style when the fish are stronger swimmers.

Each grow-out tank should be equipped with at least one airstone for degassing and oxygenation purposes (Figure 3.10). The size, number and position of the airstones will vary based on the size and shape of the tank. In circular tanks, airstones can sometimes disrupt the circular flow of water, thus disrupting the self-cleaning action of circular tanks. At the CML, two airstones are placed anywhere between the tank walls and the center drain during the early larval portion, since no circular flow is needed. However, when the fish are stronger and a circular flow in the tank has been established, a single stone placed right behind the water diffuser seems to provide sufficient aeration without disrupting the flow (Figure 3.11). Again, the ideal placement will vary and it's easy to experiment with different locations and sizes. Air flow will be fairly light.

Small fish will have fairly light demands on dissolved oxygen. However, supplemental oxygen supplied to the tank will help improve water quality and also offer a measure of insurance should water flow be interrupted. Welding-grade oxygen delivered to an ultra-fine pore diffuser located on the bottom of the tank is a good way to introduce oxygen to the tank (Figures 3.12, 3.13). If possible, locate the diffuser on the opposite

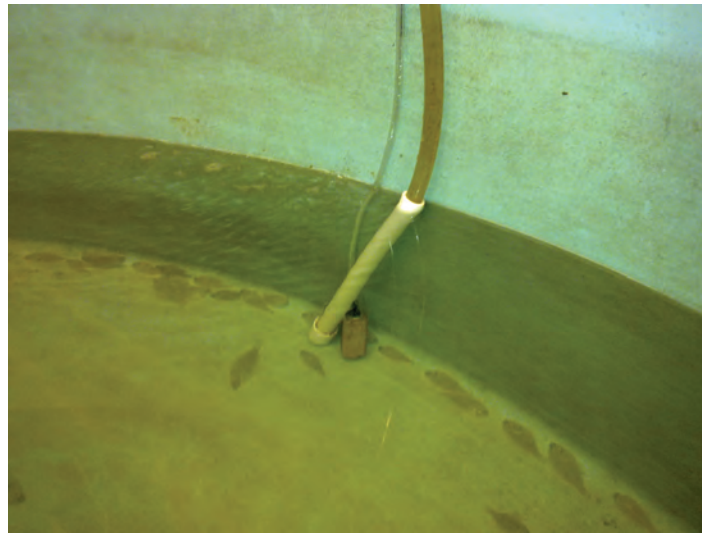


Figure 3.10 (far left): A variety of airstone sizes exist.

Figure 3.11(left): Airstone installed behind water diffuser to provide aeration without disrupting the circular flow in the tank.



Figure 3.12 (right): Oxygen cylinders with regulator installed. Note the manifold that can distribute the oxygen to more than one fish tank.

Figure 3.13 (above): Ultra-fine pore oxygen diffuser. Note the stainless steel hose clamp.



side of the tank as the water diffuser to spread out the sources of oxygen and ensure good distribution to all areas of the tank (Figure 3.14).

Drainage can be provided initially by the same system used for the honeymoon tanks. At the CML, a version of the banjo filter with a larger filter area (same 200 μm mesh) is used for the initial drain solution (Figure 3.15). As soon as the fish are big enough, the filter is switched for one with larger (approximately 500 μm) mesh. After the fish settle, the banjo filter assembly is removed, leaving a standard 2" internal standpipe. The standpipe end is screened off with a fine mesh screen (1 mm mesh) over a framework of larger plastic mesh (for stability)(Figure 3.16). The size of the screen can then be increased as the fish get larger and less prone to getting impinged.

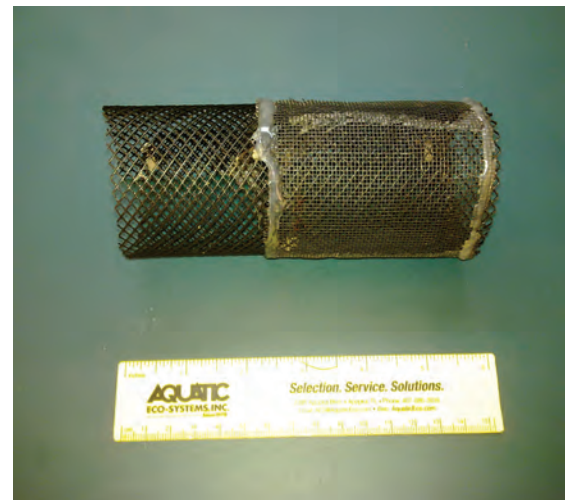
Surface scum can be a problem in the grow-out tanks, particularly when the fish are very small. Scum at the surface can trap small fish and kill them, and also hampers gas exchange at the surface. At the CML, the initial solution for surface scum is a home-made skimmer device (Figure 3.17). This is simply a $\frac{3}{4}$ " PVC pipe that has been bent into a triangular shape (with a heat gun, after having been filled with sand and the ends capped). The sand is then dumped out, and the ends of the PVC sealed with a backing material and hot glue. Where the ends of the PVC meet in the middle of a side, a "cross" fitting is installed (no PVC cement necessary)(Figure 3.18). On the top of this fitting a hole is drilled, large enough for an air fitting to fit snugly inside. The hole is drilled at an angle, so the air introduced is flowing towards the middle of the triangle. The triangle floats inside the tank, with an air hose attached to the air fitting. As the air flows over the water, it moves the surface scum through the cross fitting and into the middle of the triangle, where it is removed with a clean beaker several times a day. If extra buoyancy is required on the side of the triangle with the cross fitting, a piece of tubing can be sealed (by heating the ends almost to the melting point and pressing them flat) and attached to the underside of the triangle, providing a bit of lift (Figure 3.19). There are undoubtedly many design possibilities that can accomplish the same thing; all that matters is that the skimmer removes the surface scum.



Figure 3.14 (left): Ultra-fine pore oxygen diffuser installed in grow-out tank. Note the cloud of fine bubbles coming from the diffuser.

Figure 3.15 (below left): Banjo filter for a honeymoon tank (left) and a larger version for the early stages of a grow-out tank (right).

Figure 3.16 (below right): Standpipe screen for post-settlement flounder. Note the 1 mm mesh glued over a frame of larger plastic mesh.



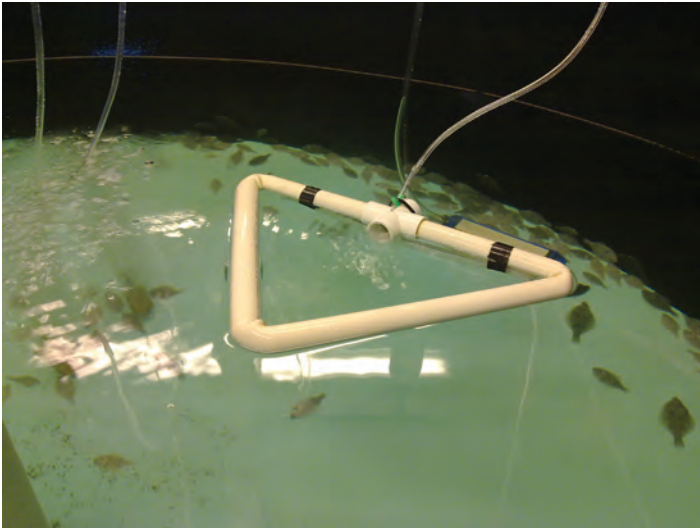


Figure 3.17 (above left): Surface skimmer installed in grow-out tank.



Figure 3.18 (above right): Close-up of surface skimmer. Note the angled hole for the air fitting.

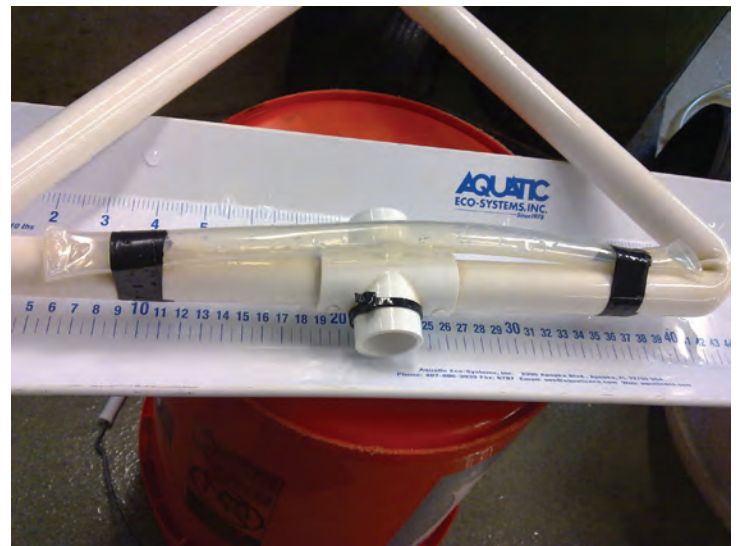


Figure 3.19 (right): Bottom of surface skimmer. Note the sealed piece of tubing for added buoyancy.

Spawning

Broodstock and Honeymoon Setup

Wild, mature, pre-spawning broodstock (at least 50 males and 50 females) should be collected a few weeks prior to the natural spawning season. For stocking projects, it is imperative that the broodstock are captured in locations as close to the study sites as possible in order to maintain the genetic integrity of the winter flounder population to be enhanced. This is because the origin of the broodstock affects zygote characteristics and development; there are latitudinal differences in growth rates for winter flounder (Buckley et al. 1991; Butts and Litvak 2007a,b). Only healthy adults should be kept for broodstock as egg quality and survival to metamorphosis are correlated to both paternal (Butts and Litvak 2007a) and maternal health (Buckley et al. 1991).

At UNH, winter flounder broodstock are collected by commercial trawlers. Short (30 min) tows are best as fish will be less stressed. All live, adult, pre-spawning winter flounder caught immediately are placed in holding tanks on board the boat. These holding tanks usually are Xactic containers (simply 1m³ insulated plastic boxes) filled with seawater. A deck hose is used to refresh the water regularly so that water temperature remains low and dissolved oxygen remains high. As long as the water temperature and dissolved oxygen are stable, then the fish can adjust to being captured and transported from the fishing grounds to the hatchery.

The broodstock obviously should be of the highest quality possible, and hopefully as near to running ripe as possible (Figures 3.20 and 3.21). Usually the broodstock are given two to three days in the holding tanks to recover from the capture and transport process, and then distributed to the honeymoon tanks. Each honeymoon tank starts with one female and two males, but that can be adjusted as desired. Use the ripest specimens first, giving the others more time in the broodstock tank to mature. Once the fish are in the honeymoon tanks, cover the tanks and try to avoid any undue disruptions to the fish. Additional stress can delay or cause problems with spawning.

The length of time to and the quality of the spawn depends on a lot of factors. The condition of the broodstock,

how they were captured, the temperature of the water and the facility itself may all play a part. Patience is key; it may take weeks before some fish spawn. Some hatcheries use hormones to induce spawning but we have found that volitional (natural) spawning by the fish results in a higher egg fertilization rate. Because these are adult fish, they can produce a fair amount of slime, which will clog up the fine mesh of the filter

on the tank drain. Clean the filter every day, and take a few minutes to check on the condition of the fish and whether there have been any spawns.

Figure 3.20 (right): Male winter flounder, expressing milt.

Figure 3.21 (below): Female winter flounder, extruding eggs. Note the swollen appearance of the fish from the enlarged ovary that extends almost to the tail.



Spawning and Incubating Eggs

The first sign of a spawn is usually a small amount of foam on the surface of the water when the tank is checked.

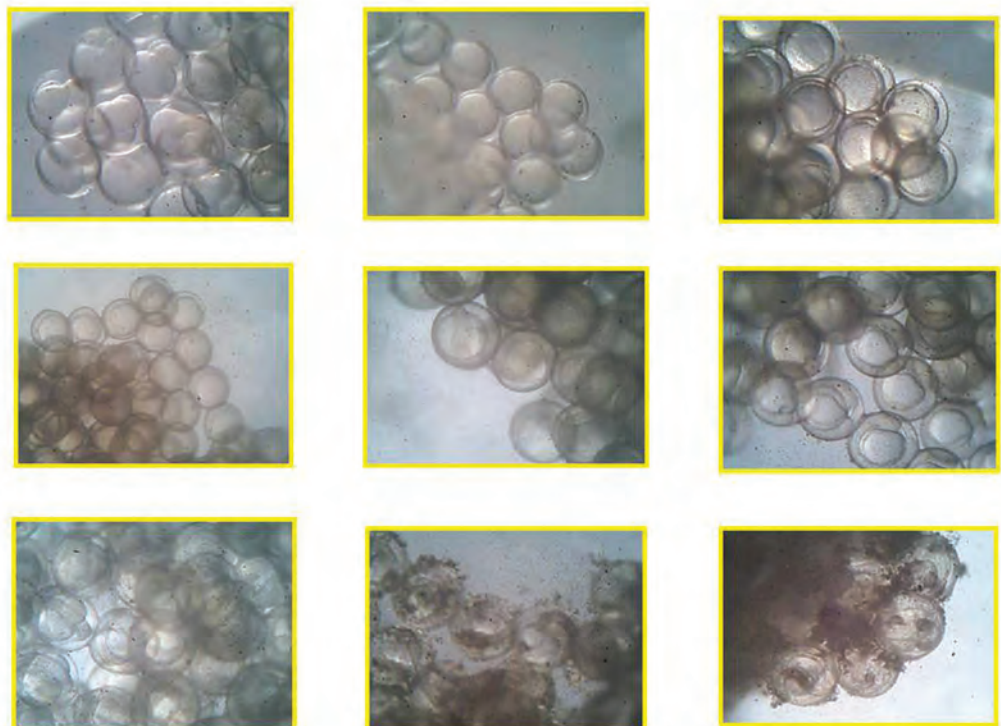
Good quality, fertilized eggs usually will take the form of a very pale orange mass in the center of the tank around the drain pipe (assuming a circular tank with circular flow) (Figure 3.22). Eggs that are non-clumping generally prove to be unfertilized and therefore useless, but they should be checked under a dissecting scope to confirm this before being discarded. If the egg mass looks good, carefully remove the adult fish. Try to chase them around as little as possible, as any frantic struggling on their part can damage the eggs. Once the adults are removed, clean the drain filter (it will probably be clogged with any unfertilized eggs) and check a sample of the eggs under the dissecting scope.

Sometimes it can be difficult to determine if eggs are fertilized within 24 hrs after spawning. Water temperature affects development rate, with colder temperatures retarding growth. When winter flounder spawn, the water is quite cold, so development is slow. Using a very clean glass bowl, scoop up a fragment of the eggs in water. Examine this sample under a dissecting scope. If the eggs are fertilized, they should be translucent and cell division should be visible. Time since fertilization will determine how far division has progressed (i.e., you could see many stages of development/cell cleavage from the two-cell stage to one far more divided (what looks like a small, dimpled golf ball)(Figure 3.23 and p. 140-142 in appendix). If the eggs are opaque or irregularly shaped (not round), then they are unfertilized. It is typical to have a small percentage (<20%) of unfertilized eggs within a batch of mostly fertilized eggs. Even if the egg mass is as much as 50% unfertilized, it is still worth incubating if space is available in the hatchery. If better spawns occur, these inferior eggs can be disposed of later. Return the egg sample to the tank.



Figure 3.22 (above): Honeymoon tank showing a mass of newly spawned eggs. Note that eggs may be floating if the clumping process has entrained air bubbles within the mass.

Figure 3.23 (below): Sequence of egg development from Day 0 in upper left to Day 14 in lower right.



When the eggs have been confirmed to be fertilized, they can be dipped in a solution of hydrogen peroxide to clean them up and reduce the chance of damaging fungus growing on them. At the CML, egg batches are dipped in a solution of 14 mL of 35% hydrogen peroxide in 20 L of clean seawater for 15 minutes. At the end of 15 minutes, the egg mass is carefully rinsed with flowing seawater for 20 minutes. During this time, any cleaning or maintenance to the honeymoon tank can be performed. When the rinse is finished, the eggs are returned carefully to the honeymoon tank for incubation. The hydrogen peroxide dip is performed only once at the CML, but it can be repeated several times if necessary or desired. Do not leave the eggs in the dip for too long, as this can damage the eggs and prevent them from hatching.

Hatching and Stocking Grow-Out Tanks

The incubation period for the eggs is highly correlated to water temperature. Eggs can hatch in as few as 9-10 days in warmer water and more than 14 days in colder water. During the incubation period, keep the water flow fairly high to enhance the water quality and keep the eggs clean. Periodic inspection under a dissecting scope will indicate how close the eggs are to hatching. When movement is observed within the eggs, hatching should begin in the next day or so. As soon as larvae are seen in the water, turn down the air and water flow. This will prevent the larvae (which at this point are barely able to swim) from getting beat up too much. Water flow should be reduced to a light stream and air flow to a very light bubble.

Figure 3.24: Newly hatched winter flounder (yolk sac larvae). Note the large yolk sac.



The grow-out tanks should be filled and started as soon as the first larvae hatch. Adding greenwater and rotifers will commence just before larvae are moved into the grow-out tanks. The fish actually have several days of yolk sac to use up before they need live feed, but (assuming there isn't a problem with rotifer supply) experience at the CML indicates the fish are less prone to ingesting air bubbles and getting stuck at the surface if food is always available (Figure 3.24). Greenwater will be supplied by adding doses of Instant Algae, a concentrated algae product from Reed Mariculture, Inc. At the CML, the 1.8 m diameter tanks require about 10 mL of Instant Algae up to three times per day to keep the water at a slight greenish tinge. The amount of rotifers to feed will also depend on the size of the tanks, as well as how many fish will be in them. Initially, shoot for approximately 5,000 rot/L. Feed can be adjusted up or down from there by examining the larvae under a dissecting scope and observing how many rotifers they have in their guts, as well as how many rotifers are in a sample of tank water.

After the first larvae have hatched out in the honeymoon tank, the majority of the hatch could happen in as little as a few hours or as long as a day or two. Frequent checks on the tank are necessary. With the setup at the CML, larvae begin forming dense swirls when the hatching is at its peak (Figure 3.25). At this



Figure 3.25: Grow-out tanks shortly after stocking with larvae. Note the large swirl of larvae and the green tinge of the water.

point, stocking of the grow-out tanks begins. Using sanitized 3L pitchers, the dense swirls are carefully collected and carefully moved into the grow-out tank. Instead of simply dumping the larvae in, the pitcher is partially submerged, turned horizontal, and pulled out from around the larvae, leaving them in the grow-out tank. This process is repeated until the denser swirls have been removed from the honeymoon tank. At this point, the honeymoon tank is left alone until more larvae have hatched and once again created dense swirls. It may take a few rounds of this process before all the larvae have been moved over, depending on the size of the hatch. At the CML, a decent-sized egg mass can populate one of the 1.8 m diameter grow-out tanks. The hatches from smaller egg masses are often combined into one grow-out tank. Obviously, the stocking of grow-out tanks in relation to egg masses will depend on the setup of the facility.

Grow-Out: Live Feed Stage

Rotifer Stage

As mentioned, the larvae have several days of yolk sac to go through before they begin eating rotifers, so keep a close eye on their progress through daily checks under the dissecting scope. The frequent checks also will allow for monitoring of the available feed in the water. If a 1 mL sample of tank water reveals 5-10 rotifers swimming around, the level of feed is correct and feeding should be continued at the current rate. If the tank water is a veritable soup of rotifers, adjust the feed amounts down. Conversely, if a sample of water reveals no rotifers, adjust the amounts up. More rotifers are always better than a dearth of rotifers.

It is crucial to have a plentiful supply of rotifers in the tank when the larvae have used up the yolk sac, as they will need to begin feeding immediately (Figure 3.26). A lack of rotifers can lead to ingestion of air bubbles, which cause the larvae to become positively buoyant, collecting on the surface and at the edges of the tanks and negatively affecting their feeding behavior. The mesh on the standpipe screen will be large enough to pass the rotifers, so the daily feedings are necessary to refresh the rotifer supply. At the CML, rotifers are fed out to the fish three times daily at 0800, 1400 and 2000 hours. Daily feedings can be adjusted as needed based on hatchery schedules and personnel availability, but it's best to spread the feeds out as much as possible to maintain a relatively steady population of rotifers in the tanks. As previously mentioned, the minimum goal for feed amounts is 5,000 prey/L, but higher would be better. At the CML, the 1.8 m

diameter tanks are fed anywhere from five million to 10 million rotifers per feeding, depending on the density of larvae in the tank and how full their guts look.

Water flow during the first two weeks should be kept at a relatively low level, both to avoid washing the rotifers out too quickly and to prevent any strong current patterns in the tank. When the fish begin to swim relatively strongly on their own, the flow can be increased slightly and a circular flow pattern can be induced to assist with future cleaning.

Figure 3.26 (right):
Feeding winter flounder larvae. Note the rotifer above the larvae.

Figure 3.27 (below):
Winter flounder, with metamorphosis nearly complete. Note the Artemia both in the water and in the fish's gut.



Greenwater Stage

Instant Algae feedings should be whatever is needed to keep the tank water colored a slight green. At the CML, the 1.8 m tanks get 10 mL of Instant Algae twice daily to achieve this goal. Obviously, amounts will vary based on tank size, flow rate, etc. The Instant Algae is fairly viscous, and the easiest way to apply it to the tanks

is by adding the required amount to a lidded container of seawater, shaking well to mix, and pouring around the tank. This greenwater treatment should continue for at least 14 days and as many as 20 days post hatch.

Artemia Stage

Transitioning of the young fish from rotifers to *Artemia* will depend both on their age and the size of the fish (Figure 3.27). As a rule of thumb, *Artemia* can be introduced after the greenwater is finished, around day 20. A fairly extended period of co-feeding may be required to successfully make the transition depending on how

early *Artemia* are introduced. During co-feeding, relatively small amounts of *Artemia* are fed out 20-30 minutes before the main rotifer feedings. For the first several days of the co-feeding period, consider feeding unenriched, newly hatched *Artemia* instead of enriched specimens. Their smaller size will make it easier for the fish to catch and consume them, which should speed the transition onto the larger, enriched *Artemia*. As time goes on, gradually increase the amount of *Artemia* and decrease the amount of rotifers until the fish are being supplied with *Artemia* exclusively.

Artemia feeding amounts will depend mainly on how much *Artemia* is available, as well as the flushing rates of the tanks. As with rotifers, it's important to always have *Artemia* available in the tank, but not so thickly that they are creating swarms of *Artemia* around the edges. Adjust the feedings accordingly. Feeding amounts for the 1.8 m tanks at the CML range from ≤ 0.5 million/feeding during the co-feeding phase to as much as



1.2 million/feeding as the only feed. The fish should be large enough at this point that the mesh on the drainage filter can be changed to the next larger size, which should be large enough to pass *Artemia* and prevent them from collecting too densely in the tanks.

Metamorphosis and Cleaning Tanks

Sometime after 20 days post hatch, the larvae will begin their metamorphosis (the timing of this process also is temperature-dependent). The body of the fish will deepen and become flat, and the eye will begin to move over the top of the head. Eventually the fish will flatten out and settle on the bottom. If possible, the bottom of the tank should be scrubbed and siphoned before the fish settle. The fish are still fairly fragile at this stage, so repeated scrubbing and siphoning of the bottom should be avoided for several days after the fish have settled. Because of the small size of the fish, their fragility, and the fact that it's almost guaranteed that some fish will have settled before the tank is siphoned, the initial cleaning can be extremely frustrating.

At the CML, the cleaning is accomplished by gently scrubbing the bottom with a scrub pad mounted on a pole, moving slowly and carefully to avoid unduly stressing the fish. When all the bottom dirt is loosened, a gentle circular current can be encouraged by swirling the tank by hand.

Though a circular current is convenient to concentrate the debris in the center of the tank and make it easier to siphon out, make the current as gentle as possible, again to avoid any undue stress to the fish. Because of the gentle current, it will take some time for the debris to settle and concentrate, so if desired other tanks can be scrubbed and swirled in the meantime. As with all equipment, the scrubbing tool should be rinsed well and sanitized between tanks. When the debris has been concentrated, it should be siphoned out. The siphon can be constructed using basic PVC parts with standard power tools (Figure 3.28). At the CML, the siphon head is constructed of 3/4" PVC connectors and endcaps, and the actual siphoning slit is cut with a cut-off wheel in a grinder (Figure 3.29). A piece of weather stripping is glued to the bottom ahead of the slit, and the siphoning is accomplished by pulling the siphon toward the user across the bottom of the tank. Since hopefully the fish have not settled at this point, there shouldn't be many picked up by the siphon. However, it's still a good idea to siphon into buckets or other containers. After the waste settles out in the buckets, any larvae that were picked up should be easy to see and dip out with a beaker or very fine mesh net.

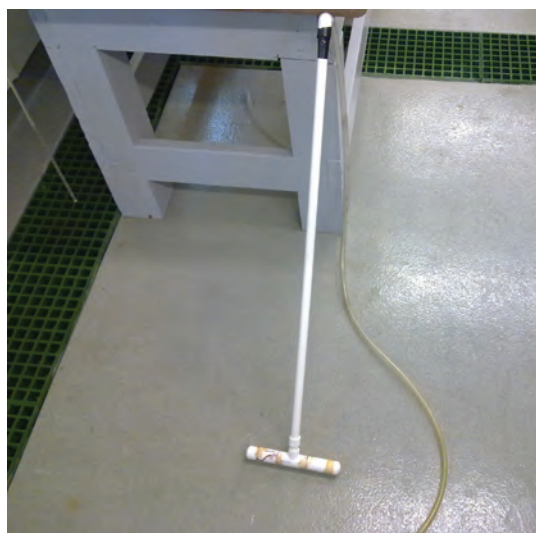


Figure 3.28 (left): Siphon used for grow-out tank.

Figure 3.29 (below): Close up of siphon head. Note the piece of weatherstripping in front of the siphon's slot.



After this initial cleaning, depending on the status of their metamorphosis, the fish should begin to settle quite rapidly. As they eat *Artemia*, they will actually be visible as a swarm of orange guts sitting on the bottom of the tank. This is a good time to analyze the density of the tanks. If there are some fish that are fully metamorphosed but refuse to settle, it could be a sign that the tank is too dense and there simply isn't enough bottom area available for all the fish. If that's the case, and assuming space permits, thin the tanks out by moving some fish around to other clean, waiting tanks. Keep an eye on feed amounts at this point, making sure that there is always plenty of *Artemia* available in the water. Once fish have settled to the bottom, aggressive behaviors like fin nipping begin. As long as tanks are not too dense and ample food is available to the fish, aggressive behavior will be minimal. After all the fish have settled, the drainage filter can be changed again. At the CML, this is the point when the banjo filter is removed entirely, and the standpipe is protected with a screen of 1 mm mesh attached over a framework of larger plastic mesh (see Figure 3.16).

Cleanings (using the technique described above) are generally performed on an "as needed" basis, particularly with regard to scrubbing the bottom of the tank. The live feed won't make tanks quite as dirty as the pelleted feed does, so simply clean and siphon when necessary. Airstones, diffusers and any removable tank parts should be cleaned at least once per week to prevent buildup.

Weaning

Once fish gut development is complete, the fish should be weaned from live feed onto a commercial, formulated feed. This will simplify culture procedures and reduce hatchery space needs and personnel time. At the CML, the general guidelines for weaning call for a co-feeding period to begin around day 40 post hatch (Table 3.1). As with the rotifer/*Artemia* co-feeding period, this period involves small feedings of the commercial diet added slightly before the regular feedings of *Artemia*. The initial diet, a 0.1 mm commercial feed, is extremely fine, almost powdery, and so it can be difficult to get through the surface layer of the tank. Obviously, the surface skimmer or similar technology should cut down on the surface tension, but the easiest way to do it is to add the feed to water and pour the mixture into the tank. For the 1.8 m tanks at the CML, a tablespoon of feed is added to a pitcher of seawater, the pitcher is mixed, and the resulting mixture is poured around the perimeter of the tank. This punches the powdery feed through the surface tension and lets it fall slowly to the bottom. This technique can be used for all the feed sizes up to 0.5 mm.

The weaning schedule can be adjusted according to the desires of the hatchery. At the CML, the weaning is accelerated as much as possible, if only to relieve the hatchery of the labor requirements of the live feed phase. Because the smaller diet contains algae, it is green in color and can be spotted in the fish's gut fairly easily. This makes it relatively simple to figure out when the fish are reliably starting to eat the weaning diet, and also relatively simple to figure out when they begin to prefer the feed over the *Artemia*. Schedules can be adjusted accordingly.

Table 3.1: 2010 winter flounder weaning schedule.

2010 WINTER FLOUNDER WEANING SCHEDULE

<u>Date</u>	<u># Days</u>	<u>dph</u>	<u>Diet</u>	<u>Diet Size(mm)</u>	<u>Prot/Fat</u>	<u>Wean at (time)</u>	<u>Artemia at (time)</u>
6/11-6/20	10	40-46	GWD 0.1			8, 1	9, 2, 8
6/21-23	3	47-49	GWD 0.1			8, 8:30, 9, 10, 11, 12	12, 3, 8
6/24	1	50	GWD 0.1 (3/4) GWD 0.2 (1/4)			8, 8:30, 9, 10, 11, 12	12, 3, 8
6/25	1	51	GWD 0.1 (1/2) GWD 0.2 (1/2)			8, 9, 10, 11, 1, 3, 8	3, 8
6/26-27	2	52-53	GWD 0.2			8, 9, 10, 11, 1, 3, 8	3, 8
6/28	1	54	GWD 0.2 (1/2) Gemma 0.3(1/2)	0.3-0.5	58/17	8, 9, 10, 12, 2, 4	4, 8
6/29-30	2	55-56	Gemma 0.3	0.3-0.5	58/17	8, 9, 10, 12, 2, 4	4, 8
7/1-7/3	3	56-58	Gemma 0.3	0.3-0.5	58/17	8, 9, 10, 12, 2, 4, 8	8
7/4-		59	Gemma 0.3	0.3-0.5	58/17	8, 10, 12, 2, 4, 8	--

The transition from live feed to a commercial pelleted diet is a pretty extreme change for a young flounder and can lead to quite a bit of stress. Mortality is not uncommon during this period. Ensure that the fish always have some form of feed and that the water quality remains good throughout the process.

The weaning period, by its nature, tends to result in a good bit of wasted feed on the bottom of the tanks. In some ways, this is desirable, because it indicates that the fish are getting plenty of weaning diet. However, that extra feed should be cleaned up at regular intervals to prevent an adverse effect on water quality. The circular water flow in the tanks should concentrate leftover feed near the center of the tank, making it relatively easy to siphon out each day. At the CML, siphoning is begun about a half hour after a feeding, to give the fish time to feed, as well as to give the feed time to spiral into the center of the tank. Because the fish are larger and stronger than during the initial cleaning, the siphon can be directed into a fine-mesh net rested on the edge of a rinser bucket (the same design used for harvesting/sieveing rotifers and *Artemia*)(Figure 3.30). Before beginning the siphoning, fill the rinser bucket with enough clean seawater to submerge the bottom of the net. This ensures that fish caught in the siphon will end up in water when they come out the other end, rather than stranded on a dry net. When the tank is siphoned, carefully con-

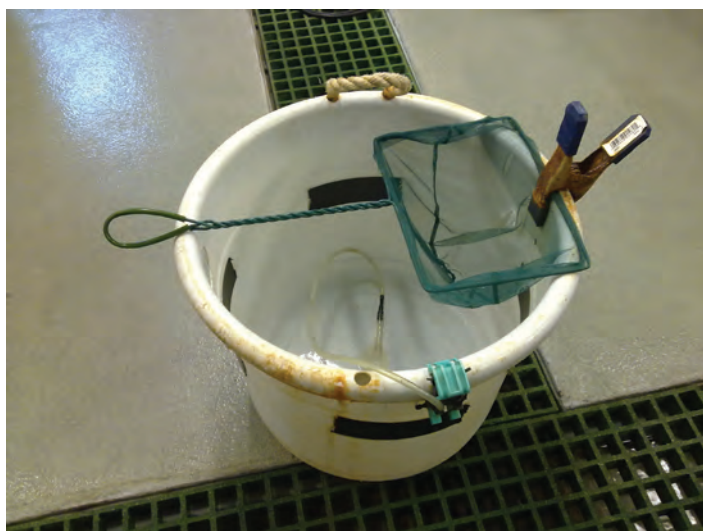
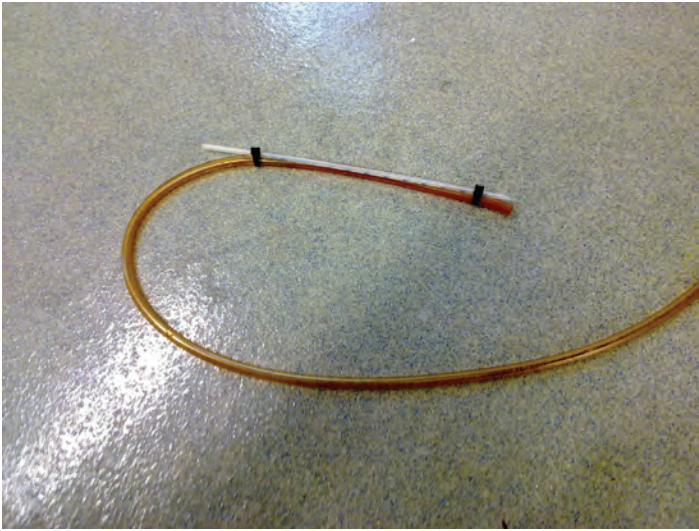
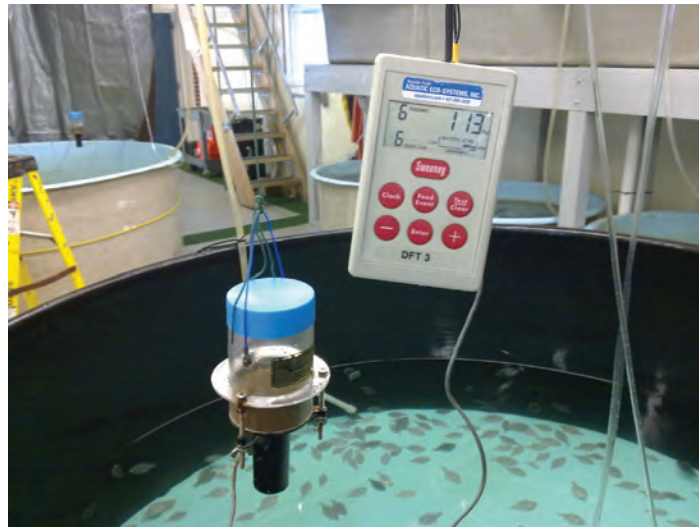


Figure 3.30: Rinser with a fine mesh net to catch fish and debris during siphoning.



*Figure 3.31 (above):
Small siphon used to
separate fish from debris
in small pitchers.*

*Figure 3.32 (right):
Vibrating feeder used
with larger feed. Note
timer mechanism
allowing for unattended
feedings.*



centrate the fish near the bottom of the net, and carefully overturn it into a pitcher of clean seawater. This pitcher can be carefully swirled and a smaller secondary siphon can be used to remove the debris without sucking up the fish (Figure 3.31). The fish can be returned to the tank carefully. The net, rinser and siphons should be sanitized between tanks. Tanks also are carefully scrubbed and carefully swirled once a week in this phase.

Near the end of the weaning period or shortly thereafter, there may be an occurrence of slime in the fish tanks. This substance has been identified only as a "biological matrix" after analysis and is produced by the fish. The purpose and cause of the production is not known at this point. This slime does not seem to be harmful in and of

itself, but its presence can cause serious problems in the tanks. First of all, the slime can actually trap fish if they're small enough, causing death. The slime can clog standpipe screens, potentially causing tank overflows, and it can clog the skimmers and coat the surface of the water, reducing gas transfer and making it difficult to get food to the fish. At the CML, attempts to eliminate the formation of slime have been unsuccessful. The only remedy is

a large-scale skimming effort. As often as every half hour, as much slime as possible is removed from the tank using a fine-mesh net. The airstones and oxygen diffuser are removed and cleaned every day to remove residual slime, and the tanks can be scrubbed more often than usual to keep them clean. Try to balance the stress of the slime with the stress of repeated scrubbing, however. Thankfully, this period of slime tends to last only about a week to 10 days.

Grow-Out: Formulated Feed Stage

As the fish grow, the size of the feed used can be increased accordingly. The 0.3 mm diet is generally the first size that can be fed out without mixing it with water, assuming the surface of the tank is relatively free of scum. At the CML, vibrating autofeeders generally are utilized after the fish are on a ≥ 0.5 mm feed to reduce labor (Figure 3.32). Regardless of the method used to feed, pelleted feed is offered to the fish more often than live feed so that its effects on water quality are spread out. At the CML, feedings happen at 0800, 1000, 1200, 1400, 1600 and 2000 hours. Feed schedules can be modified based on the needs of a particular facility.

Depending on the design of the tank, excess feed and feces will probably need to be siphoned on a daily basis. Since the fish are much stronger at this point, the flow can be increased, causing a stronger circular current and helping to concentrate debris at the center of the tank. The siphoning process becomes much easier as the fish grow, and eventually there will be no need for a fine-mesh net to catch the fish, as they will be too big to be siphoned or strong/smart enough to avoid the siphon. The tank bottom and sides should be scrubbed weekly, and the removable parts of the tank can be scrubbed then too.

The standpipe screen mesh can be increased in size as the fish grow larger, allowing easier evacuation of debris as well as less chance of clogging. The tank population may need to be thinned again at some point, as the fish grow larger. A tank that is too dense may have a large number of fish that have settled on the vertical sides of the tank. Since it requires more energy for the fish to stay there and since they don't have as much access to food from that position, it's best to avoid this situation. Thinning out the tank will free up enough space that these fish can settle on the bottom.

As the fish grow, they become substantially hardier. Still, it is necessary to keep a close eye on water quality and the various systems that affect it. Air, oxygen and water flow should be checked several times per day and, if possible, backup systems should be available to take over in case of an emergency. The fish will begin to use oxygen more quickly as they grow, and because the pelleted feed is harder on the water quality than the live feed, always make sure there is plenty of oxygen on hand.

Disease

Whenever there are a lot of fish in a relatively small area, there is a chance for disease of some sort to affect the fish. The first step to preventing this is cleanliness, of course. As previously mentioned, anyone working with tanks should have clean/disinfected hands and anything that comes in contact with the fish tank water should be sanitized. If possible, it's a good idea to have a foot bath filled with disinfectant at the entrances to the facility. Blowers for the air system should be placed in as clean a location as possible. And, of course, the incoming water should be treated as much as possible (UV sterilization, mechanical filtration, etc.).

The most common disease issue at the CML is directly related to the temperature of the incoming water. When the water temperature rises above approximately 17°C, the fish (in a relatively crowded state) start to get stressed, opening the door to opportunistic bacterial infections. There also are rare cases of infections by viruses, though fortunately those have not been common in winter flounder culture.

Treatment of bacterial infections is generally a round of medicated feed, usually medicated with oxytetracycline. At the CML, the medication is dissolved in gelatin, which is then evenly applied to the feed and mixed well. This creates a soft pellet evenly coated with medication. This feed is fed to the fish over 10 days, regardless of any improvement in symptoms before that time. It's a good idea to have all the ingredients for medicated feed on hand well before the temperatures start to rise, as the number of fish lost will be drastically reduced if they can be medicated immediately. Because the feed will be medicated in-house, there's a lower limit as to what size feed can be medicated. Feed smaller than 0.3 mm is extremely difficult to medicate evenly, so it naturally behooves the culturist to get fish onto 0.3 mm feed or larger as soon as possible, just in case.

Malpigmentation

One of the unresolved issues with raising flatfish is the occurrence of unnatural pigmentation, called malpigmentation, in a subset of the fish. Some hatchery-reared flatfish will either develop dark pigmentation on the blind side (non-eyed side) and/or lack normal pigmentation on the eyed side. Malpigmentation likely is caused by a nutritional deficiency in the larval stage, as the diet of hatchery-reared fish does not include the full nutritional spectrum of natural zooplankton that wild larvae would consume. Apart from a lack or surplus of pigmentation, there are no other morphological differences between malpigmented and normally pigmented flatfish. In winter flounder, malpigmentation typically is manifest as a lack of pigmentation on the eyed side (Figure 3.33). These fish are not able to adjust their pigment to blend into backgrounds (i.e., camouflage); therefore, malpigmented fish should not be released for enhancement as their chances for survival are much lower than normally pigmented fish.

Figure 3.33 (below), 3.34 (below right): Juvenile winter flounder. Note several malpigmented fish.



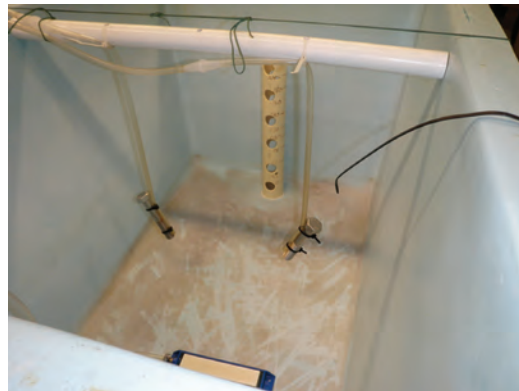
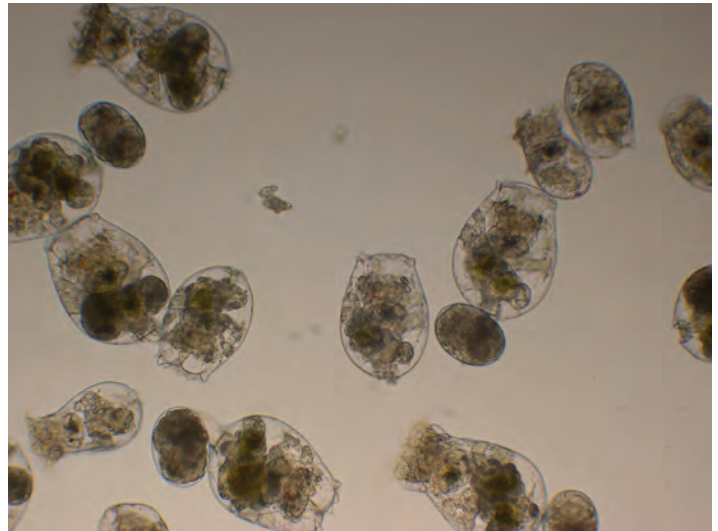
Chapter 4: Rotifer Culture CML Style

Rotifers (*Brachionus* sp.) (Figure 4.1) are used as the first feed for larval winter flounder. The basic concept is simple: raise rotifers, harvest, enrich and feed them to the larvae. At the CML, we use a static culture system that is rolled over every three days. A brand new tank is inoculated with rotifers (Day 0), grows and is harvested for two days (Day 1 and 2), and moved to a new tank ("rolled over") on the morning of Day 3. With proper care, densities of well over 1,000 rotifers/mL are easily attainable and maintainable, and the three-day cycle prevents build up of waste products that can hamper the growth of the population.

Figure 4.1 (right): Rotifers. Note individuals with eggs.
(Photo courtesy of Nick King)

Figure 4.2 (below): Rotifer tanks at the CML. Note the bottom drain with valve.

Figure 4.3 (below right): Inside view of rotifer tanks, showing weighted air lines, oxygen diffuser and graduated standpipe. Heater not pictured.



Tank Setup

Rotifers can be grown in pretty much anything, as long as the final setup meets a few basic criteria. We use rectangular fiberglass tanks that hold about 250 L. The necessary volume will of course depend on the available space at the facility, number of required rotifers, etc., but 250 L works well for the number of fish grown at the CML (Figures 4.2 and 4.3). It's large enough to raise the required number of rotifers (200 million + per tank is easily attainable), but small enough to allow for easy arrangement in the facility and lessen the requirements for a large reservoir of heated water (for rinsing). For the

method of culture described here, you will need a minimum of two tanks: one for the rotifers and an empty one that can be filled and readied for rotifers when they're rolled over. A minimum of three tanks (two with rotifers, one empty standing by for rollovers) are used at the CML in order to raise enough rotifers for our needs. More would be better, but keep in mind that one or several empty tanks will need to be available and ready at all times for roll over (depending on scheduling). Obviously, more tanks means more rotifers will be available for feeding, but also considerably more labor for the hatchery.

For ease of use, it's important that the tanks be equipped with a bottom drain of some kind, either molded into the fiberglass or achieved through the use of a bulkhead fitting and associated PVC hardware. The drain should be located such that complete drain-

age of the tank is possible. Smaller tanks, of course, simply can be upended to remove the last bit of water. A smooth finish on the interior of the tank will make it easier to clean, and some method of graduation (either by marking the sides of the tank or with a graduated standpipe) will make population and harvesting calculations easier (Figure 4.3). Drainage of the tank should be controlled by an easily operable valve. Because of the valve, there is no strict requirement for a standpipe inside the tank. However, using a pipe with large holes cut in the side (or even a stub that extends only a ½" to 1" into the tank) can prevent the layer of waste on the bottom from being sucked into the drain during harvest.

This will yield cleaner harvests that require less rinsing. The height of the harvest valve should be at least 18" above the floor to accommodate pitchers easily for harvesting and to ensure easy emptying during rollovers.

Good circulation is a necessity for any kind of high-density rotifer culture. One of the most cost-effective ways to accomplish this is with compressed air, provided either by individual air pumps or some sort of central blower system. How the air is introduced into the tank will depend on the individual scenario, but anything from weighted tubing to PVC frames on the tank bottom will work. The idea simply is to avoid dead spots in the tank. The setup at the CML uses two 3/8" diameter tubes hung from a crossbar over the tank, weighted down with stainless steel bolts (Figure 4.3). Large bubbles are preferable for air delivery, provided either by open tubing ends or fittings. Air stones will work, but are not ideal because they can produce a lot of foam. Foam can strip rotifers from the water, provide a contamination problem, and make cleaning the tanks into more of a chore. If your blower is in a damp location or you have unexplained contamination problems, a desiccant cartridge plumbed into the air line may help.

For high-density culture, supplemental oxygen is necessary to maintain dissolved oxygen at ideal levels. Oxygen cylinders can be rented from local gas supply companies (welding grade oxygen is fine and relatively cheap), and the regulators and other required hardware is readily available from those suppliers or from third parties. Effi-



Figure 4.4: Ultra-fine pore oxygen diffuser. Note the stainless steel hose clamp.

At the Coastal Marine Lab



cient use of oxygen requires a good diffuser. An ultra-fine pore diffuser such as the ones produced by Point Four Systems, Inc., will produce a cloud of extremely small bubbles, which will maximize oxygen transfer to the system water and reduce waste (Figure 4.4). It is important to always maintain a reliable supply of oxygen for any high-density culture. Even a relatively modest density of 600 rotifers/mL can completely strip the oxygen from the water in a matter of hours. Hoses should be high-quality and routed in such a way that eliminates sharp curves that could lead to kinks. Everywhere a hose is pushed onto a fitting should be secured with good-quality hose clamps. A failure in the oxygen system can lead to a total loss of rotifers. If space and finances allow it, engineer some redundancy into the system.

The ideal temperature for rotifers will vary depending on the strain, but it's safe to say that some method of heating the water will be needed. Temperatures at the CML are generally kept around 25-27 °C. This is well above room temperature, so each rotifer tank is kept heated by two separate 300 W aquarium heaters. One would probably suffice, depending on the air temperature in the room, but using two provides a bit of backup. Separate circuits for each heater would be ideal, but often is unattainable without a lot of expense.

The method of culture described here also requires a supply of warm water that is used mainly for rinsing rotifers during the harvest, but can also be used to top off tanks that have been harvested to a relatively low volume, but aren't ready to be rolled over yet. At the CML, the warm water reservoir is simply a large plastic tank, approximately 180 gallons, and heated with a single 300 W aquarium heater. A small pump attached to a length of ½" tubing is placed in the tank and left plugged in to circulate the water. When warm water is needed, a longer piece of tubing is attached to the pump and directed to wherever needed.

Healthy rotifer cultures actually contain various types of bacteria that exist in a symbiotic relationship with the rotifers. Still, it's best to have your initial water and harvest tanks as clean as possible. Tanks should be disinfected with a bleach or iodine solution and carefully rinsed before use, and anything that comes in contact with the tank water should be as sterile as possible, including hands. We use seawater that has been sterilized with UV, then filtered to 1 µm. Before rotifers are added, the water is bleached overnight, then neutralized the following day. Basically, use the cleanest water you can.

Water Preparation and Sanitation

Culture water for all forms of live feed at the CML is a separate supply from that which feeds the fish tanks. The water is pumped through a standard pool sand filter, passes through a UV sterilizer, and is then filtered through cartridge filters of 5 μm and 1 μm in size. The 1 μm filtered water then is used to fill the static systems. In addition, standard household bleach is used to sanitize the various live feed tanks in the facility. Though there are populations of beneficial bacteria that grow in concert with rotifer populations, it's helpful to start with the cleanest water possible. Household bleach is readily available and easy to work with, but contains stabilizers that extend the time of potency. Because of these stabilizers, aeration is often unable to neutralize the chlorine, and a sodium thiosulfate solution must be used instead of or in combination with aeration to neutralize the bleach. At the CML, water is prepared with 0.25 mL of liquid bleach per liter of seawater. This results in a concentration of approximately 1.25 ppm of chlorine, which is actually fairly low. A higher concentration may be needed if the culture water is not filtered to 1 μm and sterilized with UV radiation before sanitization.

Dry chlorine or pool shock treatment also can be used to sanitize culture water. These products contain about 70% chlorine, and 1/8 teaspoon per five gallons of seawater yields a concentration of 1-3 ppm. These products must be stored carefully in a dry place to maintain potency, and potency decreases rapidly when mixed in seawater. At the CML, liquid bleach is preferred partly because of its availability and partly because of its versatility around the facility.



Though aeration can eventually strip the chlorine from water, theoretically leaving it fit for culturing organisms, an addition of sodium thiosulfate provides an extra measure of confidence that the chlorine has been removed. At the CML, sodium thiosulfate crystals are dissolved in warm tap water at a concentration of 100 g/L. Usage of this solution is simple: to neutralize X mL of bleach, use 0.5X mL of the sodium thiosulfate solution. This is mathematically more than is necessary to neutralize the

bleach, but it provides a safety margin for neutralization and seems to have no ill effects on the organisms.

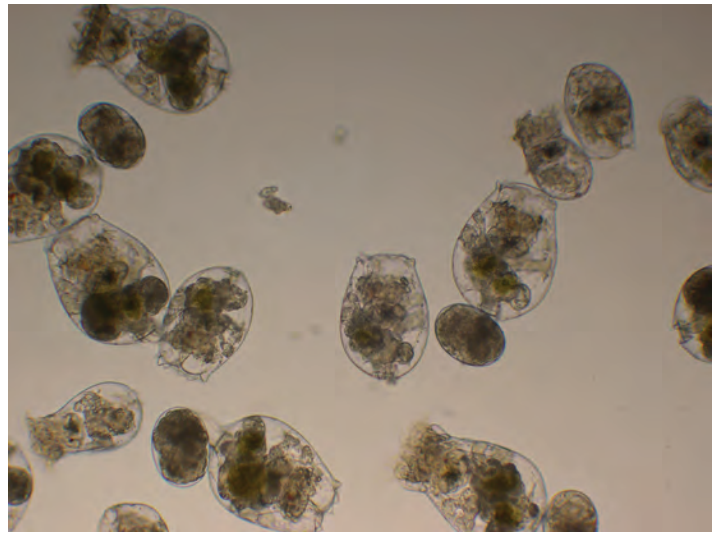
When setting up tanks for the first time, turn the aeration system down to a minimal level after adding the dose of chlorine. This will allow maximum sanitation in the time allowed and prevent any reduction in potency caused by aerating the water. The aeration will be turned back up when the sodium thiosulfate is added to aid in neutralization and also for circulation and degassing during the actual culture period.

Counting

In order to properly maintain a population of rotifers, it is important to have a fairly accurate idea of how many rotifers are in the tank. Counting under a microscope also gives you an opportunity to observe the swimming rotifers and note any behavioral oddities, as well as the fecundity (egg production) of the population, which can give an idea on its general health.

Materials needed:

- Small beaker or other clean container
- 1 mL pipet and pipet pump
- Counting cell (Sedgewick Rafter)
- Dissecting scope/low-powered microscope
- 10% buffered formalin or denatured alcohol with drop per
- Multi-clicker or some other kind of counting device
- Rotifer Data Sheet (see Appendix)



Methods:

1. Using the small beaker or other clean container, take a small sample (about 200 mL or so) from somewhere near the center of the culture tank, or wherever there is a well-mixed area.
2. Using the pipet, stir the beaker until the sample is uniformly distributed, then take a sample from the beaker. Use a 1 mL sample if the tank is likely to have less than 500 rotifers/mL. If the tank is likely to be more dense than that, use 0.5 mL samples to save time and aggravation. If the culture is known to be extremely dense, a 0.2 mL sample can be used instead. Smaller samples than that are difficult to accurately measure out.
3. Squirt the sample onto a counting slide and observe the live sample. The water should be fairly clean and the rotifers should be fairly active. There may be small, dark pellets present along with the rotifers. These are fecal pellets; their presence is not cause for concern. Also take note if there is excessive feed in the water, which will show up as dark flecks.
4. Fill the dropper with formalin/alcohol. Place 1-2 drops (3-4 drops if using a 0.2 or 0.5 mL sample) on the counting slide. Spread the sample out until it fills the entire slide area. This may take several tries due to the surface tension of the water. For 1 mL samples, it will be necessary to stir it a bit to distribute the formalin and completely kill the rotifers.
5. Count the sample using a microscope, tallying the numbers on the multi-clicker/ other device. Count the number of rotifers without eggs and also the number of rotifers with eggs. Further detail (single vs. multiple-egged rotifers, males vs. females, etc.) is possible, but experience indicates that it is not strictly necessary for this method.
6. Add the two numbers, then divide the "eggs" number by the total to get the "%eggs" on the data sheet. Record the total number of rotifers in the "Rot/mL 1" on a data sheet. If the counted sample was a 0.5 mL sample, multiply the total by 2 before recording it, and if it was a 0.2 mL sample, multiply it by 5.
7. Repeat the process, being sure to stir the beaker well before taking another sample of rotifers. If there is a difference of more than 200 rotifers, count another sample. Keep at it until there are two counts that are within 100-150 rotifers to ensure some degree of confidence.
8. Add Rot/mL 1 and Rot/mL 2 together and divide by 2 to get "Avg rots/mL." Multiply by the volume of the tank (read whatever graduation marks are on the tank) and multiply by 1,000 to get the total rotifers. Round it off (i.e. 312,200,000 becomes 312.2 on the data sheet).
9. Dump the beaker of rotifers out. DO NOT return it to the rotifer tank.
10. Repeat steps 1-9 for any other tanks.

Feeding

There are various rotifer diets on the market, and they also can be grown on a combination of baker's yeast and live microalgae. At the CML we use Ori-Culture, a commercial diet developed by Skretting. It is an algae-based diet, supplemented with fish oils and various vitamins to provide a complete diet for rotifer culture. Water quality is much better with this diet than with yeast-based diets, and the cultures are cleaner and easier to deal with on a daily basis. The dark green fecal pellets often seen in the counting process are a product of this feed. Ori-Culture is an expensive feed, but our results indicate that it's worth it.

The correct feed amount is extremely important to the health of the culture. Insufficient feed can have several deleterious effects, such as low fecundity, low activity level and excessive foaming. Conversely, excessive feed can cause severe degradation in water quality, as well as wasting a lot of money. So it's important to pay attention during the counting process, both to get accurate counts of the population and to catch any overfeeding situation before it gets out of hand.



For this method, the amount of feed that a rotifer tank gets per day depends on the age of the tank. A tank at day 0 gets 0.4 g Ori-Culture/million rotifers/24 hrs; day 1 gets 0.5 g Ori-Culture/million rotifers/24 hrs; and day 2 gets 0.4 g Ori-Culture/million rotifers/24 hrs. The rotifers are moved on the morning of day 3, and therefore the 0800 feeding becomes the first feeding of the new tank.

Materials needed:

- Ori-Culture
- Scale, appropriately sized for the amount of feed you'll be using
- 3L pitcher or similar container
- Beaker/small container and a spoon to dispense the food
- Stick blender (a cheap one will last about one season; good ones cost several hundred dollars)
- Something to remove excess foam (jar lid or similar works fine)
- Calculator (not strictly necessary but makes things easier)

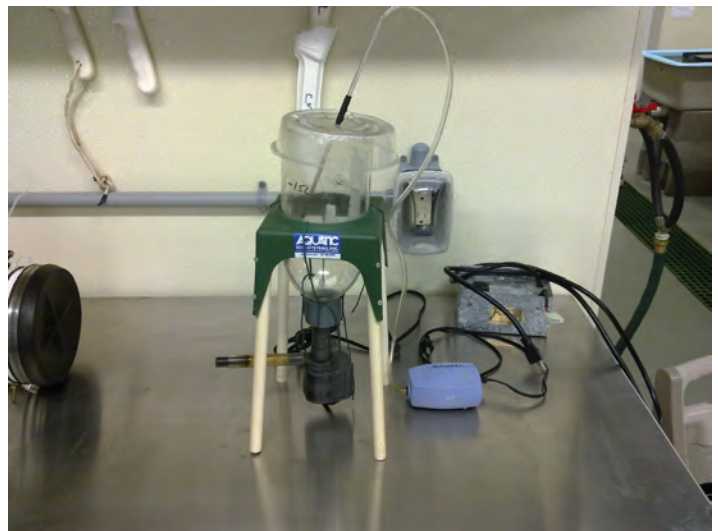
Methods:

1. Determine feed. Feed is determined by the age of the tank. Day 0 cultures get 0.4 g Ori-Culture/million rotifers/day; day 1 get 0.5 g Ori-Culture/million rotifers/day; and day 2 get 0.4 g Ori-Culture/million rotifers/day. Feeding times are dependent on the individual hatchery, but it's best to feed the rotifer cultures less feed at more frequent intervals than more feed a few times per day. At the CML, feedings are at 0800, 1200 and 1600 by hand, then an auto-feeder is used to distribute another two or three feedings throughout the night.
2. Using a scale and a clean, dry beaker, measure out the feed.
3. Fill a pitcher with about 1500 mL of tap water and begin to mix with the blender on high. When a vortex forms, dump the feed into the vortex and continue to blend for one to two minutes. Stop and let the feed hydrate for 30 seconds, then blend for an additional minute.
4. Dump the feed into the appropriate tank. If there is a lot of foam, scoop it out into the sink first, using the jar lid.
5. As mentioned, feedings during the day at the CML are at 0800 (or as early as possible after counting and determining feed), 1200 and 1600. A simple auto-feeder feeds two or three more times during the night. The actual times of the feedings don't really matter — the goal is to split up the feedings so that the doses are smaller and have less of an effect on water quality. Night feedings can be done by a night staff or an auto-feeding device, which could be anything that can hold the liquefied feed, keep it cold and reliably deliver it to the tanks.

Auto Feeders

If the facility in question does not have a 24 hour staff, an investment in some kind of autofeeder technology, which can be used for both the live feed culture and the fish culture, can make life a lot simpler. For rotifers, simple technology and materials can feed several tanks reliably through the night. The autofeeder at the CML consists of two parts, the storage reservoir and the drip reservoir (Figure 4.5). The storage reservoir is simply an upside-down 3L soda bottle with the bottom chopped off. The top of the reservoir and the stand that it is suspended in originally was a small hatching cone for *Artemia* cysts; as long as the reservoir can be kept in an upright position, anything will do. Attached to the threaded portion of the soda bottle is a small aquarium pump, which is sealed with caulking around the inlet. Again, any small pump that can handle a decent head will work. Air is supplied to the storage reservoir through a small electric air pump, which delivers air through a flexible tube and into a piece of rigid tubing that goes through the top of the reservoir. The tubing is prevented from cavitating the pump by a small piece of tape on the rigid tubing, just above the top. This prevents the rigid tubing from extending too far through the top. The outlet of the pump is attached to a length of hose that leads up to the drip reservoir. The liquid feed in the reservoir is chilled by floating a frozen bottle of water in it.

Figure 4.5: Storage reservoir for the rotifer autofeeder. Shown: reservoir, pump, air pump, timer power supply. Timer controller not pictured.



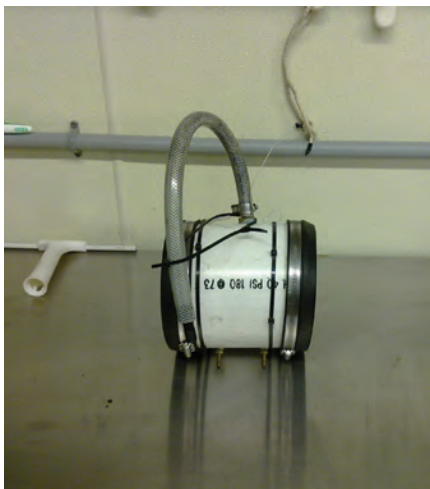


Figure 4.6: Drop reservoir for rotifer autofeeder. Feed delivery lines not pictured.

At the CML, the drip reservoir is constructed from a piece of 6" PVC pipe that happened to be on hand (Figure 4.6). The ends are capped and two hose barbs are threaded into the bottom. A third, larger hose barb is threaded into the top and connected to the hose from the storage reservoir. Two smaller hoses lead from the bottom hose barbs into the rotifer tanks to be fed. The drip reservoir essentially can be any container that can be suspended above the tanks and is large enough to hold about 1.5-2 L of liquid, as long as there's some way to get the feed into

it from the storage reservoir and out of it to the tanks. Unless the storage reservoir is equipped with a type of pump that won't back siphon (or a small foot valve), make sure the feed enters the drip chamber at the top, otherwise it will simply drain back into the storage reservoir. At the CML, the drip reservoir is suspended levelly about 18" above the rotifer tanks, and the storage reservoir is on a shelf almost at the height of the top of the rotifer tanks.

The function of the autofeeder is controlled by a timer/powersupply combination from Aquatic Ecosystems. It is the same set-up that the CML uses to control the vibrating feeders supplying the fish tanks. The timer generally is set up to feed three times. Each feeder is different, so careful calibration (through a lot of trial and error) is required to arrive at a feeding duration that will deliver one-third of the reservoir's contents to the drip reservoir at each feeding. Operation is simple: before leaving for the day, make up enough blended rotifer feed to cover three feedings, add enough water to arrive at the volume required by the autofeeder, and dump it slowly (to avoid excessive foam) into the storage reservoir. Hook up the air hose, ensure that air is bubbling into the feed, and place the frozen water bottle into the reservoir. Route the drip hoses into the rotifer tanks, and it's ready. When the timer goes off, the pump will deliver a volume of the mixed feed into the drip reservoir where it will be evenly distributed to the rotifer tanks.

Water Quality

Water quality (specifically, dissolved oxygen and temperature) should be recorded every day to keep an eye on trends and compare them with growth rates and fecundity. Instructions for measuring dissolved oxygen with the YSI 200Meter are included in the Appendix. Because this method uses static systems, salinity will probably not change much from day to day, but it's a good idea to check it every few days, particularly if there are large tidal variations that may come into play when filling new tanks. As mentioned, the ideal temperature for the rotifers will depend on the strain, but at the CML the temperature is kept between 25°C and 27°C. Obviously, changes to the heaters will take a while to affect the actual water temperature, so check often until the optimal heater setting is finalized.

If the salinity of the available sea water is subject to large shifts due to tidal influences, try to fill new rotifer tanks when the salinity is at least 20 ppt. Rotifers can handle abrupt shifts in salinity of up to 10 ppt, but it's best to try to time rollovers and tank fills so that the salinity difference is as small as possible.

Determining Amount to Harvest for Fish Feedings

Materials needed:

- Several pitchers (3L size works well)
- 210 µm sieve and 55 µm sieve, 4-6" in diameter (constructed from PVC)(Figure 4.7)
- Setup for enriching

Methods:

1. Divide the number of rotifers you need by the number of rotifers/mL in the rotifer tank that you're harvesting. For example: if 4 million rotifers are required for a feeding and you have a tank at 1,350 rotifers/mL, divide $4,000,000 / 1,350 = 2,962$ mL or 2.9 L. So the harvest amount would be 2.9 L.
2. Collect about 2 L of clean seawater in a clean container. This water should be cool if possible.
3. Using another clean container(s), drain the appropriate amount of rotifers. It is important that whatever aeration exists in the tank be running at the time of harvest, as this will help keep the rotifers evenly mixed and the harvests more accurate.
4. Set up the sieves so that the 210 µm is set on top of the 55 µm, and the whole thing on top of or near a drain (Figure 4.8).
5. Pour the rotifers slowly through the sieves. If they are dense, you may need to stop one or two times and rub/tap the bottom of the sieve to get the water to go through.
6. Rinse the pitcher.
7. Shake/swirl the 50 µm sieve to remove as much liquid as possible, then overturn it over the clean pitcher, and use the clean seawater to rinse the rotifers into the pitcher. Leave enough room in the pitcher to dump in the enrichment (anywhere from 0.5-1 L, plus extra room to minimize splash-over from the aeration).
8. The pitcher of rotifers now can be set aside to enrich for several hours, or fed out immediately if required. If they are to be enriched, put the harvested rotifers in a pitcher and aerate them with an open-ended tube. It's best to have some way of keeping the rotifers relatively cool during the enrichment, either with ice-packs or a moving water bath. The rotifers can be relatively dense during the enrichment period (6 hours or less) as long as they are adequately aerated. At the CML, rotifers are routinely enriched at densities of approximately 2,400/mL.
9. Repeat the process as needed.

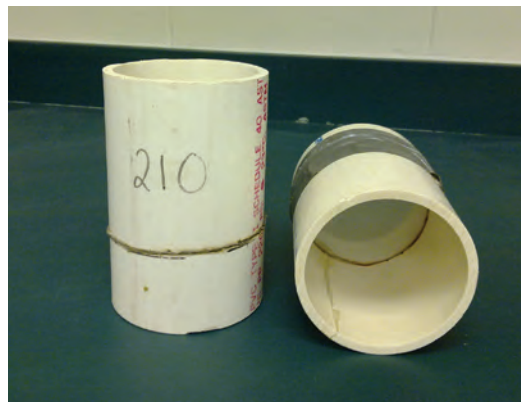


Figure 4.7 (above): Small sieves for harvesting rotifers to be fed out.

Figure 4.8 (right): Sieves set up and placed over a drain for harvest.



Enrichment

Rotifers by themselves are essentially empty sacks and contribute very little to the proper nutrition needed by growing larval fish. They can be enriched by aerating them in a solution of whichever nutrients are needed. One method is to use live algae, which delivers many if not all of the required nutrients, but requires the culture of the algae. This can be expensive, time-consuming and difficult, so at the CML a commercial product is used instead. The product is called Ori-Green, made by Skretting (same company that makes the rotifer feed). As with the rotifer feed, it's expensive, but the convenience and complete nutrition profile make it worthwhile.

Materials needed:

- Ori-Green
- Scale
- 3L pitcher or similar container
- Beaker/small container and a spoon
- Stick blender (a cheap one will last about one season; good ones cost several hundred dollars)
- Calculator (not strictly necessary but makes things easier)

Methods:

1. The bag of Ori-Green has a guide as to how much to use, but amounts can be adjusted based on need.
2. Weigh out the correct amount of Ori-Green based on the number of rotifers harvested and the density of the rotifers in the enrichment container.
3. Put about 1 L of tap water in a pitcher.
4. Using a stick blender, get the water moving, then dump in the enrichment.
5. Blend for 2 minutes, let it hydrate for 30 seconds, and blend for another 30 seconds.
6. Dump the enrichment into the pitcher of aerated rotifers that was set up following the harvest. Repeat for any other pitchers of rotifers.
7. Enrich the rotifers until the next feeding time (approximately 6 hours if feeding on the CML schedule of 0800, 1400, 2000 hours).
8. At feeding time, sieve the rotifers out using the 210 μm and 55 μm sieves. Rinse the sieved rotifers off of the 55 μm sieve into a pitcher, preferably using sea water that's close to the temperature of the fish tank water. The pitcher of rotifers can then be fed out to the fish. Repeat as necessary.

Rolling Over Rotifers

According to the CML method of static cultures in tubs, the tubs need to be taken down and the rotifers moved on the third day. At the CML, this is referred to as “rolling over” or simply “moving.” Waste products build up in the water and can hamper reproduction at this point, and depending on growth rate and how many rotifers are being harvested, the population may be becoming too dense. The tanks can certainly be rolled over sooner if needed (i.e., to solve a scheduling conflict or rearrange tanks). However, it’s best to let them go the full time to reach the maximum numbers. At the CML, the rollover is completed as early in the day as possible, so that the 0800 feeding actually serves as the background feed in the new tank.

Materials needed:

- Sodium thiosulfate solution (mixed at 100 g/L)
- Rinser/separator with air fitting on bottom (Figures 4.9 and 4.10)
- 250 μm sieve with filter material (Figures 4.11 and 4.12)
- 50 μm filter bag (sewn or pre-made) (Figure 4.13)
- Hoses to fit warm water reservoir and rotifer harvest valve
- Prepared background feed
- Sanitized bucket
- Cleaning supplies

Figure 4.9 (right): Rinser tub. Note drainage hole and attachment for air hose.

Figure 4.10 (below): Inside of rinser. Note air fitting on bottom.

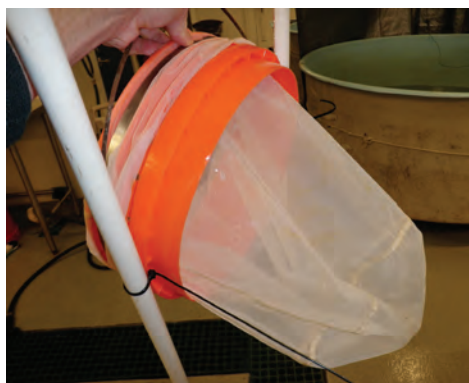
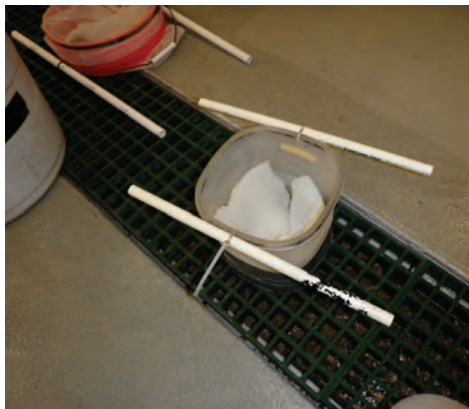
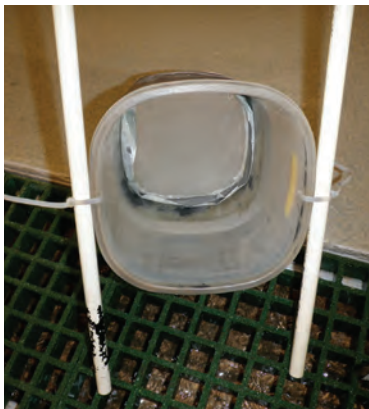
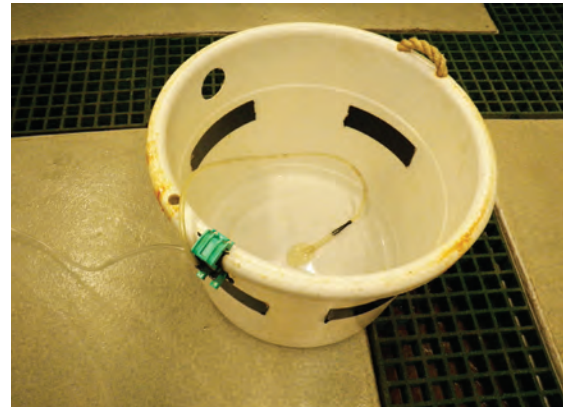


Figure 4.11 (far left): 250 μm sieve.

Figure 4.12 (top left): 250 μm sieve with filter material installed.

Figure 4.13 (lower left): Bag sieve. Note PVC pipes to suspend it over the rinser.

Methods:

1. The new rotifer tank will be filled with chlorinated water and will need to be neutralized with sodium thiosulfate ("thio"). The water in the tub will have been sterilized with a specific amount of bleach, based on the volume. Chlorine is much more deadly than thio, so err on the aggressive side when neutralizing it.
2. The aeration in the new tank will be turned way down. During the sterilization process, this helps prevent the loss of chlorine to the atmosphere. Turn up the aeration system in the new rotifer tank to a gently rolling boil (it will be left this way for culturing). This helps to circulate the thio and also can bubble off any chlorine that didn't get neutralized. Make sure it's not violently bubbling as this will damage the rotifers.

Wait 15 minutes for the water to neutralize.

3. While the water is neutralizing, assemble the separator/rinser with the 250 μm coarse sieve on top and the 55 μm fine sieve on the bottom, placed over a drain or some place that can receive the waste water. Hook up the airline in the rinser if so equipped (Figure 4.14).

4. Be sure that the warm water reservoir is clean, neutralized and ready to go.

5. Collect the necessary hoses:

- a. Heated sea water rinse — a smaller diameter is preferable, or some way to control the flow, unless there is an unlimited supply of heated seawater.

- b. Rotifer harvest- a larger diameter (1" at the CML) hose can be used here if the rinser is monitored and the rotifer water is relatively clean. This helps get the job done as quickly as possible.

- c. Garden hose — for cleaning the old tank (tap water).

6. Begin the take down

- a. Begin running warm water from the reservoir into the 55 μm sieve until the water level in the separator reaches the bottom of the sieve. At this point, the flow from the warm water reservoir can be choked off a bit if it is necessary to conserve water.

- b. Holding the end of the harvest hose higher than the tank it is attached to, open the harvest valve on the rotifer tank about $\frac{3}{4}$ of the way.

- c. Lower the end of the hose. The cleanliness of the harvest can be helped by letting the first couple seconds of flow go directly down the drain, particularly if the tank design tends to accumulate scum at the bottom of the standpipe. Then hold the hose in the 250 μm sieve and let it flow.

- d. Rock the 55 μm sieve back and forth gently if necessary to improve circulation inside the sieve. It's easy to see if more circulation/slower rotifer flow is needed because the water level inside the 55 μm sieve will actually be higher than that of the outer tub. If so equipped, the air bubbles coming up from the bottom of the outer tub also should help the circulation and draining.

Figure 4.14: Rinser and sieves assembled in preparation for harvest.



7. During the take down:

- a. When the tank gets down to about 100 L remaining (remember to keep the hose end high when you get up to check, so the flow of rotifers stops), unplug the heaters if there are any in the tank. Aquarium heaters without safety devices can overheat and explode if they are out of water for too long. If it's easier, close the valve and put the hose down to do this. When the heaters are off, pick up the hose end and turn the valve back on.
- b. During a rollover is a good time to adjust the population size as needed. If the rotifers merely are in a holding pattern (i.e., getting established, but not needed to feed fish at this point), it's best to knock the population down so that the new tank doesn't start out quite as dense. This helps maintain healthy rotifers and also saves on rotifer feed. At the CML, rotifers in a holding pattern generally are knocked back to a density of approximately 500-600 rots/mL, depending on anticipated need. Obviously, this is determined volumetrically, and when the appropriate volume has been harvested, the rest is dumped.
- c. Depending on the tank design, the flow of rotifers might stop while there is an inch or so of water in the tank. Generally that's helpful, as it keeps the slime and scum from the bottom of the tank from getting into the harvest sieve.
- d. When the tank is empty, put the end of the harvest hose in the drain. Lift the 250 μ m sieve out of the rinser, letting it drain first. Increase the flow on the warm rinse water hose all the way and put it directly in the 55 μ m sieve. This will rinse the rotifers. At this point, the rinsing rotifers can be left relatively unattended, as long as the warm water hose is secure in the sieve. The flow from the warm water hose, combined with the aeration in the rinser, circulates the rotifers during the rinse.



8. During the rinse, there should be plenty of time available to feed out the background feed and ready the new tank. There probably also will be enough time to clean out the old tank, but that will depend on the amount of warm seawater available for rinsing. Assuming the rinse time is not limited by the amount of warm seawater, aim for a rinse of about 10 minutes. If the harvested tank was particularly infested with ciliates or just exceptionally dirty, the rinse can be extended, but keep in mind that the rotifers are very dense at this point and should be moved to the new tank as quickly as is reasonable.

- a. Background feed — The rotifers need some food in the new tank when they're introduced. The amount will be determined by how many rotifers are estimated to remain after the move. For instance, if the old tank has 100 million rotifers in it and the intent is to keep them all, feed as if there will be 100 million in the new tank ($100 \text{ million} \times 0.4 \text{ g/million} = 40 \text{ g/day}$; divide by the number of feedings per day to get the amount per feeding). Err a bit on the low side, since a small number of rotifers are always lost during the process, and it would be undesirable to overfeed. Blend the feed up as usual and dump it in. If the population will be knocked down during the rollover, just feed according to the estimated population after the move.

- b. Cleaning the tank — The best method of course will depend on the specific tanks and environment in question, but the basic goal is to get things as clean as possible. Everything that was in the tank needs to get hosed off and scrubbed so that there is no slime or film on it, including the walls of the tank. It is not necessary to use detergent or anything at this point. Any sanitation that needs to take place will be accomplished by the bleach solution when the tank is refilled. There is some evidence that rotifers do best when some of the old culture water is transferred to the new tank, as it helps to promote the colonization of beneficial bacteria. At the CML, this has never been tried, so clearly it's not strictly necessary.
9. The move — Use a very clean bucket for transferring the rotifers to a new tank. At the CML there is a bucket dedicated to this task and used for nothing else. If necessary, sanitize the bucket with bleach solution or an acid bath, and rinse well.
- Remove the warm water rinse hose from the 55 μm sieve and put a couple inches of water into the bucket.
 - Lift the 55 μm sieve out of the water by the handle and twist it back and forth gently to let most of the water drain out. Get as much liquid out as possible (getting it completely out is impossible). Rinse down the sides of the sieve a few times with the warm seawater to make sure all the rotifers are concentrated in the middle. Try not to be too violent with this step, as the rotifers will be damaged.
 - CAREFULLY dump the rotifers into the bucket. The easiest way is to pinch the sieve just above the concentrated rotifers and carefully turn it upside into the bucket, then release the sieve to let the rotifers run out. Use the rinse hose to rinse the rotifers off the sieve, put it aside, and rinse the rotifers off the side of the bucket into the water.
 - Take the bucket and carefully dump it into the new rotifer tank. Rinse the sides down again with the warm water hose and dump it into the tank again if it looks like there are lots of rotifers remaining in the bucket.
10. Clean everything up and put it away for next time. Remember to rinse the inside of the harvest hose well before putting it away and store it so that it doesn't retain water inside.
11. Allow the new tank about an hour to circulate, then count the rotifers again, using the same procedure as before. Determine the feed amounts as before, dividing the total amount by the number of feedings per day. Remember that the background feeding in the new tank takes the place of the 0800 feeding. Resume the regular feeding schedule after that.



Rotifer Troubleshooting and Some Notes

- If the rotifers are moving around sluggishly in the live sample, it usually means the oxygen is low or there is something else fundamentally wrong with the water quality. Check the DO and temperature, and make whatever adjustments are necessary to get back to the ideal measurements. Also check that the air is at a rolling boil, which will insure good circulation in the tank. Don't go overboard with the air, as violent bubbling will damage or kill the rotifers. Assuming everything looks okay, sluggish rotifers might also just be hungry.
- If the water is extremely dirty/smelly and the rotifers look like they're covered in scum, it's time to move the tank. Hopefully, this is on schedule. If the water is only slightly dirty and the rotifers look alright otherwise, consider underfeeding a bit. Also make sure the water quality is good. "Iffy" water quality can stress the rotifers, which causes them to eat less, which causes the feed to build up in the water.
- If the daily count on a tank reveals a low egg percentage (less than 15%), it is a sign that something is wrong with the tank. If it's a young tank, it may just be a result of the move, since the physical trauma of the rollover simply can knock eggs off of the rotifers. Otherwise, again, check the water quality. If it's an older tank with low eggs, consider increasing the feed a bit, assuming the water is relatively clean. If you have reason to suspect that something is off about the water, you can roll the rotifers over into a new tank, hopefully to get them into some higher-quality water to recover.
- If the rotifers aren't moving at all and you can see no internal movement (and you haven't put formalin on them yet), then the tank is dead for whatever reason. It's important to figure out what went wrong and look closely at the remaining tanks to ensure that it won't happen again.



Chapter 5: *Artemia* Culture CML Style



Figure 5.1: Artemia nauplii (Instar II). Note size comparison between the larger Artemia and the one small rotifer shown in the lower left. Photo courtesy of Nick King.

Artemia (brine shrimp)(Figure 5.1) are the second live feed for the winter flounder larvae. Again, the concept is simple: hatch, enrich and feed out. *Artemia* culture at the CML is done with a strictly batch procedure, preparing one day's worth at a time. Dried cysts are available from several suppliers and most of the commercially available ones come from the Great Salt Lake. At the CML, the preferred brand is the "silver" grade 85% hatchout variety from Argent Laboratories. There are other, higher grades (higher hatchout percentages) available, but the silver grade seems a good compromise between performance and price. Higher grades get pricey very quickly.

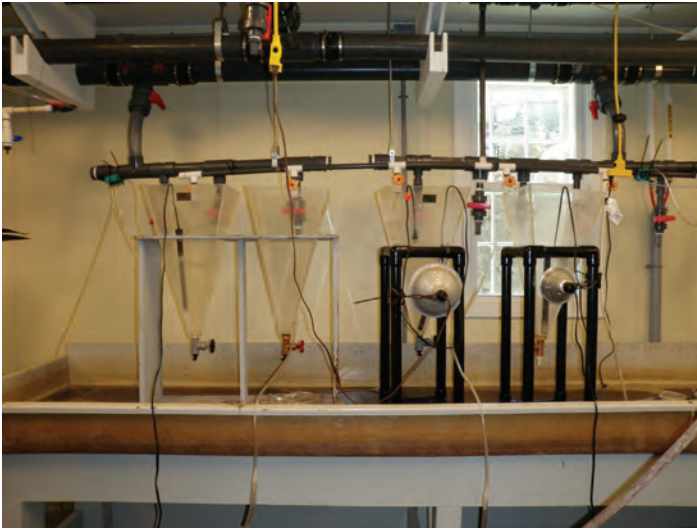
The amount of *Artemia* cysts that need to be hatched depends of course on the amount of living *Artemia* needed to feed the fish larvae. At the CML, a 1.8 m diameter tank

fully stocked with fish gets a minimum of one million *Artemia* three times per day. The amount of *Artemia* hatched out simply depends on the number of tanks that need to be fed. Experience suggests that with the silver-grade *Artemia*, 30 g of cysts, decapsulated, will yield approximately five million enriched *Artemia* when all is said and done. However, because *Artemia* cysts are harvested from the wild, there will always be some variability, and counts should be done to quantify the yields of a particular facility in a particular year.

Hatching Setup

Tank Requirements:

Artemia can be hatched out in almost any container with some form of circulation. They are actually popular as "pets" for children (and adults) due to the ease with which one can coax life from a cyst that most closely resembles a grain of sand. However, the ideal hatching container for *Artemia* cysts is a conically shaped one because it encourages good circulation throughout the hatching process, as well as providing a fairly convenient method of harvest. The preferred container at the CML is a clear acrylic hatching cone, approximately 15 L capacity, mounted either in premade PVC stands or home-made wooden stands (Figures 5.2 and 5.3). A gate valve is installed at the bottom, at the point of the cone. The valve should be located high enough above the floor so that various containers can fit underneath it, for convenience. It's best to have enough of these containers that one can always be ready to go filled with heated, sanitized water. Alternatively, the cysts can be added to cones with cold water and have the water heated after the fact if necessary, but this extends the hatching time and therefore is undesirable in a hatchery. The Argent cysts used at the CML recommend a density ≤ 10 g/L; usually they are hatched at < 4 g/L.



Lighting Requirements:

Artemia need a source of light for successful hatching. At the CML, light is provided with simple clip-on spotlights, available from hardware stores. Because these lights are not necessarily intended for wet environments, care must be taken when locating them and they should be secured as strongly as possible. If the hatching tanks are opaque, light can be provided from above. Overhead room lights, however, probably will not be enough for hatching *Artemia*, and an additional source should be provided. If the clip-on lights are utilized, it's best to use compact fluorescent bulbs. Standard incandescent bulbs can transfer a lot of heat to the hatching water, causing the temperature to climb out of the ideal range.

Aeration Requirements:

During the hatch, the cysts must remain in constant suspension, which of course requires good circulation. The easiest way to achieve that, as with the rotifers, is with aeration. At the CML, the hatching cones are aerated by introducing air into the bottom harvest valve, which then can be opened a small amount to let bubbles into the cone (Figure 5.4). This produces an adjustable supply of large bubbles that keep the cysts in suspension and the dissolved oxygen at saturation. Aeration also can be supplied by a rigid tube that exits near the bottom of the cone, as long as it produces fairly large bubbles. An airstone is not preferred for *Artemia* hatching because the small bubbles can cause foam, can get lodged in swimming appendages, and can even be ingested by the *Artemia*. If your blower is in a damp location, or you have unexplained contamination problems, a desiccant cartridge plumbed into the air line may help. The air should be turned up relatively high for hatching, as good oxygen saturation and good circulation is necessary. Think "dancing tanks" when determining the correct amount of air, particularly if the hatching tanks are on individual stands; they should move around a bit under the force of the bubbles. There may be some splash-over depending on tank design.



Figure 5.2 (left): Hatching cones set up at the CML.

Figure 5.3 (above): Individual hatching cone. Note clip-on light and aquarium heater.

Figure 5.4 (below): Hatching cone set up and ready to begin hatching. Note the high aeration from the bottom valve.



Temperature Requirements:

The ideal temperature for *Artemia* hatching is generally 25-28°C. If this is above room temperature in the facility, each hatching cone will require its own heat source. At the CML, this is provided by standard 300 W aquarium heaters, one in each cone. Redundancy would be best, of course, but space is limited in the small cones, so one heater will suffice if the temperature is monitored several times daily.

The method of culture described here also requires a supply of warm seawater, which is used mainly for rinsing *Artemia* between the hatching and enriching phases, but can also be used to fill cones/tanks that don't have time to come to temperature with the help of their own heater. Obviously, the same reservoir utilized for rotifer culture can be used for *Artemia* duties. At the CML, the warm seawater reservoir is simply a large plastic tank, approximately 180 gallons, and heated with a single 300 W aquarium heater. A small pump attached to a length of ½" tubing is placed in the tank and left plugged in, so it works to circulate the water. When warm water is needed, a longer piece of tubing is attached to the pump and directed to wherever it's needed. This water is sanitized with bleach and neutralized when needed.

Figure 5.5: Enrichment tank. Note two air sources and aquarium heater. Oxygen diffuser not pictured.

Enrichment Setup

After hatching, the *Artemia* need to be enriched so that they are more nutritious for larval fish. Although a conical bottomed tank would once again be ideal for circulation and harvesting purposes, it's less of a requirement for the enrichment phase. At the CML, *Artemia* are enriched in clear, cylindrical, fiberglass 'k-walls', approximately 75 L in volume with a flat bottom and a valve extending from the center of the bottom (Figure 5.5). Circular tanks are preferable due to the lack of dead spots in the circulation. *Artemia* generally can be enriched in approximately double the volume of water in which they were hatched, so the number of hatching cones in action can help determine how many enrichment tanks should be available. If the space and tanks are available, it's a good idea to split up the enrichment into two or more tanks. If something goes wrong during the enrichment period and causes one of the tanks to crash, the fish will still get *Artemia* for that day, albeit at a reduced amount. In addition, because the *Artemia* are hatched and active when they are placed in the enrichment tank, the tank should already be at temperature. At the CML, this means that a k-wall is filled, sanitized and heated one day before it's needed; this way the tank has plenty of time to come to temperature and sanitize.

Lighting Requirements:

As with the hatching process, light also is required for the enrichment. Again, this can be provided by any means available. At the CML, light is provided by a halogen work light pointed at the tanks. Obviously, opaque tanks will require an overhead source. In either case, the light should be far enough away so that the heat produced will not affect the temperature of the water.



Temperature Requirements:

Some method of heating the water will be necessary during the enrichment. Anywhere from 28-30°C will work for enriching the *Artemia*, though at the CML the temperature is maintained closer to 28°C. This will slow the growth of the *Artemia* just slightly and make them easier for the fish to catch and eat.

Aeration Requirements:

Good aeration is required for a successful enrichment. The air should be introduced near or at the bottom of the tank to encourage good circulation and gas exchange. At the CML there are two air sources in each tank: an open rigid tube that is suspended approximately 10 cm above the bottom of the k-wall and air introduced through the bottom valve of the k-wall. This provides a bit of back-up in case one fails and also helps prevent the *Artemia* and the enrichment product from finding their way into the valve and getting stuck there. Air from the bottom valve is sufficient, as long as the circulation it promotes is good, with no dead areas or settling zones. As far as how much air is required, during enrichment the water in the tank should resemble a rolling boil. It should not boil violently as this will damage and kill the *Artemia*.

The enrichment products, as well as the activity of the *Artemia* as they metabolize them, can degrade the water quality rather quickly in the enrichment tank. Therefore supplemental oxygen is a good insurance policy, if not an outright necessity. As with the rotifer culturing, welding-grade oxygen delivered to an ultra-fine pore diffuser located near the bottom of the tank is the best way to get oxygen into the culture water.

Cultured juvenile winter flounder



Water Preparation and Sanitation

Culture water for all forms of live feed at the CML passes through an additional filtering system compared to the water supply that feeds the fish tanks. The water is pumped through a standard pool sand filter, passes through a UV sterilizer, and is then filtered through cartridge filters of 5 µm and 1 µm in size. The 1 µm filtered water then is used to fill the static systems. In addition, standard household bleach is used to sanitize the various live feed tanks in the facility. Household bleach is readily available and easy to work with, but contains stabilizers that extend the time of potency. Because of these stabilizers, aeration is often unable to neutralize the chlorine and a sodium thiosulfate solution



must be used instead of or in combination with aeration to neutralize the bleach. At the CML, water is prepared with 0.25 mL of liquid bleach per liter of seawater. This results in a concentration of approximately 1.25 ppm of chlorine, which is actually fairly low. A higher concentration may be needed if the culture water is not filtered to 1 μ m and sterilized with UV radiation before sanitization.

Dry chlorine or pool shock treatment can also be used to sanitize culture water. These products contain about 70% chlorine, and 1/8 teaspoon per five gallons of seawater yields a concentration of 1-3 ppm. These products must be stored carefully in a dry place to maintain potency, and potency decreases rapidly when mixed in seawater. At the CML, liquid bleach is preferred partly because of its availability and partly because of its versatility around the facility.

Though aeration can eventually strip the chlorine from water, theoretically leaving it fit for culturing organisms, an addition of sodium thiosulfate provides an extra measure of confidence that the chlorine has been removed. At the CML, sodium thiosulfate crystals are dissolved in warm tap water at a concentration of 100 g/L. Use of this solution is simple: to neutralize X mL of bleach, use 0.5X mL of the sodium thiosulfate solution. This is mathematically more than is necessary to neutralize the bleach, but it provides a safety margin for neutralization and seems to have no ill effects on the organisms.

When setting up tanks for the first time, turn the aeration system down to a minimal level after adding the dose of chlorine. This will allow maximum sanitation in the time allowed, and prevent any reduction in potency caused by aerating the water. The aeration will be turned back up when the sodium thiosulfate is added to aid in neutralization and also for circulation and degassing during the actual culture period.

Water Quality

Both the hatching cones and the enrichment tanks are drained on a daily basis, so there won't really be any trends to watch for in water quality measurements. However, daily measurements should be performed to ensure that the heaters and oxygen systems are all working as they should be. Temperature should be 25-28°C for the hatching tanks and 28-30°C for the enrichment tanks. Salinity is fine as long as it's between about 15 and 30 ppt. Dissolved oxygen should be maintained as close to 100% saturation as possible in the hatching tanks. The absolute measurement will depend on the temperature of the water. In the enrichment tanks, keep the oxygen above 7 mg/L. Pay particular attention to the dissolved oxygen in the enrichment tanks, since the enrichment process can degrade water quality fairly quickly.

Decapsulation of *Artemia* Cysts

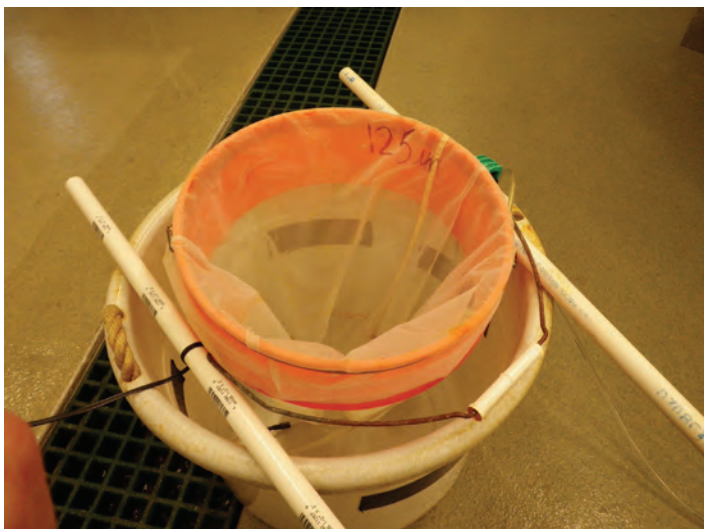
Cysts in their dried state have a hard, virtually indigestible layer called the chorion that envelops the cyst. Besides being indigestible for the larval fish (and potentially causing gut obstructions and death), the chorion can cause lower hatch rates as well as a potential avenue for contamination of the culture. It is also the chorion that causes unhatched cysts to float, as a small amount of air gets trapped underneath it. It's in the best interest of the culturist to get rid of the chorion, a process called decapsulation. Aside from essentially sanitizing the cysts, decapsulation also can increase hatch rates. It also has the advantage of making even unhatched cysts somewhat nutritious for the fish, since the whole thing is now completely digestible. In addition, decapsulated cysts are more likely to hatch in the enrichment than standard cysts.

A dry *Artemia* cyst is actually not spherical at all, but instead resembles a deflated soccer ball with a pronounced dimple in the side. This dimple makes it difficult for the chorion to be removed completely, so decapsulation actually becomes a two-step process: 1) hydrate the cysts in seawater and then 2) decapsulate them. At the CML, hydration is done in a hatching cone filled with clean seawater at 25°C, but any container with good circulation will work. Aeration should be high to promote good circulation.

Decapsulation is a relatively simple process requiring easily attainable items, but is time consuming. Luckily, decapsulated cysts can be stored in the refrigerator for up to a week, so it's best to make a big batch at the beginning of each week and store it for several days usage.

Due to the chemicals involved, it's best to keep safety in mind when decapsulating cysts. The bleach is of course just household bleach, with all the precautions that entails. However, the sodium hydroxide (in the form of drain cleaner) is incredibly caustic. Heavy gloves should be used when making up the stock solution and care should be exercised even when dealing with the diluted form. Also, the decapsulation reaction is strongly exothermic (gives off heat), so it's best to have some way to keep things cool during the process. At the CML, the chemicals are chilled in the fridge prior to the reaction and the reaction vessel itself is cooled in a flowing seawater bath. A container of ice would work just as well.

The only chemical in the process that can be somewhat difficult to locate is the 100% sodium hydroxide (NaOH). It is commonly called lye and the easiest way to obtain it is to find a bottle of "100% lye" drain cleaner. Lowe's sells such a brand, known as Roebic Crystal Drain Opener. Sodium hydroxide also is available at chemical supply companies. If you go the drain cleaner route, be absolutely sure that the label says "100% lye," as any other additional ingredients will throw off the reaction.



Preparing the stock solution of sodium hydroxide (NaOH):

Make a 50% solution of NaOH by dissolving 50 g of NaOH crystals in 50 mL of clean tap water. Be prepared for this mixture to let off quite a bit of heat as it is mixed. Scale up the amounts as desired (at the CML, 100 g NaOH dissolved in 100 mL of water is enough for the entire *Artemia* stage of fish culture). Store the finished solution in the fridge and cool it down.

Figure 5.6: Rinser with bag sieve. Note: For decapsulation and harvest of newly hatched Artemia, a 100 µm sieve is used instead.

Materials needed:

- scale
- 50% NaOH solution
- Bleach
- Source of clean seawater (UV sterilized is best)
- Clean beakers for chemicals, spatulas, pipets for measuring liquids, graduated cylinders
- Dry *Artemia* cysts
- 100 µm bag sieve (Figure 5.6)
- Rinser
- Source of aeration, with a long enough hose for convenience and a rigid tube at the end for combination stirring and aeration
- Sodium thiosulfate solution, mixed at 100 g/L
- Garden hose, for various rinsing and cleaning duties
- Container for cold storage of decapsulated cysts, properly sized for whatever decapsulation/hatching schedule is desired

Methods:

1. Add the required amount of cysts (up to 1 g of cysts/30 mL of seawater) to the prepared hydration vessel and ensure the aeration and temperature are at the proper levels. Hydration should take about an hour. Do not exceed two hours, since this can have detrimental effects on the hatch rates.
2. Measure out the chemicals. Per gram of cysts, measure out:
 - a. 0.24 mL of the 50% NaOH solution
 - b. 4.76 mL of clean seawater (UV treated is best)
 - c. 10 mL of household bleach
3. Carefully mix the NaOH solution with the clean seawater. Mix well until it is thoroughly combined. Place the mixture in the fridge to chill down. The bleach also can go in the fridge if there's room.
4. Assemble the rest of the equipment and put it in place. Things happen relatively quickly during the reaction, so it's best to be prepared. The required items are:
 - a. Rinser — The same one used for rotifers will suffice, just remember to clean it well before and after this process.
 - b. 100 µm bag sieve — This will be used both for harvesting the hydrated cysts and to hold the newly decapsulated cysts during the rinse.
 - c. Source of aeration — The cysts need to be aerated and stirred during the decapsulation process, so some method of delivering air to the container is nec-

- essary. At the CML, this is simply a hose from an available air valve, with a rigid tube on the end for easy stirring. A small electric air pump also will work.
- d. Source of rinse seawater — Following the decapsulation reaction, all residual chemicals will need to be removed, so a flowing source of clean seawater is required.
 - e. Sodium thiosulfate solution — This will neutralize any residual chlorine that is not removed by rinsing. A relatively large supply of this is necessary to neutralize the large amount of bleach used in the reaction, so make sure the stock bottle has enough. Approximately 5 mL per gram of cysts decapsulated is a good number to start from (the same as ½ mL per mL of bleach used in the reaction).
 - f. Retrieve the measured NaOH/seawater mixture and the measured bleach and have them ready to go.
 - g. Pitcher or other decapsulation container — This should be large enough to accommodate all the chemicals and the cysts with plenty of room to spare.
5. Harvest the hydrated cysts. Unplug the heater and, using a piece of hose if necessary, drain the contents of the cone into the 100 µm bag sieve. Because of the small amount of liquid available to later wash the cysts into the decapsulation container, it's helpful to try to keep the pile of cysts as compact as possible while they are being drained into the sieve.
 6. Pinch the sieve above the piles of cysts and turn it over into the decapsulation container. Using both the NaOH/seawater mixture and the bleach if necessary, rinse the cysts into the decapsulation container. Be careful not to lose too much of the chemicals.
 7. Quickly move the pitcher to the aeration source and constantly stir the mixture as it's aerated. This is the actual decapsulation reaction. As the chorion dissolves, the color of the mixture will change from brown to a grayish color and finally to a fairly bright orange color. The behavior of the cysts in response to the stirring and aeration also will change as they become negatively buoyant. Do not let the reaction continue for long after the orange color is reached or the chemicals will begin eating away at the cysts themselves. Because of the variability of cysts from year to year, it's impossible to say how long this will take, so keep a close eye on the reaction. For reference, the last run at the CML took about two minutes to decapsulate.
 8. Quickly rinse the majority of the chemicals from the 100 µm sieve, then place it over the rinser where it can easily be reached by the rinse water source. Pour the decapsulated cyst mixture into the sieve and immediately begin rinsing with clean seawater. Also use the clean seawater to rinse the decapsulation container into the sieve. Rinse it well, as it will be used later. Continue rinsing for at least 15 minutes after the seawater has filled up the rinser and begun to overflow.



9. When the rinsing is complete, concentrate the cysts at the bottom of the sieve, lift the sieve out of the water, and upend it over the clean decapsulation container. Use the clean seawater to rinse the cysts into the container and add enough water to circulate. Add the appropriate amount of sodium thiosulfate solution (1/2 the amount of bleach used). Return to the aeration source. Before placing the air tube into the cysts, rinse it to get any residual chlorine off. Aerate and stir the cyst mixture for approximately five minutes to neutralize any residual chlorine on the cysts.
10. Return the cysts to the 100 μm sieve, place it over the rinser, and rinse again for approximately five more minutes to remove the sodium thiosulfate solution.
11. When the rinse is complete, concentrate the cysts at the bottom of the sieve again and use the clean seawater to rinse them into the storage container. Add enough water to make an easily dividable amount for the week's feedings. At the CML, decapsulated cysts are prepared in three-day batches, so 3 L of water is added to the container, allowing easy doses of 1 L each.
12. Rinse everything well, including the cone/tank used for hydrating the cysts. Store the chemicals carefully.

Counting *Artemia*

It is important to count the *Artemia* to determine the yield of cysts from that particular lot (hatched *Artemia*/gram of cysts). Counting *Artemia* is accomplished using the same basic method as counting rotifers (see Chapter 4). However, counting a smaller volume (0.1-0.2 mL) will make the job much easier, as the *Artemia* tend to be fairly dense when in the feed-out pitchers. In addition, it will be necessary to wait approximately 30 minutes for all the *Artemia* to die after adding the formalin.

Winter flounder larvae with Artemia in its gut.



At the CML, *Artemia* is counted each day after the hatching phase for the first week to ensure confidence in the yield of the cysts. When the yield has been established with some amount of certainty, counts just after the hatching phase can be discontinued. Counts are also performed on the enriched *Artemia* to get an estimate of the amount being fed to the fish tanks. The number of enriched *Artemia* will generally be less than the newly hatched *Artemia* that went into the enrichment tanks. However, large losses (>20%) in the enrichment process are signs of a problem and should be investigated. Daily counts of the *Artemia* hatches are not strictly necessary once the yield of the procedure has been established. However, at the CML, daily counts of enriched *Artemia* are continued throughout the *Artemia* phase (time permitting).

Hatching *Artemia* Cysts

Hatching out *Artemia* cysts essentially consists of dumping some ingredients into a container and waiting 24 hours. If the tanks are well designed and circulation is good, there should be no problems with dissolved oxygen levels, but until that is confirmed a daily measurement is necessary. Absolute measurements of dissolved oxygen will vary, but the saturation percentage should be right around 100% with the vigorous bubbling. The decapsulated *Artemia* are prepared ahead of time and should be stored in the fridge. When dosing the cysts into the hatching cone, remember to shake the container very well and pour relatively quickly to get the correct number of cysts, since decapsulating them makes them negatively buoyant and they'll fall out of suspension quickly.

Materials needed:

- Container of decapsulated cysts in water
- Sodium thiosulfate solution mixed at 100 g/L
- Measuring utensil, dry beaker or small container
- Sodium bicarbonate (standard baking soda is fine)
- Two clean pitchers

Methods:

1. One of the hatching cones should be prepared, meaning it has been filled with clean water, heated and bleached. Before doing anything else, neutralize the bleach by adding the appropriate amount of sodium thiosulfate solution.
2. Turn the air up to a fairly high bubble. The tank should be moving around a bit. Make sure the light source is on.
3. Using a dry, clean beaker and a dry, clean utensil, measure out the appropriate amount of baking soda. Dump it into the hatching tank. This helps to buffer the pH as the cysts hatch.
4. After the cone has neutralized for about 10-15 minutes, the cysts can be added. Get the jug of cysts from the fridge and two clean pitchers.
5. Shake the jug well (but not violently) to disperse all of the cysts. Working quickly to avoid settling, remove the cap and pour the required amount into one pitcher.
6. Use the other pitcher to drain about 2 L of water from the harvest valve at the bottom of the hatching tank.
7. Swirl the cysts around and pour quickly into the cone.
8. Use the water from the cone to rinse out the pitcher and get all of the cysts into the cone.
9. Rinse both pitchers. Return the cysts to the fridge.
10. A full hatch will take at least 24 hours. To get the maximum amount of hatched *Artemia*, let the hatch go for as long as possible. At the CML, new cones of *Artemia* are started first thing in the morning and harvested in the late morning/early afternoon of the following day.

Harvesting Hatched *Artemia* Cysts and Enriching

Remember that any water that touches the *Artemia* between the hatching cone and the enrichment tank must be the warm water from the reservoir to prevent thermal stress. So make sure the reservoir has sufficient volume, is the right temperature, and has been properly neutralized with sodium thiosulfate.

Materials needed:

- Rinser
- 100 μm bag sieve
- Sodium thiosulfate solution, mixed at 100 g/L
- Warm water reservoir hose
- Hose for harvesting hatched *Artemia*
- Cleaning supplies, dish detergent
- Pitchers
- Ori-Green enrichment

Methods:

1. The hatching tank that was started the previous day should be orange and bubbling briskly.
2. One of the enriching tanks should already be prepared (i.e., filled with bleached sea water and heated). Neutralize it with the appropriate amount of sodium thiosulfate and turn the aeration system up to the proper level. Ensure that the light source is on. Allow the tank to neutralize for 10-15 minutes.
3. Assemble the sieve/rinser by placing the 100 μm sieve into the rinser.
4. Ready the hose from the warm water reservoir, choke down the flow a bit, and place it in the rinser tub.
5. Turn off the air in the hatching tank.
6. If possible, position the light source so that it is concentrated at the bottom third of the hatching tank (if transparent).
7. Remove the heater if present, wash with detergent, and rinse very well. Put it aside.
8. After a few minutes, you can begin harvesting. Attach a piece of clean hose to the harvest valve on the tank and hold the other end above your head. Turn the valve on all the way to drain the *Artemia* into the hose. If there's a lot of junk in the first few inches of the hose, dump it directly down the drain. Otherwise, the hose can go straight into the 100 μm sieve and all the *Artemia* drained out. If there is a layer of floating garbage (hatched shells as well as any foam or debris) at the surface of the water in the hatching tank, do not add that to the sieve. Just dump it directly down the drain.
9. Position the rinser over a floor drain. Move the warm water hose from the rinser tub directly into the sieve and let the *Artemia* rinse. Rinse for 5-10 minutes after the water begins flowing out of the rinser. Make sure the sieve is sitting level so no *Artemia* will be lost.
10. Clean the hatching tank using a sponge or scrub pad of some sort and some form of detergent (dish detergent is used at the CML). Make sure the inside of the valve is clean. Rinse well.

11. Turn the light off and replace the heater (if necessary), but don't plug it in.
12. Ensure that the enrichment tank is at the correct volume. It should be at least twice the volume of the hatching tank. More volume is better, as long as it can be circulated adequately. Ensure that the heat source, light source, oxygen source and aeration are all working properly and set at the correct levels.
13. When the *Artemia* are finished rinsing, grab two pitchers. Slowly lift the sieve out of the rinser, using the warm water to rinse the *Artemia* down to the bottom of the sieve so they are concentrated. Fill one pitcher with warm water. Gather the sieve above the pile of *Artemia*, invert it over the second pitcher, and use the warm water hose to rinse the *Artemia* into the pitcher. Return the warm water hose end to the reservoir.
14. Dump the pitcher carefully into the enrichment tank. Use the pitcher of warm water to rinse the rest of the *Artemia* into the tank.
15. Rinse everything, including the inside of the harvesting hose.
16. Measure out the appropriate amount of Ori-Green and blend it up in 1.5 L of water. Let it sit for 30 seconds to hydrate, blend it briefly again, and dump it in the enriching tank.
17. For safety's sake, check the oxygen at least twice during the next 24 hours.
18. If necessary, prepare the hatching cone by filling it with seawater, bleaching it with the appropriate amount of bleach, turning the heater on, and setting the aeration to a low level.

Harvesting Enriched *Artemia*

The next stop for the enriched *Artemia* is into the fish tanks so the *Artemia* can be rinsed with whatever cool clean seawater (similar temperature to what the fish are raised in) is available, which will slow their metabolism and prevent them from growing too big for the fish to eat. With this method, the entire day's worth of *Artemia* is harvested at once, then spread out into pitchers (one pitcher per fish tank) and aerated. If possible, the pitchers should be kept chilled to slow down the metabolism of the *Artemia* and prevent them from getting too large for the fish to comfortably eat.

Materials needed:

- Rinser
- 125 μ m bag sieve (Figure 5.7)
- Source of clean seawater
- Hose for harvesting enriched *Artemia*
- Cleaning supplies, dish detergent
- Pitchers

*Figure 5.7:
125 μ m bag
sieve. Note
PVC pipes to
suspend it in
the rinser.*



Methods:

1. Assemble the sieve/rinser again and place it by the enrichment tank. Run a source of clean, cool seawater over to the tank as well.
2. Unplug the heater in the tank (if necessary) and turn off the oxygen. Remove the oxygen diffuser from the tank.
3. Attach a harvesting hose to the harvest valve of the tank. Open the valve and observe

what flows into the hose. If the tank was set up correctly with air coming out of the bottom valve, it should be pretty clean and can go into the sieve. If there is a bunch of junk that had settled in the valve, dump it down the drain before putting the hose in the sieve.

4. Put the clean seawater hose into the sieve. Secure both the harvest hose and the seawater hose to the sieve in such a way that they can't fall out, but flow is not reduced. Continue draining and rinsing.
5. When the tank is empty, turn off the valve and remove the hose. Remove the heater and any aeration devices from the tank. Wash everything well with de-

tergent and set aside.

6. Clean the tank with detergent and a sponge or scrub pad. Rinse very well. Replace the heater, oxygen diffuser and any aeration devices that were removed.
7. After the *Artemia* have rinsed for 10 minutes or so, grab a clean pitcher. Slowly lift the sieve out and twist/shake while rinsing the *Artemia* down into the bottom. With the sieve suspended, rinse the concentrated *Artemia* for a minute or two to clean them up even more. Gather the sieve above the pile of *Artemia*, upend over the pitcher, and use the seawater hose to rinse the *Artemia* into the pitcher.
8. At this point, the pitcher of *Artemia* can be split up according to how many tanks of fish need to be fed. For instance, if there are four tanks of fish, stir the pitcher of *Artemia* well and distribute it evenly into four new pitchers, stirring frequently. Then, top off each pitcher with an amount of water that is easily divisible by the number of feedings in a day. For instance, fill the pitcher to 3 L if there are three feedings in a day (1 L per feeding). The pitchers should be aerated throughout the day and, if possible, kept cool. At the CML, the pitchers are placed in a flowing seawater bath.
9. Rinse the sieve/rinser and put it away.
10. Prepare the enrichment tank for its next use by filling with seawater, sanitizing and heating.

CML seen
from the
UNH Pier.



Chapter 6: Stocking

The exact details for stocking fish will vary with location. The goal is to tag and release as many as 50,000 juvenile, hatchery-reared winter flounder in one location in each state. The release strategy, in terms of selecting the optimal site, season and fish size-at-release, will be determined from the ecosystem analyses (Chapter 2). The following is a general outline of what will happen. These guidelines are just that – a set of suggestions that will vary as more is known about a potential release area and as technology advances marine stock enhancement science. UNH personnel will be involved with all aspects of stocking in both N.Y. and Mass.

Pre-Release

Fish tagging

All released juveniles must be tagged to differentiate them from wild fish in order to measure the effects of the release. Many tagging methods have been used successfully with winter flounder juveniles. The type of tag used will depend somewhat on the fish size-at-release. For age 0 winter flounder, Visible Implant Elastomer (VIE) tags (Northwest Marine Technologies, Inc.) are preferable because they are quickly applied with an air injection tagging system, can be used on winter flounder as small as 2.5 cm TL, and are externally visible (Figure 6.1). Small t-bar tags also are a possibility (Figure 6.2). Experiments using these tags on age 0 winter flounder currently are in progress at UNH.

Regardless of type of tag, all hatchery-reared fish will be tagged at least 48 hrs prior to stocking to allow a recovery period. Immediately prior to release, all fish will be inspected to ensure that tags are still in place. For either elastomer or t-bar tags, two different fluorescent colors (orange and pink) will be used to distinguish two different release treatments (acclimated vs. direct release) in each region. A sample of 100 fish will be held in the laboratory to confirm tag retention.



Figure 6.1 (far left): Elastomer tagged juvenile winter flounder.

Figure 6.2 (left): T-bar tagged juvenile winter flounder.

Health Inspection/Certification

Only normally pigmented, healthy juveniles will be used for this project. Before release, a USDA-certified veterinarian will inspect the fish and only fish deemed healthy will be released.

Pre-Release Survey

To establish the pre-release baseline population structure of winter flounder at the release site, survey work will begin three weeks prior to stockings. Surveys will be conducted weekly for two weeks, then in the third week (the week pre-stocking) surveys will be conducted daily for two to three days immediately prior to the release. Both beam trawl and beach seine will be used on each sampling occasion (see sampling methods in Chapter 2). The catch from all fish sampling will be identified and counted. All winter flounder caught will be checked for tags. Abundance of winter flounder will be estimated as catch-per-unit-effort (CPUE), given as number caught per m² sampled. On each sampling occasion, bottom temperature, salinity, dissolved oxygen and depth will be measured too.

Release

Transport to Release Site

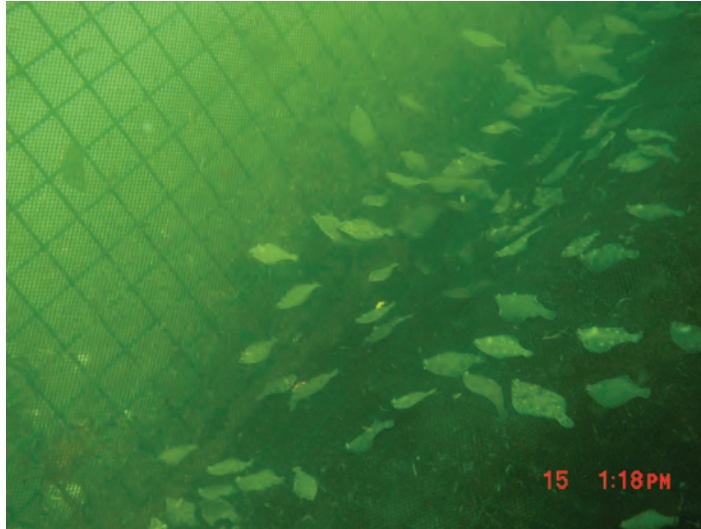
Tagged fish will be transported in insulated containers filled with seawater and supplied with oxygen at stocking densities of $\leq 400\%$ (ratio of fish ventral surface area to container bottom surface area). Depending on the release site, this will be done using a combination of trucks and/or boats. It is important to handle the fish quickly and gently with dip nets and always make sure the dissolved oxygen range is 8-12 mg/l. If transporting by boat, periodically flush the containers with new seawater using an ambient temperature deck hose or with buckets. The fish will be stressed during this process so it's important to monitor them closely to make sure temperature and dissolved oxygen are stable.

Acclimating at the Release Site

For this project, two release methods will be tested in both N.Y. and Mass.: acclimating fish at the release site and direct (no acclimation) release. Conditioning winter flounder in acclimation cages during the first 48 hrs at the release site aids the fish in adjusting to their new environment and reduces the foraging delay of released fish. Caged fish maintain higher site fidelity than fish that are not caged. However, if the acclimation cages are benthic (sit on the bottom), they also will attract green crabs, a major predator of juvenile winter flounder present in most New England estuaries. Juvenile winter flounder have been successfully maintained in floating, in-situ cages (away from bottom-dwelling green crabs), but this release strategy has not been scientifically compared to a direct release (no acclimating or caging), and the benefits are largely unknown. Comparing these release strategies is one of the goals of this project.

Acclimated fish (orange tags) will be stocked first into covered, floating cages anchored at the release site. After a two-day period, these acclimated fish will be released from the cages by opening the bottom and letting the flounder swim to the bottom (Figure 6.3). At the same time, all remaining fish (direct release – pink tags) will be released at the same location by gently transferring the fish from the hatchery straight into the water at the release site.

*Figure 6.3:
Hatchery-
reared
winter floun-
der being
released
from ac-
climation
cages.*



Post-Release

Surveys and Data Collection

Because in the first days immediately following release, fish are most likely to emigrate or be preyed on (Furuta et al. 1998; Sparrevohn and Stottrup 2007; Fairchild et al. 2009), sampling for released fish will begin the day after release, and will occur daily for the first two weeks and at weekly intervals for the following 10 weeks. Both beam trawl and beach seine will be used on each sampling occasion (see sampling methods in Chapter 2). The catch from all fish sampling will be identified and counted. All winter flounder caught will be checked for tags. Abundance of winter flounder will be estimated as catch-per-unit-effort (CPUE), given as number caught per m² sampled. On each sampling occasion, bottom temperature, salinity, dissolved oxygen and depth will be measured too.

To compare the feeding ecology of hatchery-reared and wild fish, we will dissect out and preserve stomachs from a representative sample of 10 recaptured fish from each release treatment and 10 similarly sized wild fish, at weekly intervals. Individual fish will be euthanized with an overdose of tricaine methane sulfonate (MS-222, 100 ppm), formalin will be injected directly into the stomach using a syringe, and whole fish will be stored in containers filled with 10% buffered formalin until analyses at UNH. At UNH, prey taxa will be identified, to the lowest possible taxonomic level, using a dissecting microscope. The number and total weight of each taxa will be recorded.



Data Interpretation and Analyses

By testing and comparing two different release strategies in each region, we will be able to evaluate the overall success of the releases by:

- **Estimating the mortality (survival) of released fish.**

Instantaneous natural mortality rates (M) will be calculated from survival estimates (S). For the first two weeks after release, the time interval will be one day, yielding daily mortality estimates. From weeks 3-12 after release, sampling will be done weekly, yielding weekly mortality estimates. A longer term, monthly instantaneous natural mortality rate estimate (M_{mo}), will be calculated using catch curve analyses based on weekly abundance data (Ricker 1975).



- **Describing the diet transition in released fish.**

An Index of Relative Importance (IRI) will be calculated for each prey taxa, for both hatchery-released fish and for wild fish. These data will allow us to determine how quickly cultured fish adapt to natural (wild) prey, if there are any differences in diet transition of fish from different release treatments, and whether there is a need for any additional pre-release conditioning.

- **Studying the movements of released fish.**

Movement away from the release location, here considered emigration, will be quantified using recapture data. The magnitude of movement will be estimated from the mean of all straight-line distances between recapture locations and release location. The rate of movement will be estimated from distance traveled per unit time. Data will also be grouped by size class, release treatment and month to determine how these three variables affect distances moved and rates of movement.

Chapter 7: Summary

Once all the various components of the winter flounder release have been analyzed, we will determine the impacts of the project. This will entail a final workshop open to all project participants, scientists, regulators and interested parties, and a summary proceeding published through Sea Grant. If this project and restocking strategy are successful, this will not be just another scientific research exercise — this model will be applicable to other New England fishing communities to expedite the recovery of winter flounder populations. The goal is that both Martha's Vineyard and East Hampton implement responsible winter flounder enhancement programs, and that they become the model (demonstration sites) for other coastal communities, states and regulators.



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Appendix

Data Sheets: Beam Trawl Data Sheet

2004 TRAWL DATA SHEET

[illegible]

Data Sheets: Beach Seine Data Sheet

2004 SEINE DATA SHEET

DATE	LOCATION				SEINE #		
	START TIME						
TIME OF LOW TIDE					SEINE DISTANCE		
	END TIME						
CORES TAKEN? (y/n)							
COMMENTS							
SPECIES	TOTAL N	LENGTH	TAGS?	SEX	LENGTH	TAGS?	SEX

Data Sheets: Benthic Sample Inventory Sheet

[illegible]

Data Sheets: Hatchery Data Sheet

[illegible]

Data Sheets: Rotifer Data Sheet

Rotifer Tubs 2010

[illegible]

Winter Flounder Culture Chapters: Howell and Litvak 2000

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Surface water sources, especially those from established ecosystems, will contain pathogens and potential predators. Pollution may also be a concern for rain, surface, and groundwater sources that are in proximity to a pollution source. Various screens and sterilizers are available to reduce predators and pathogens, but treatments for pollution will depend on the nature of the pollution, and may not be treatable.

Regulations for the removal of water from either a groundwater or surface-water source exist at multiple levels in almost all countries. This is true for marine or freshwater sources. Additional regulations govern discharge of water from aquaculture facilities. Property owners should check with local authorities and government agencies regarding rights and permits for development of the water source.

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See also SITE SELECTION.

WINTER FLOUNDER CULTURE

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OUTLINE

Collection and Maintenance of Broodstock
Collection and Incubation of Eggs

Collection
Incubation Systems
Incubation Conditions
Larviculture
Development and Size
Larval Rearing Systems
Photoperiod and Light Intensity
Larval Stocking Densities
Green Water
Larval Feeding
Larval Growth Rates
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Bibliography

The winter flounder, *Pseudopleuronectes americanus*, is a right-eyed flounder (family Pleuronectidae) found along the east coast of North America from Labrador, Canada to Georgia, USA. The species is typically found on mud and sand substrates, at depths ranging from the shallow subtidal to 37 m (1). Maximum size is usually 2.25 kg and 65 cm, although they occasionally grow to over 3 kg (2). Among the flatfish species found along the coast of New England, winter flounder are the heaviest per unit length (3). The species has been exploited both commercially and recreationally for well over a century. Their stocks have been in decline over the past 20 years, and the species is currently considered overexploited (4).

The first propagation of winter flounder occurred in the late 1880s, at three government hatcheries located in Massachusetts and Maine, and operated by the U.S. Fish and Fisheries Commission. The work was undertaken in an effort to rebuild declining wild populations, and tens of millions of early larvae were released before the last of these hatcheries closed in the early 1950s (5,6). Although the success of those efforts was probably minimal due to the small size of the larvae released, some of the basic culture techniques developed through those efforts are still in use today.

Recent declines in the fishery, combined with a demand for high quality flatfish, have once again stimulated interest in the culture of various flounder species, including winter flounder (7). Although a pilot attempt to rear winter flounder in New Hampshire had only minimal success (8), advances since then, by a number of researchers, have improved the ability to culture this species. Researchers and culturists have also relied heavily on the wealth of information available on production techniques for other flounder species, including turbot (9,10), Japanese flounder (11), and summer flounder (12).

The purpose of this entry is to review what is known about winter flounder culture. Regrettably there are some topics for which little information is available (e.g., broodstock management, growout systems). In some cases, techniques come from our own recent studies, which are not yet published. Nevertheless, we are confident that the techniques work, and since we are anxious to give the reader the most recent information, we have included some of these results and observations.

COLLECTION AND MAINTENANCE OF BROODSTOCK

Currently broodstock are being kept by Huntsman Marine Science Centre in Saint Andrews, NB, Canada, and by Sambro Fisheries, Sambro, NS, Canada. Save for a few fish kept at Sambro that were grown by Litvak's laboratory in 1994–1997, these broodstock are wild caught. For the most part, researchers have relied on wild-caught adults to produce the early life-history stages needed for their studies (13–25). Brood fish are collected by trawl net, gillnet, fyke net, or by divers in the weeks preceding the natural spawning season, and returned to the laboratory. Ripe females are identified by their swollen ovaries, which indicate hydration, and spermiating males are identified as those which release milt upon slight abdominal pressure. Maintenance of captive adults for short periods of time is relatively easy. Although females fast during the spawning season, males readily consume clams, squid, chopped menhaden, silversides, clam worms, earthworms, and chopped capelin during the spawning season (16,26–29).

Wild winter flounder adults caught prior to spawning will usually undergo gametogenesis when provided with photoperiods and temperatures that are "normal" to their place and time. Spawning times vary from December to June, beginning earlier in the southern part of the fish's range and later in the northern parts of the range (30,31). Final maturation and spawning are commonly induced through hormonal manipulation. Smigielski (32) experimented with a variety of hormones, including human chorionic gonadotropin (HCG), pregnant mare serum gonadotropin (PMSG), deoxycorticosterone (DOCA), oxytocin, and freeze-dried carp pituitary extract. Smigielski's best success, in every case, was with freeze-dried carp pituitary extract, at doses of 0.5 or 5 mg/454 g female body weight. The extract was mixed in an isotonic (to fish blood) solution of sodium chloride as a carrier, and injected intramuscularly on a daily basis until spawning. Fish receiving the higher dose spawned after only three injections, while those receiving the lower dose required six injections. All but one female (which was later determined to be sexually immature) hydrated and spawned, and all resultant eggs and larvae were normal. Water temperature appeared to be a critical factor in producing ovulation, as the majority of fish did not hydrate at temperatures above 6 °C, even under hormonal treatment.

More recently, Harmin and Crim (33,34) conducted additional research on hormonal induction of maturation and spawning. Intraperitoneal injections of gonadotropic releasing hormone analog (GnRH-A), 20 µg per kg

body weight, three times per week, resulted in a few females ovulating at temperatures as low as 0 °C, and accelerated ovulation and increased spawning reliability in prespawning flounders maintained at 5 °C. Harmin and Crim were able to induce spawning in February, which was three months prior to normal spawning season in their area (Newfoundland). Furthermore, egg and larval quality (as indicated by rates of fertilization, hatching, and larval survival) was good after this accelerated spawning. The researchers also observed that when using intramuscular implants of GnRH-A (100–120 µg slow release, or 40 µg fast release) there were rapid and predictable ovulatory responses from the fish.

Harmin and Crim (35) have also used GnRH-A injections to treat prespawning males. Maturing fish treated during winter (December/January), with a single injection of either 20 or 200 µg/kg body weight, showed increased levels of testosterone and 11 ketotestosterone within 12 hours and these levels remained high for several days. Single injections advanced spermiation in some individuals, but only small amounts (<50 µL) of milt were produced. By March, following GnRH-A treatment, all males were spermiating. In fish that were injected twice, there was a significant increase in sperm production and milt volume.

Although wild fish are easily collected and spawned in the laboratory, the development of a winter-flounder aquaculture industry will require the establishment of captive adult populations (broodstock) to produce a reliable supply of high-quality eggs. There are several disadvantages associated with the use of wild brood fish. Perry et al. (22) have shown, for example, in brood fish collected from areas that differed in habitat quality, that habitat affected the viability and health of resultant embryos and larvae. Fish from anthropogenically contaminated areas produced eggs with high incidence of chromosome damage and mitotic abnormalities. Similarly, brood fish collected from different spawning locations in Long Island Sound, CT, and Narragansett Bay, RI, spanning about 200 km, produced larvae with different mean sizes at hatching and different biochemical content (20). This was important, because there was a direct correlation between these variables and survival through the first month. In a related study (21), it was found that spawning time and female size affected the composition and viability of the eggs and larvae. Larger females produced larger eggs, and mean egg weight from the population decreased as the spawning season progressed. Therefore, large, early spawning fish produced the largest eggs, and these in turn had the highest viability.

COLLECTION AND INCUBATION OF EGGS

Collection

The fecundity of winter flounder has been described by a number of authors for a number of areas (2,36–38) and generally ranges from 100,000 to 3.3 million eggs per female, depending on female size. Ripe winter-flounder

eggs are spherical, range in diameter from about 0.71 to 0.96 mm diameter (39), have a specific gravity of 1.085–1.095 (40), and are adhesive (15). Thus, eggs form demersal clumps when extruded. Artificial fertilization is typically done using the "dry" technique, in which the ripe eggs are stripped from the female into a dry container. Typically all the eggs from a female are extruded by a single stripping. A mixture of milt and clean seawater is then added to the eggs, and the mixture is swirled for several minutes to ensure fertilization. Fertilization percentages are generally quite high and range from 78 to 93% (20,21). Because high egg mortalities have been attributed to clumping (15), the fertilized eggs are often treated with a diatomaceous earth solution to prevent them from adhering to one another. Newly fertilized eggs are spread into a polyethylene pan, then covered by a dense slurry of diatomaceous earth solution (50 g diatomaceous earth in 1 L of sterile seawater). The mixture is swirled for five minutes and then rinsed to remove excess earth (15). Litvak's lab has switched to Pyrex trays instead of polyethylene pans because plastic is prone to scratches, making them harder to disinfect after use and increasing the risk of bacterial outbreak. Alternatives to this procedure include the "plating out" of eggs into single layers on panels of either glass or fine plastic screening, which allows each egg more contact with the seawater (Klein-MacPhee, unpublished).

Very ripe females spawn volitionally when placed together with males in small (100 L) spawning tanks supplied with ambient (6°C, 30 ppt) flowing seawater (25). No attempt is made to control photoperiod or temperature, but the amount of ambient light entering the spawning tanks is reduced by covering them with black window screens. Stocking ratio of males to females is 3:1. Depending on ripeness, the fish spawn within 1 to 10 days, and spawning always occurs at night. Although winter flounder are reputedly batch spawners (30), our experience is that each female releases most of her eggs in a single spawning. Occasionally, however, smaller groups of eggs are released on subsequent days. After spawning is completed, the adults are removed from the tank. Eggs are allowed to clump and are then moved to 100 L tanks supplied with flowing seawater. This technique of volitional spawning usually resulted in very high (>95%) fertilization rates (Howell, unpublished). Although spawning behavior was not observed by King and Howell (25), the spawning behavior of captive winter flounder has been described (41).

Incubation Systems

A variety of embryo incubation systems have been used successfully. Smigielski and Arnold (15) used simple "incubation baskets," which were 15 L rectangular plastic containers into which windows, covered with 505 µm plastic screening, had been cut in the sides and bottom. Baskets of fertilized eggs were then incubated in flowing seawater troughs. Similar systems have been used by other researchers (16,20,22). Six-liter acrylic hatching jars, supplied with filtered seawater at ambient temperatures and salinities (4–6°C; 31–33 ppt) have

also been used successfully (42–44). Static methods, in which embryos are incubated in small, temperature-controlled containers, and in which a portion of the water (25–50%) is periodically (every 1 to 3 days) replaced with filtered, ultraviolet treated seawater, have also been used extensively (24,45,46). Stocking densities of embryos in static systems have ranged from 40–1250 per liter (16,19,43). Litvak's lab incubates eggs at a density as high as 5000 per liter in Pyrex trays. These relatively low densities prevent ammonia buildup and oxygen depletion. With containers placed in a flowing seawater trough, Smigielski and Arnold (15) stocked eggs at 17,000–34,000 per liter. King and Howell (25) allowed entire egg masses to incubate in 100 L, flowing seawater tanks. Stocking density of eggs is estimated as 10,000–30,000 per liter.

Incubation Conditions

Winter flounder embryos are relatively eurythermal, but survival to hatching is generally higher at temperatures less than 10°C. Williams (47) incubated eggs over a range of water temperatures from –1.8 to 18°C and found that viability was high over a wide temperature range. Percentage of eggs surviving to hatch at low temperature (less than –1°C) was variable, ranging from 0 to 79%. Survival to hatching was consistently higher than 75% for embryos incubated between 0 and 10°C and consistently less than 75% for those incubated at temperatures between 10 and 15°C. It was noted, however, that egg mortality may have resulted from microbial infection rather than directly from temperature. The upper lethal thermal limit was given as 15°C. Buckley et al. (19) reported hatching percentages of 70–85% at 4, 7, and 10°C, and noted that percentage was significantly higher at the lower temperatures. They also noted that hatching percentage was unaffected by the acclimation temperatures of the adults, which were 2 and 7°C. Rogers (16) experimentally investigated various combinations of incubation temperature (3–14°C) and salinity (0.5–45 ppt). She found that viable hatching was highest at 3°C, lowest at 14°C, and was similar at 5, 7, and 12°C. Moreover, she found that temperature and salinity interacted, such that the highest viable hatch (78%) occurred at 3°C over a salinity range of 15–35 ppt, while at temperatures above 3°C, the optimal salinity range decreased to 15–25 ppt.

Embryonic development has been described (14) and is typical of other flatfish species. Rate of embryonic development is temperature dependent (16,47). Rogers (16) reported that time from fertilization to 50% hatching can last from 17 to 31 days (mean of 25 days) at 3°C and from 5–10 days (mean of 7 days) at 14°C. Near the end of the embryonic period, larvae move within the egg capsules, and this movement assists with rupturing the capsule at hatching (13).

Solutions of penicillin G and streptomycin (0.02 mg/mL of each) have been used prophylactically to disinfect embryos (24,45,48,51). These antibiotic solutions were added to the culture water 24 hours after fertilization, or periodically, as water in the cultures was changed.

LARVICULTURE

Development and Size

Good descriptions of larval development, from hatching to the end of the second month, are available (13,49). For a thorough review of the older literature on early life-history stages, the reader is referred to Martin and Drewry (50). Newly hatched winter flounder larvae range in total length from about 3.5 to 3.8 mm (13,45,46). Acclimation temperature of the adults, incubation temperature of the embryos, and geographic origin of the broodstock can affect both length and weight at hatching, as well as the biochemical composition of the larvae (17,18). Size at hatching affects survival potential, with larger larvae having a higher probability of surviving through the first month of life (19,20).

Time to yolk sac absorption is dependent on temperature, and generally occurs at 7 days posthatching (dph) at 12°C (48), 9–10 dph at 5°C, 12–14 dph at 4°C, and 14 dph at 2°C (17,19).

Larval Rearing Systems

Winter flounder larvae have been reared in static, flow-through and *in situ* systems. In static systems, which are used in research, but not aquaculture, the larvae are reared in containers with no flowing water. To prevent the buildup of nitrogenous waste products and to ensure adequate amounts of dissolved oxygen, a portion of the water is replaced either daily, or every second or third day. Generally, one-third to one-half of the volume is changed on each occasion. Because larvae that have been raised in static systems have been used for experiments, container sizes are relatively small, ranging from 0.4 to 40 L. The containers are typically dark walled and made of plastic or glass. Water used is typically filtered (0.45 µm) and sterilized by ultraviolet light. Antibiotics (an equal mixture of penicillin G and streptomycin) have been added (25 ppm) to static larval culture systems (42–44). Light aeration is provided, and overhead lights are used for illumination, which facilitates the visual feeding of the larvae. The advantage of static systems is that temperature is easily controlled by placing the container in a constant temperature room or water bath. An additional advantage is that prey organisms are not washed out of the system. Static systems have been used extensively (15–25, 42–45).

Flow-through systems, in which larvae are reared in 100 L circular tanks, supplied with 5 µm filtered, ultraviolet light-treated seawater at ambient temperatures and salinities (5–10°C, 28–33 ppt) have also been used successfully (25). Flow rate to the tanks is 1–2 L (0.25–0.50 gallon) per minute. Loss of larvae is prevented by a high surface area, screened (80 µm) outflow that is located in the middle of the water column. The advantage of the flow-through system is its larger volume and improved water quality. Maintenance of desired prey levels is achieved by feeding the fish multiple times per day. Litvak's laboratory also uses a flow-through system, except they use an upwelling cylindroconical tank (48). Both experimental-sized (100 L) and production-sized cylindroconical tanks

(1,000 L) have been used to successfully produce metamorphosed fish.

An *in situ* system to monitor the growth and survival of winter flounder larvae has also been used (52). It was a large (11.5 m³) open-mesh enclosure, suspended from a surface floatation collar, deployed in a subestuary of Narragansett Bay, RI. Mesh size (505 µm) was small enough to prevent the escape of the larvae and large enough to allow their natural food to enter. The system was stocked with 1,000 four-week-old, laboratory-reared larvae, and these were held in the enclosure for two weeks in April and May. Results of the experiment with this system were very encouraging. Physical conditions (temperature and salinity) within the enclosure were optimal, and prey (copepods, rotifers, polychaete larvae, barnacle nauplii, and cladocerans) concentrations were high (10–8700 per liter). Of the fish placed in the enclosure, 76.8% survived and all metamorphosed. Daily specific growth rate was 10.7% dry weight and 1.9% standard length. The advantage of this system is the ability to raise large numbers of larvae at relatively low cost. Perceived disadvantages are the lack of control of the rearing conditions (e.g., temperature, prey availability) and the danger of losing the system in storm conditions.

Photoperiod and Light Intensity

Downing and Litvak (unpublished data) found that there was no effect of light intensity (5 vs. 100 lux) on larval winter flounder growth and survival. Continuous light, however, significantly improved both growth and survival of winter flounder larvae (48). Larvae raised under continuous light showed a five-fold increase (50 vs. 10%) in survival to metamorphosis, compared with those raised under ambient photoperiod (48). The continuous light treatment also reduced the time to metamorphosis by five days. Both our laboratories now use continuous light in rearing winter flounder larvae (25,48).

Larval Stocking Densities

Because static systems have been used for experimental studies, the number of larvae stocked per liter has varied with experimental design. Numbers range from 2 larvae/L (44) to nearly 300 larvae/L (23). More typically, 10–40 larvae/L are stocked (19,25,42,43). In flow-through systems, stocking density is as high as 100/L (Howell, unpublished).

Green Water

The addition of cultured microalgae to larval rearing tanks ("green water" treatment) has been widely accepted as a technique for commercial marine finfish production. Cultured microalgae has occasionally been added to winter flounder larval culture systems as a "water conditioner." Species of microalgae used have included *Nannochloropsis* sp., *Isochrysis galbana*, *Dunaliella tertiolecta*, and *Tetraselmis soucui* (20,21,23,25,42,43). Buckley et al. (20,21) inoculated larval tanks prior to rotifer introduction with dense cultures of *Tetraselmis* sp. at a rate of 1 L per 36 L of aquarium water. King and Howell (25), working in static systems, determined that when

3 L (200,000 cells per mL) of *I. galbana* were added to 20 L larval cultures every third day, the larvae in green water grew significantly faster than those in clear water. The importance of microalgae to winter flounder larvae has been further documented in a recently completed experiment in which microalgal species (*Nannochloropsis*, *Dunaliella*, or *Tetraselmis*) were provided at 3 L of dense (200,000 cells/mL) microalgae per 36 L tank per day, for varying lengths of time (one to four weeks) following yolk absorption (Bidwell, unpublished). Results of the study indicated that larvae cultured in green water for at least two weeks had significantly higher growth rates than those provided either no microalgae (control) or those given microalgae for only one week. Moreover, the larvae provided with microalgae for at least two weeks were more completely metamorphosed after four weeks than those which received either no microalgae or microalgae for only two weeks. We strongly recommend the use of microalgae during larval culture.

Larval Feeding

Hatchlings spend the first several days feeding endogenously as they absorb nutrients from their yolk sac. First feeding occurs within one day of absorption of the yolk sac (19,53). Total lengths at first feeding range from about 4 to 4.4 mm, depending on adult acclimation temperature and larval incubation temperature (19). Winter flounder larvae are continuous, visual, daylight feeders (18). As with virtually all small marine fish larvae, first feeding winter flounder larvae require small (<200 µm) live food items. This need has been met by feeding the larvae the cultured rotifer (*Brachionus* sp.), field collected zooplankton, or a mix of rotifers and wild zooplankton. Feeding rates of rotifers range from 2000 to 5000/L/day (24,25,51).

A 1:1 or 1:2 mixture (wild plankton:cultured rotifers) has been used by Buckley et al. (20,21) and Klein-MacPhee (42,43), respectively. Feeding rates of these mixtures ranged from 1000 to 3000 prey per liter. The exclusive use of small (48–200 µm) wild zooplankton (principally copepod nauplii) has also been used successfully (17,18,25). Initial feeding rates ranged from 2000 to 2100 prey per liter. Larger sized (up to 500 µm) wild plankton are used as the larvae grow (17,25,45). King and Howell (25) found no difference in growth or survival between larval winter flounder fed wild zooplankton and cultured rotifers that had been enriched with the microalgae *I. galbana*.

Rotifers or wild zooplankton are typically given for the first four to five weeks of feeding, but the larvae need larger food particles as they continue to grow. In most instances, this need has been met by feeding nauplii of brine shrimp (*Artemia salina*) to the late stage larvae. *Artemia* feeding is typically started at four to six weeks after hatching (22,23,41–44,51); however, this is dependent on larval size and *Artemia* can be fed to the larvae as early as 21 dph (48). Klein-MacPhee et al. (42,43) tested commercially available brine shrimp from different geographical locations and found that the geographic origin of the brine shrimp affected the growth and survival of late stage (42–71 dph) winter flounder. They suggested that the poor performance of some larvae may have resulted from the presence of various pesticides,

which were relatively high in some of the brine shrimp strains tested. The differences may have also have been due to differences in the nutritional value of the different strains, particularly in the amount of long chain polyunsaturated fatty acids (e.g., 20:5n-3 series) that were present. In a related study Shauer and Simpson (44) found that *Artemia* are able to bioconvert short-chain fatty acids (18:2n-6, 18:3n-3) to longer chain forms (20:5n-3, 22:6n-3). Further, winter flounder juveniles accumulated the long-chain fatty acids, and were able to convert 18:3n-3 gotten from the *Artemia* to 20:5n-3 and to lesser extent, 22:6n-3.

Because cultured rotifers and brine shrimp may lack the essential fatty acids required for optimal growth and survival, many fish culturists, including those of winter flounder, enrich these live prey organisms. This is usually done through the use of commercially available emulsions (e.g., SelcoTM products, Inve Aquaculture) or by feeding the rotifers and brine shrimp microalgal species that have the desired fatty acids.

The amount of food required by winter flounder larvae held at 8°C, from yolk absorption to metamorphosis, has been documented (18). It was found that (1) all larvae fed less than 100 prey/L died within two weeks; (2) the number of prey consumed increased curvilinearly with prey density, particularly in fish that were five to seven weeks old; (3) daily specific growth in dry weight was similar at prey concentrations ranging from 500 to 3000/L. Mean daily specific growth in dry weight was 5.72% at 500 prey/L, 7.68% at 1000 prey/L, and 8.62% at 3000 prey/L; (4) mortality rates decreased as prey density increased, and (5) complete digestion of gut contents occurred within 5–8 hours. In the same paper, Laurence developed a bioenergetic model that simulated the effects of a number of variables, including temperature, prey density, and larval size on the ability of larvae to obtain the food energy needed to meet the needs of experimentally determined growth and metabolism. Results of the model simulations indicate that (1) the amount of time feeding must change as the larvae develop, and this in turn is related to prey density. Depending on concentration, larvae need to feed from about 3 to 18 hours per day; (2) wild larvae need a minimum of 300–800 prey/L to meet their feeding needs within the 12 hour of light that is normally available to them; and (3) the theoretical number of nauplii or older stage copepods needed to be eaten per day increased with the dry weight of larvae (from about 25 to 200 nauplii, or from 2 to 16 older-stage copepods) over the size from first feeding to metamorphosis.

Larval Growth Rates

The growth of winter flounder, from hatching to metamorphosis has been extensively studied, and can be influenced by temperature (17–19), prey concentration (18), culture conditions (25), and nutritional quality of prey (42,43). Laurence (17) studied the growth of winter flounder larvae from yolk absorption through metamorphosis at 2, 5, and 8°C. Larvae were fed wild zooplankton (principally copepod nauplii) at a rate of 2000/L/day. Mean daily specific growth (dry weight) was 10.1, 5.8, and 2.6% at 8, 5, and 2°C, respectively. He also provided regression

equations that related larval dry weight to weeks after yolk absorption. Growth in mass was found to be exponential. Growth rates using different units of measurement are also available. Chambers and Leggett (45) reported that the average daily growth rate, from hatching to metamorphosis was 0.068 mm/day at 6.9°C. King and Howell (25) reported specific growth rates (length increases per week) of larvae reared using different combinations of green and clear water and wild zooplankton and rotifers. Rates were 15.4% (green water/wild zooplankton), 14.2% (green water/rotifers), 12.2% (clear water/rotifers), and 9.6% (clear water/wild zooplankton). Bertram et al. (24) found that there was some variation in growth rates between individual larvae and that growth in length was nonlinear. Increase in length was rapid up to 30 days posthatch, then slowed, or even decreased at metamorphosis. In a related finding, Laurence (17) found that oxygen consumption increased as the larvae grew, decreased at metamorphosis, and then increased again. Jerald et al. (23) described the development of daily growth increments of otoliths and provided a growth equation (length) for laboratory-reared fish up to 50 days posthatch. The best fit of the growth data was achieved using a Gompertz-type curve. The length-weight relationship for laboratory-reared larval winter flounder has been reported (54).

Age and Size at Metamorphosis

Mean age at metamorphosis, which is functionally defined as the migration of the left eye to the right side of the head and loss of pigmentation on the blind side (45), has been reported by a number of investigators, and ranges from 49 to 64 days posthatch at incubation temperatures ranging from about 7–10°C (17,18,23,24,45,46,51). At a higher mean incubation temperature of 15°C, metamorphosis occurred from 26 to 33 days posthatch (55), while at a lower incubation temperature of 5°C, metamorphosis was delayed until 80 days posthatch (17). Mean length at metamorphosis has also been reported by a number of investigators, and ranges from 6.1 to 10.1 mm total length (23,24,45,46,51). Laurence (17) found that all larvae maintained at 2°C died before reaching metamorphosis. Length at metamorphosis is less variable than age at metamorphosis and that larvae that metamorphose at a later age do so at a larger size (45,46).

GROWOUT

Weaning of Juveniles

Lee and Litvak (55) used wild young-of-the-year winter flounder juveniles to develop a weaning protocol. They were able to wean wild juvenile flounder onto dry feed (BP Nutrition™, Aquaculture Research Centre, Stavanger, Norway) by cofeeding live *Artemia* over one week. In a study using recently metamorphosed laboratory-reared winter flounder juveniles, they further examined the weaning protocol and also tested two different diets: nonsalmonid and salmonid starter feed (56). The study found that the locally produced inexpensive salmonid pellet (Hi-Pro™, Corey Feed Mills, Fredericton, NB, Canada) performed as well as the specialty marine

dry pellet (Nippai SFI-3, CATVIS, Hertogenbosch, The Netherlands). However, they did see a slight decrease in growth rates immediately after the switch to dry diets, suggesting that there is room for improvement in their weaning protocol during this critical stage.

Juvenile Rations and Diets

Little information is available on how much food juvenile winter flounder need, and for this reason, fish in captivity are normally fed *ad libitum*. Juveniles fed a diet of chopped bivalve (*Mya arenaria*) siphons *ad libitum*, and held at 20°C (4°C above their normal seasonal limit) consumed between 193 and 973 mg food/day but lost weight because of the temperature stress (57). Fish at cooler temperatures (12° and 16°C) consumed more food per day (1,118–2,088 mg/day) and gained weight. Maintenance ration was calculated to be about 1.5% wet-body weight per day at 12–16°C, and gross caloric conversion efficiencies ranges from 13.9 to 36.8% (57).

Little work has been completed on juvenile diets. The only published work that we are aware of is that of Hoornbeek et al. (8), who found that wild-caught juveniles would feed on a bound mixture of frozen shrimp, herring meal and oil, and a vitamin premix. That diet was 38% protein. Research is currently being conducted at the Department of Fisheries and Oceans (Cheryl Hebb and John Castell, DFO Saint Andrews Biological Station, NB, Canada) on diet formulation for on-growing juvenile winter flounder. Winter flounder do not seem to have the capability to spare protein and require at least 50% protein in their diets. However, there have been promising results suggesting that winter flounder protein requirements may be partially satisfied with either a portion of soy meal or canola protein (John Castell, personal communication). Clearly, the capability to digest and utilize plant protein would be a boost to winter flounder's potential for aquaculture.

Juvenile Growth Rates

Winter flounder juvenile growth rate compares well with other commercially grown flatfish (56). Daily specific growth rates of recently metamorphosed fish have reached 3.11% weight gain per day (Table 1). This high growth rate was maintained through their first year of growth (Casey and Litvak, unpublished data) in which the specific growth rate exceeded 2.8% per day. From this research, it is clear that the culture of winter flounder juveniles will have to be conducted in warm or heated water to be profitable. In a further study of photoperiod manipulation, Casey and Litvak (unpublished data) found that winter flounder juveniles, like larvae, grow fastest under continuous light.

Producing Adults

To our knowledge, winter flounder have yet to be grown from egg to market size in captivity. Growth to market size, which we assume would be about 30 cm total length, takes between two and four years in the wild, depending on latitude (30). Presumably this time could be shortened in an aquaculture setting, where fish would be provided with optimal diets and warmer year-round temperatures (8).

Table 1. Daily Specific Growth Rates for Juvenile Winter Flounder (*Pseudopleuronectes americanus*) Reared at Different Temperatures

Reference	Age (years)	Source ^a	SGR ^b (%lg/d)	SGR (%wt/d)	Temperature (°C)
Frame (57)	1	W	—	0.27	12
	1	W	—	0.47	16
	1	W	—	-0.23	20
Hoornebeek et al. (8)	0-1	H	0.09	0.21	1-15
		W	0.24	0.71	10-15
Lee and Litvak (55)	0	W	0.12	0.53	5.3
Lee and Litvak (56)	0	H	1.36	3.11	15.0
Casey and Litvak (unpublished data)	1	H	0.67	2.30	10.0
	1	H	0.82	2.81	15.0
	1	H	0.79	2.71	20.0

^aH = hatchery; W = wild-caught tested in the laboratory.

^bSpecific growth rate = $(\ln(\text{final weight}) - \ln(\text{initial weight})) / \text{time} \times 100\%$.

Adult winter flounders held in captivity and provided with *ad libitum* amounts of clams and cubed beef liver daily consumed 2% of their body weight per day. Gross caloric conversion efficiency ranged from 0.10 to 0.22 (27).

Growout systems, although not developed, will probably be similar to those used for other flatfish species, including land-based tanks or raceways and net pens. There is no reason to suspect that winter flounder could not be raised in recirculating systems. Litvak (unpublished observation) has conducted preliminary experiments on cage grow out of winter flounder. A plastic coated, wire mesh cage, with a flat bottom, was suspended from a 10 m octagonal collar. Winter flounder were placed in the cage in the fall and their weights taken in December. The greatest weight gain was 40% in this period. Fish grown in the cage were 1.8 times heavier than were wild-caught fish of similar size, suggesting that the yield (fillet weight) could be higher from cultured fish than from wild-caught winter flounder.

DISEASE AND PARASITES

Diseases and parasites of wild winter flounder have been reviewed by Klein-MacPhee (30). Because winter flounder spawn and hatch during a cold part of the year, disease problems do not appear to be as great as in some warmwater species. Prophylactic antibiotics are often administered to egg and larval cultures as a precautionary measure (42,43). Fin erosion of captive juveniles has been observed (56), and smaller individuals appeared to be more susceptible to this disease. Most mortalities of wild caught juveniles held in the laboratory for extended periods of time resulted from systemic bacterial infections and parasites (8). *Vibrio anguillarum* was isolated during the periods of heaviest mortality. Symptoms included the cessation of feeding, fin rot, and hemorrhagic areas on the ventral surface. The most effective treatment was mixing furazolidone into the feed, provided at a rate of 12 g/100 g of fish per day, for 10 days. Two myxosporidian parasites were also identified from these fish. In Howell's laboratory, cultured juveniles have occasionally developed an unidentified fungal infection. This has been effectively

treated by placing the fish in a dilute (250 ppm) hydrogen peroxide solution for 20 minutes. Treatment is repeated three times over 10 days.

ECONOMIC VALUE AND PRODUCT POTENTIAL

Winter flounder market value varies seasonally, depending on quantities being harvested and on the size, catch location, and name given by the broker. Current price (May 1998) for whole winter flounder in New York City is between US\$5.50 and US\$6.00 per pound, which is more than \$2.00 higher than either yellowtail flounder and cod. Winter flounder is also sold fresh dressed and filleted. Considering that this fish is eurythermal, euryhaline, and extremely hardy, it is an excellent candidate for the live fish markets of Asia and the developing live fish markets in North America. Another favorable attribute of the species is that it is very cold tolerant. Because they produce a set of antifreeze polypeptides (58), they survive even in ice-laden seawater at temperatures below -1°C. For this reason, they are one of the few candidates for flow-through, or net pen aquaculture at high latitudes, particularly in Canadian waters. Attempts have also been made to transfer the antifreeze producing genes from the winter flounder to the genome of the Atlantic salmon, thereby making the salmon more cold tolerant (59).

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See also BRINE SHRIMP CULTURE; FLOUNDER CULTURE, JAPANESE;
 HALIBUT CULTURE; LARVAL FEEDING—FISH; PLAICE CULTURE;
 SOLE CULTURE; SUMMER FLOUNDER CULTURE.

Winter Flounder Culture Chapters: Fairchild 2010

Chapter 6

Culture of winter flounder

Elizabeth A. Fairchild

The winter flounder, *Pseudopleuronectes americanus*, is a commercially and recreationally important right-eyed flatfish found along the northwestern Atlantic coast, ranging from Georgia, United States, to Labrador, Canada (Scott and Scott 1988; Figure 6.1). It is a long-living flatfish and can reach up to a maximum age and length of 15 years and 65 cm, respectively (Bigelow and Schroeder 1953; Fields 1988). Maximum weight can be as much as 3 kg, resulting in the thickest fillets of all native New England flatfish (Bigelow and Schroeder 1953; Lux 1973).

Winter flounder are federally managed in the United States as three separate stocks, and in Canada as three separate divisions. As with most groundfish species, catches have declined precipitously in recent years in both the countries. The goal of the current management program is to reduce fishing mortality to levels that will allow stocks to rebuild above minimum biomass thresholds, and then remain at or near target biomass levels. While it is hoped that these more stringent fisheries regulations will allow winter flounder populations to rebuild to historic levels, recovery may take decades. Culturing winter flounder for market and rearing juvenile fish for stock enhancement are viable options for reducing fishing pressure and rebuilding this species (Waters 1996; Litvak 1999; Howell and Litvak 2000), and both have been the focus of research for well over 100 years. As early as 1890, wild broodstock were captured in fyke nets near Woods Hole, MA, and spawned in government hatcheries (Bean 1890). Many of the basic techniques for culturing winter flounder have been developed since then, though on a small, experimental scale. The reader is referred to Litvak (1999) and Howell and Litvak (2000) for a more thorough review of winter flounder aquaculture techniques developed before 1999.

6.1 Life history and biology

The biology and ecology of winter flounder have been well studied (see reviews by Klein-MacPhee 1978; Able and Fahay 1998; Pereira 1999; Collette and

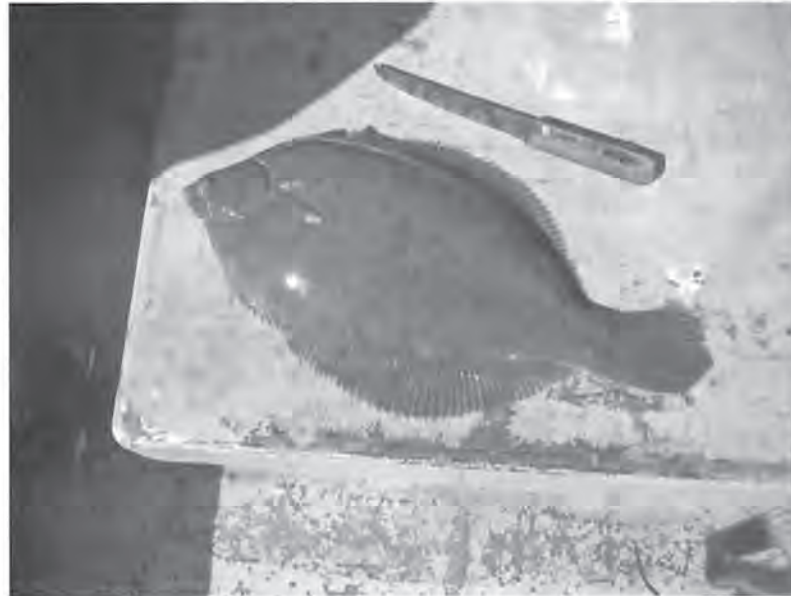


Figure 6.1 Adult winter flounder *Pseudopleuronectes americanus*.

Klein-MacPhee 2002). Habitat characteristics of winter flounder vary among life stages and affect their distributions. Winter flounder reside in depths from the shallow subtidal to 37 m (McCracken 1963). Winter flounder are eurythermal, euryhaline, and freeze resistant (Pearcy 1962; Duman and DeVries 1974). Growth rates are highly correlated to temperature. In the wild, fish reach maturity at 26–30 cm, depending on location (O'Brien *et al.* 1993); maturity appears to be a function of size rather than age (O'Brien *et al.* 1993). The peak spawning season for winter flounder varies by latitude, but generally lasts 2–3 months in the winter and spring (see DeCelles and Cadrin 2007). Southern populations may initiate spawning as early as December (Lobell 1939), while peak spawning in Newfoundland does not occur until May (Kennedy and Steele 1971). Except for the Georges Bank (GB) stock which spawns offshore, reproductively isolated adult populations typically undergo onshore migrations into specific estuaries or coastal embayments where spawning occurs (Lobell 1939; Perlmuter 1947; Saila 1961) and the young-of-the-year (YOY) remain for their first two years before moving offshore (Pereira *et al.* 1999).

6.2 Broodstock husbandry

Wild adult fish are readily available, and so, most researchers capture, spawn, and release their broodstock each year. Broodstock are captured easily by otter trawl (Smigielski 1975; Stoner *et al.* 1999; Butts and Litvak 2007a, 2007b; Fairchild *et al.* 2007), fyke nets, fish traps (Ben Khemis *et al.* 2000; Plante *et al.* 2002; Plante *et al.* 2003; Mercier *et al.* 2004), and SCUBA (Burton and Idler 1987; Shangguan and Crim 1999). Ripe females are easily recognizable by their

distended ovaries, which encompass the entire ventral side of the fish during spawning season. Spermiating males can be identified by the extrusion of milt when gentle pressure is applied to the abdomen.

Because winter flounder are divided into distinct localized populations associated with the specific bays and estuaries along the northwestern Atlantic (Klein-MacPhee 1978), broodstock origin affects zygote characteristics and development. Fish from anthropogenically contaminated areas produced eggs with a high incidence of abnormalities (Perry *et al.* 1991). In addition, the stock origin of parental fish is important since there are latitudinal differences in growth rates (Buckley *et al.* 1991a; Butts and Litvak 2007a, 2007b). For instance, larvae sired by Georges Bank males developed faster and were significantly larger during both early and late larval development than those sired by Passamaquoddy Bay, NB, males (Butts and Litvak 2007a, 2007b). It is paramount that broodstock are healthy as egg quality and survival to metamorphosis are correlated to both paternal (Butts and Litvak 2007a) and maternal health (Buckley *et al.* 1991b). Both maternal size (Chambers and Leggett 1996) and paternal sperm volume (Butts and Litvak 2007b) are positively correlated to egg size and fertilization success, respectively.

Though cultured flatfish are not immune to diseases caused and/or exacerbated by bacteria, viruses, nutrition, and environmental factors (Mulcahy 2002), few immunological studies have been conducted with winter flounder. One detected disease is nodavirus (Nervous Necrosis Virus) which was found (albeit infrequently) in wild winter flounder from Passamaquoddy Bay, NB, Canada (Barker *et al.* 2002b). Nodavirus could pose a potential threat to the successful development of a commercial winter flounder aquaculture industry since an outbreak would be pandemic and cause high mortality, especially in cultured larvae and juveniles (Gagne *et al.* 2004). Care must be taken to prevent bringing contaminated broodstock into the hatchery. In order to develop appropriate biosecurity measures, a nonlethal method for screening broodstock for nodavirus should be developed (Gagne *et al.* 2004).

Wild winter flounder are known to carry ectoparasites such as *Gyrodactylus* spp. and *Trichodina* spp. which can easily spread to other fish in a crowded, hatchery environment; these ciliates can be removed by treating fish in a formalin bath (1:4,000) for 1 hour, once/week for 3 weeks (Barker *et al.* 2002a). To prevent infections, topical antibiotics like 1% methylene blue solution (Plante *et al.* 2002, 2003) or 10% iodine solution (Fairchild, unpublished data) are applied externally to small wounds that probably occurred during capture of broodstock.

To date, no one has domesticated winter flounder broodstock. This is imperative for the success of a commercial-scale operation. Through domestication, fish can be selected for fast growth and disease resistance, among other desirable characteristics.

6.2.1 Broodstock system design and requirements

Determining optimal broodstock requirements for winter flounder is the focus of several research laboratories. Because manipulating photoperiod and

temperature do not appear to strongly affect gametogenesis in adult winter flounder (Duchemin *et al.* 2004), typically adults are kept at ambient temperatures and provided with a natural photoperiod (Plante *et al.* 2002, 2003). This is the case with the broodstock at the Institut des Sciences de la Mer de Rimouski (ISMER) in Québec, where fish are kept in tanks at a density of 5–6 kg/m² (or the equivalent of 20 fish/m²) in 1 m² rectangular tanks, supplied with 29 ppt saline water at a flow of 5–10 L/min (Plante *et al.* 2002, 2003). At the University of New Brunswick Saint John (UNBSJ), broodstock are kept in a 2,700-L closed recirculation system in which temperature and salinity are maintained at 2–4°C and 28–30 ppt, respectively (Butts and Litvak 2007a, 2007b). Winter flounder can withstand such cold water temperatures since they synthesize antifreeze proteins and secrete them into their blood during the winter (Duman and DeVries 1974; Fletcher *et al.* 1985). At UNBSJ, approximately 15% of the water is replaced daily, and photoperiod mimics natural conditions during the spawning season at a light intensity of 10 lx (Butts and Litvak 2007a, 2007b).

Plante *et al.* (2002) examined the effects of two salinities on the survival, growth, and stress of wild winter flounder broodstock. Fish were acclimated to either a brackish salinity (15 ppt) or seawater salinity (29 ppt), and reared for 5 months. There were no differences in growth, somatic condition, hepatosomatic index, and gonadosomatic index of fish reared in different salinities. However, fish in the higher salinity treatment had higher mortality and stress indicators (plasma cortisol and plasma osmolality) than fish in the brackish salinity treatment, rendering these fish more vulnerable to opportunistic infections. Adults were captured inshore. This is interesting to note since most winter flounder stocks spawn inshore in estuaries and coastal embayment where waters may be less saline (Saila 1961; McCracken 1963). Presumably, GB broodstock, which spawn offshore (Pereira 1999), would fare better in more saline waters.

In a study to determine whether captive winter flounder broodstock are chronically stressed, Plante *et al.* (2003) compared the condition and stress responses of newly captured wild broodstock with a captive, wild broodstock kept in the hatchery for 13 months. To simulate an acute stressor, fish were dipnetted, held out of water for 3 minutes, marked, and returned to the tank. Sampling to measure hemoglobin, hematocrit, and cortisol occurred 1 hour after the acute stress event. Winter flounder held in captivity for over 1 year showed higher condition indices and similar energy reserves compared to wild fish, suggesting that chronic stress was not present in the captive broodstock. In addition, they had the same cortisol stress response as the wild fish and, it did not decrease over time, indicating that their endocrine systems were not exhausted (Seyle 1973). Though the captive broodstock fed well and was not chronically stressed, Plante *et al.* (2003) still observed an unexplainable 30% mortality loss.

6.2.2 Broodstock diet and nutrition

The maintenance ration for adult winter flounder weighing 584–801 g is 7.9 kcal/g/day (Tyler and Dunn 1976). Adults are omnivorous and will feed on a variety of items including chopped clams, squid, menhaden, and silversides

(Smigielski 1975; Stoner *et al.* 1999). At ISMER, broodstock are fed daily 1.5% of their biomass with pellets consisting of 60% commercial feed (6.0 mm Corey Feed Mill, Ltd.), 25% frozen capelin or Atlantic herring, and 15% frozen amphipods (Plante *et al.* 2002). This particular formulated pellet has 38% moisture content and 14.6 kJ/g energy content (Plante *et al.* 2002, 2003).

Mature winter flounder cease feeding during the reproductive season (Smigielski 1975). Feeding resumes for female fish once spent and for males when the spawning season is over (Stoner *et al.* 1999), though some males will continue to feed throughout this period (Smigielski 1975). However, just before and following the spawning season are nutritionally critical sensitive periods to broodstock (Burton 1994). Generally, if females are deprived of food either before or immediately after spawning, they will not be gametogenic in the subsequent spawning season (Tyler and Dunn 1976; Burton and Idler 1987; Burton 1994). This can be reversed by feeding to satiation during the months following the subsequent season when females would have spawned (Burton 1991). Alternatively, if the nutritional condition of the females is high (>1.20 condition factor), they have a higher probability of withstanding starvation and being able to spawn the following year (Burton 1994).

6.2.3 Controlled spawning

Winter flounder can be induced to spawn using several hormones; the most reliable ones are freeze-dried carp pituitary extract (CPE) (Smigielski 1975) and gonadotropic-releasing hormone analog (GnRH-A; Harmin and Crim 1992). CPE mixed at doses of 0.5 or 5 mg/454 g female body weight (BW) in a solution of sodium chloride and injected intramuscularly daily (3 or 6 days depending on dosage) results in spawning (Smigielski 1975). GnRH-A either administered in saline injections or through a sustained-release cholesterol pellet, stimulates the reproductive system of both male and female fish throughout the year except for in postspawned, sexually regressed fish (Harmin *et al.* 1995a).

Harmin and Crim (1992) successfully induced spawning in female winter flounder using either 100–120 μ g GnRH-A/slow-release pellet, 40 μ g GnRH-A/quick-release pellet, or 20 μ g/kg BW GnRH-A in saline injected 3/week. Though all three techniques were effective, the repeated handling of fish for the saline injections caused more stress, and resulted in higher mortality than the implanted pellets. In mature female fish, GnRH-A accelerated ovarian growth by increasing plasma estradiol-17 β and testosterone levels (Harmin *et al.* 1995a). In prespawning female fish, the hormone stimulated ovulation through germinal vesicle migration by increasing plasma testosterone levels (Harmin *et al.* 1995a). This hormone is most effective and reliable when used as the fish approach the time of the natural spawning season (Harmin and Crim 1992). In addition, in this scenario, both egg and larval quality are higher (Harmin and Crim 1992).

Male winter flounder begin spermiating as much as 5 months before females ovulate (Shangguan and Crim 1999); however, milt production remains low until the females begin spawning. When administered in the fall, GnRH-A stimulates the growth of the testes by increasing plasma androgen levels in male winter

flounder (Harmin *et al.* 1995a). Injecting males with either 20 or 200 µg/kg BW GnRH-A caused plasma levels of testosterone and 11-ketotestosterone to increase within 12 hours and remain elevated for several days (Harmin and Crim 1993). The hormone can also cause males to spermiate several months early (Harmin *et al.* 1995a) yet only small amounts (<50 µL) of milt could be collected (Harmin and Crim 1993). Rather than increasing sperm production during the spawning season, GnRH-A treated males (110 µg/kg BW GnRH-A) had a greater volume of milt but more diluted and earlier termination of the spawning season than in non-treated males (Shangguan and Crim 1999). Hormonal treatments of GnRH-A probably do not increase total spermatozoa production since spermatogenesis occurs well before the spawning season (Harmin *et al.* 1995b). Sperm quality, based on sperm motility and egg fertility, is unaffected throughout the spawning season by use of GnRH-A (Shangguan and Crim 1999).

Initial research on the cryopreservation of sperm has been tested and shown to be successful in fertilizing winter flounder eggs (Rideout *et al.* 2003). They tested three cryoprotectants (dimethyl sulphoxide, propylene glycol, and glycerol) and two diluents (sucrose based and saline based) mixed (9 parts diluent:1 part cryoprotectant) in a factorial experiment. Sperm were diluted 1:3 with each of the six extenders, and frozen and stored on liquid nitrogen. All the diluents and cryoprotectants were successful in cryopreserving winter flounder sperm, and no toxic effects were apparent on sperm. Sperm preserved with propylene glycol (regardless of diluent type) had the highest motility recovery, followed by dimethyl sulphoxide in sucrose diluent, dimethyl sulphoxide in saline diluent, and glycerol (regardless of diluent). The postthaw motility of winter flounder sperm was not affected by adding ovarian fluid to the seawater used to activate sperm swimming behavior. In addition, cryopreservation of the sperm did not adversely affect the hatching success or larval development of winter flounder. Though this study indicates that cryopreservation of winter flounder sperm is a viable method for fertilizing eggs, these techniques need to be developed further.

Despite reports that female winter flounder rarely spawn spontaneously in the laboratory (Harmin and Crim 1992), at the University of New Hampshire (UNH), fish are reared from a volitionally spawning wild broodstock (King and Howell 1997; Fairchild *et al.* 2007). Mature fish are collected during the natural spawning season and transferred to 0.9 m diameter tanks provided with flow-through, ambient, ultraviolet filtered (0.5 µm) seawater, and covered with dark screens to filter any visual disturbances in the laboratory. Typically, one female and two or more males are stocked into each tank. Usually, within 1 week, the fish will spawn during the night (Howell and Litvak 2000). When females do not volitionally spawn, luteinizing hormone-releasing hormone (LHRH), injected at a dosage of 10µg/kg, is effective (Butts and Litvak 2007a, 2007b).

There are only two published accounts of winter flounder spawning behavior in captivity. Winter flounder are nocturnal spawners; spawning events occur from sunset to dawn with the majority happening near 21:00 hours (Breder 1922; Stoner *et al.* 1999). A spawning event appears to be initiated by a male pursuing a female (Stoner *et al.* 1999). Instead of fleeing, the female stays close to the sediment, then the pair rapidly swim in an upward tight spiral as eggs and

milt are released, then return to the bottom (Breder 1922; Stoner *et al.* 1999). It is not uncommon for several males to participate in spawning (Breder 1922). Stoner *et al.* (1999) noticed that in 151 spawning events, only 22.5% were paired spawnings; the remainder involved two to six males.

Female winter flounder are batch spawners; only one group of oocytes matures each year (Burton and Idler 1984). By filming spawning events in a large (121 KL) aquarium, Stoner *et al.* (1999) observed that individual females spawned multiple times over a period of a week, and they estimated that the average female spawned 40 times during the reproductive season. However, at UNH, females either release all their eggs within one night, or release a small batch followed by all the remaining eggs during the subsequent night (Howell and Litvak 2000).

6.2.4 Collection of eggs and egg incubation systems

Female winter flounder fecundity ranges from 0.4 to 3.3 million eggs (Topp 1968). Egg diameter ranges from 0.74 to 0.85 mm, with a mean of 0.80 mm (Smigielski and Arnold 1972). Once spawned, the demersal, adhesive eggs harden into masses, which can range from 33–71 g (Ben Khemis *et al.* 2000, 2003). It used to be thought that low survival of winter flounder embryos was due to the eggs clumping together (Smigielski and Arnold 1972), so diatomaceous earth was, and still is, commonly used to prevent adhesion. For example, at ISMER, females are strip spawned into plastic containers, and the eggs are fertilized with sperm from several males (Ben Khemis *et al.* 2000, 2003). A suspension of diatomaceous earth in seawater (50 g/L) is added, and the eggs and sperm are mixed by gently swirling the container. After 5 minutes, the eggs are rinsed, 100–150 mL more diatomaceous earth suspension is added, and the eggs are rocked until egg membrane hardening is completed (approx. 30 minutes after fertilization). Five hours later, the eggs are rinsed in a 500 µm sieve, treated with iodine, and incubated.

Inhibiting egg clumping is not necessary for successful fertilization and larval survival. Fairchild *et al.* (2007) simply remove spawned out adult fish and tank covers from their spawning tanks, and allow the eggs to incubate naturally in the existing tanks. Despite eggs clumping together, high fertilization and hatching rates are standard (Howell and Litvak 2000). However, because other particles may adhere to the eggs too, daily inspection of the egg masses is necessary to ensure fungus is absent. If fungal growth appears, eggs can be dipped in an iodine bath (25 ppm solution) to disinfect. Butts and Litvak (2007a, 2007b) strip spawn females, collect the eggs, and add cryopreserved sperm. The gametes are swirled together and then sterilized seawater (8 °C, 28 ppt) containing antibiotics (13 mg/L each of penicillin G and streptomycin sulphate) is added. This gamete solution rests for 20 seconds, then sperm are rinsed out, sterilized seawater is added, and the eggs are incubated.

In recirculating and flow through systems, incubating eggs are subjected to a 0L:24D photoperiod, supplied with UV-sterilized, 0.5 µm filtered sea water at a rate of 1–4 L/min, and provided gentle upwelling aeration (Ben Khemis

et al. 2000, 2003; Mercier *et al.* 2004; Fairchild *et al.* 2007). Eggs have been incubated successfully in flat-bottomed, fiberglass, flow-through tanks (Fairchild *et al.* 2007), baskets within a water table maintained at 7°C and 28 ppt (Mercier *et al.* 2004), and in 60 liters, black, cylindroconical, fiberglass tanks at 7°C and 27 ppt (Ben Khemis *et al.* 2000, 2003). In all these systems, hatching occurred 9–10 days after fertilization. Winter flounder eggs also can be incubated successfully in static systems in temperature controlled rooms using the same water quality and photothermal conditions (Chambers and Leggett 1987; Chambers *et al.* 1988; Butts and Litvak 2007a, 2007b). In these, 25–75% of the water is replaced every 1–3 days to maintain high water quality. Stocking density ranges from <1000 to >30,000 eggs/L (Howell and Litvak 2000).

6.3 Larval culture

6.3.1 Larval system design and requirements

Upon hatching, larvae are transferred into rearing systems. At ISMER, photoperiod remains 0L:24D until mouth opening, and then is switched to a different lighting regime to facilitate visual feeding of the larvae (Ben Khemis *et al.* 2000, 2003; de Montgolfier *et al.* 2005). At UNH, a 24L:0D photoperiod is used once hatching begins to bring the photophilic larvae to the surface for easier collection (Fairchild *et al.* 2007). At UNBSJ, photoperiod also is switched to 24L:0D as larval growth rates and survival to metamorphosis under continuous light are significantly higher than those reared under ambient lighting conditions (Litvak 1999). In addition, time to metamorphosis is shortened by 5 days (Litvak 1999). Light intensity, however, does not affect larval growth or survival (Litvak 1999). A variety of different rearing systems are used by researchers (Table 6.1); all contain UV-sterilized, 0.5–10 µm filtered sea water and gentle aeration, usually in the center of the tank to create an upwelling effect, thereby keeping food suspended in the water column and oxygen levels high. In nonstatic systems, water flow is very light (0.1–0.2 L/min; Ben Khemis *et al.* 2000, 2003) since newly hatched winter flounder larvae are not strong swimmers (Sullivan 1915). In static systems, 50% of the water is changed every 1–3 days depending on the size and density of the larval tanks (King and Howell 1997; Butts and Litvak 2007a, 2007b).

6.3.2 Larval food and feeding

Greenwater

The addition of microalgae to larval rearing systems (termed “greenwater”) is used to enhance water quality, provide both food for larvae and rotifers, and prevent “larval wall syndrome” (Mercier *et al.* 2004). King and Howell (1997) showed that first feeding winter flounder larvae grew faster (SGR = 14.2% vs. 12.2%) and significantly longer (8.7 vs. 7.0 mm TL) if greenwater was added to

Tank specifications				Lighting regime			Source		
Density (larvae/L)	Size (L)	Color	Shape	Material	Temp (°C)	Salinity (ppt)		Photoperiod	Intensity
10	20	gray	cylindroconical	polyethylene	8–15	30–32	24L:0D	35 watt	King and Howell 1997
10	40	black		glass	7		14L:8D	40 watt	Chambers and Leggett 1987
11	35	clear	circular	glass	7		14L:8D	40 watt	Chambers <i>et al.</i> 1988
13	38	black			8	28–30	12L:12D		Laurence 1975, 1977
30	24	black	rectangular	plastic	6–9.5	30–31	12L:12D		Klein-MacPhee 1982
50		clear	circular	glass	8	28	24L:0D	50 lx	Butts and Litvak 2007b
60	120	dark green	cylindroconical	fiberglass			12L:12D	400 lx	Ben Khemis <i>et al.</i> 2003
77		clear	circular	glass	8	28	24L:0D	1100 lx	Butts and Litvak 2007a
100	2000	black sides, green bottom	circular	fiberglass	7–15	14–31	24L:0D	30 µE/s/m ²	Howell and Litvak 2000; Fairchild <i>et al.</i> 2007
250	120	dark green	cylindroconical	fiberglass			12L:12D	400 lx	Ben Khemis <i>et al.</i> 2003
250	55	black sides, white bottom	cylindroconical	polyethylene	10	28	12L:12D	7.1 µM/s/m ²	Mercier <i>et al.</i> 2004

culture water for the first 5 weeks at a concentration of 1 liter *Isochrysis galbana* (200,000 cells/mL)/6 liters culture water. In addition, larvae in the greenwater treatments initiated first feeding before larvae in the clearwater treatments.

There is no standard protocol on greenwater methodology. In the past, fish culturists always used live algae, typically grown on-site. For example, at UNH a variety of species including *Nannochloropsis* sp., *Isochrysis* sp., *Dunaliella tertiolecta*, and *Tetraselmis suecica* were cultured and added (1 liter algae/100 liters culture water) twice daily (Fairchild *et al.* 2007). With the advancement in algae processing, fish culturists now have a variety of algal products to use. At ISMER, *Chlorella* sp. is added at 1 liter algae/50 liters culture water each morning along with 100 mL of an algal paste solution (1 g diluted in 450 mL seawater; Mercier *et al.* 2004). Ben Khemis *et al.* (2000) also uses *Chlorella* sp. but at a concentration of 1 liter algae/120 liters culture water/day administered in the morning. Other culturists (Ben Khemis *et al.* 2003; de Montgolfier *et al.* 2005) add only Starter Formula Algae Paste (Innovative Aquaculture Products Ltd., Skerry Bay, NB) every morning until weaning. Butts and Litvak (2007a) use Instant Algae[®] (*Pavlova* sp.; 40,000 cells/mL of culture water; Reed Mariculture Inc., United States). In a preliminary study, Fairchild (unpublished data) found that winter flounder larvae cultured in Instant Algae[®] (*Nannochloropsis* sp.) exhibited good if not better growth rates than the fish in live algae or control (no algae) treatments, plus had superior survival. One drawback was that only fish cultured in Instant Algae[®] had abnormal pigmentation. However, the proportion of abnormally pigmented fish was relatively low compared to the higher survival of fish in this treatment.

Live feed

Newly hatched winter flounder larvae have an endogenous yolk sac, which is absorbed, depending on the culture temperature, 4–6 days post hatch (dph; Ben Khemis *et al.* 2000; Mercier *et al.* 2004; Fairchild, unpublished data). Due to their small gape size and undifferentiated stomach, winter flounder larvae require live prey (first *Brachionus* spp., then *Artemia* spp.) once yolk sac absorption has occurred (Ben Khemis *et al.* 2000). To retain prey in the larval tanks, water flow can be ceased during the feeding (= daylight) period, and resumed at night for complete water replacement (Ben Khemis *et al.* 2000, 2003). Typically, rotifers are provided daily at a concentration of 2–5/mL (Bertram *et al.* 1993, 1997; King and Howell 1997; Litvak 1999; Ben Khemis *et al.* 2000, 2003; Mercier *et al.* 2004; Fairchild *et al.* 2007). Protocols differ between facilities. Some disperse the daily ration of rotifers in a single morning feeding (water flow is shut off for a period of time so prey are not lost from the system), while others disperse it in multiple feedings per day. Because rotifers lack some of the essential fatty acids required for optimal growth and survival for winter flounder, they typically are enriched with a combination of the following: Starter Formula Algae Paste, live algae (e.g., *Pavlova lutheri* or *Isochrysis* sp.), Culture Selco (INVE Aquaculture, Salt Lake City, UT, USA), DHA Selco (INVE Aquaculture), and Microfeast[®] MB-30. To receive maximum nutritional benefits, rotifer enrichment occurs ≥ 4 hours before being fed out to the winter flounder larvae at UNH

(Fairchild *et al.* 2007). Despite the water in rotifer cultures being substantially warmer than that of winter flounder cultures, there is no need to acclimate the rotifers to this colder temperature prior to feeding. Mercier *et al.* (2004) found no substantial differences in any of the growth performance and nutritional condition parameters measured between winter flounder larvae fed rotifers from a normal rotifer culture temperature (24°C) and those acclimated overnight to the fish culture tanks (10°C).

Ben Khemis *et al.* (2000) tested whether a high performance microencapsulated diet could be used as a total or partial replacement of rotifers in first-feeding winter flounder larvae. They compared the growth, survival, and nutritional condition of larvae fed either (i) enriched rotifers, (ii) enriched rotifers and microencapsulated diet, (iii) only microencapsulated diet, or (iv) not fed (starved). Fish from the latter two feeding treatments experienced high mortality and no survival beyond day 10 posthatch indicating that newly hatched winter flounder larvae are unable to digest microencapsulated diets. Larvae fed a mixed diet had slower growth than larvae fed exclusively rotifers; however, the RNA/DNA ratios between the two treatments were similar by stomach differentiation (5.5 mm TL).

As the larvae develop and undergo stomach differentiation, rotifers are gradually replaced by *Artemia* over several days. At both UNH and ISMER, newly hatched, unenriched *Artemia* are offered at first (Ben Khemis *et al.* 2003; Fairchild *et al.* 2007). Once larvae readily eat the *Artemia*, Fairchild *et al.* (2007) discontinue the rotifers and only offer *Artemia* enriched with DC DHA Selco (INVE Aquaculture) at a concentration of 3/mL/day. Alternatively, Butts and Litvak (2007a) cofeed rotifers with *Artemia* enriched with Microfeast® MB-30 and algae (*Isochrysis* sp., Tahitian strain, and *Pavlova lutheri*).

Weaning onto formulated feeds

Weaning winter flounder from live to formulated feeds usually occurs with the completion of gut development and metamorphosis. Mean age at metamorphosis (when the left eye migrates to the right side of the head and loss of pigmentation occurs on the blind side; Chambers and Leggett 1987), is temperature-dependent; it can range from as little as 26 dph at 15°C (Lee and Litvak 1996a) to as many as 80 dph at 5°C (Laurence 1975). Mean size at metamorphosis is less variable; it ranges from 6.1 to 10.1 mm TL (Chambers and Leggett 1987; Chambers *et al.* 1988; Perry *et al.* 1991; Bertram *et al.* 1993; Jearld *et al.* 1993).

Weaning is initiated by a period of cofeeding a starter diet (150–450 µm) and *Artemia* to the fish for 5–7 days, then a wean onto the commercial diet over 7–14 days (Lee and Litvak 1996a; de Montgolfier *et al.* 2005; Fairchild *et al.* 2007). Ben Khemis *et al.* (2003) found larvae as small as 5–6.6 mm TL could be weaned onto a commercial microencapsulated diet with no adverse effects on growth rate or time to metamorphosis as long as an extended transitional cofeeding period was used. They recommend feeding the larvae the commercial diet 4 times/day, followed by rotifers for at least 8 days. Once fish are ≥6.6 mm TL, they can be weaned onto a microencapsulated diet.

6.3.3 Genetics for culture versus enhancement

Winter flounder are sexually dimorphic with females growing faster and larger than males (Nash and Geffen 2005). Many flatfish species exhibit a combination of genetic and environmental sex determination in which certain exogenous parameters (e.g., temperature, pH) interact with the genetic factors that influence sex determination, thereby influencing the male to female sex ratio (Nakamura *et al.* 1998). If winter flounder sex determination is affected by these factors, it would occur when fish are <41 mm TL as this is when the gonads differentiate (Fairchild *et al.* 2007). At UNH, cultured winter flounder cohorts do not deviate from a 1:1 sex ratio, with the exception of fish produced in 2003 (Fairchild *et al.* 2007; Fairchild unpublished data). The causes of the 2003 male dominated population are unknown, but temperature effects were unlikely. Research continues at UNH to determine if winter flounder sex determination is affected by variables like temperature, stocking density, photoperiod, and other stressors. If winter flounder sex determination is affected by such variables, there would be some economic advantage to produce a faster-growing, larger, female monoculture for growout; this could be achieved by manipulating environmental cues. In contrast, if fish are produced for stock enhancement, it is necessary that cultures result in an equal mixture of males and females so as not to disrupt the natural sex ratio. In addition, rearing all females using meiotogynogenetic techniques (Howell 1995; Luckenbach *et al.* 2004) warrants research.

6.4 Nursery culture and growout

6.4.1 Juvenile system design and requirements

Juvenile winter flounder can either remain in the larval tanks or be moved to different tanks for growout. de Montgolfier *et al.* (2005) found no differences in growth and condition of juveniles reared in plankton kriesels and in rectangular raceways. Litvak (1999) recommends rearing juvenile in suspended cages within tanks to isolate the small fish from bottom-accumulating debris and to facilitate cleaning the tanks without disturbing the small fish. For juvenile culture, both aeration and water flow are increased, and drains are covered by small-size (500 μ m) mesh to prevent small fish from being lost from the system. At ISMER, flow rates are 1.4 L/min (de Montgolfier *et al.* 2005). At UNH, oxygen is added to each tank to ensure dissolved oxygen does not fall below 7.0 mg/L in densely stocked tanks (Fairchild, unpublished data). Juvenile growth rates are highly variable and temperature-dependent (see Litvak 1999; Howell and Litvak 2000). Juveniles can be stocked as high as 300% (measured as ventral fish area to bottom tank area ratio) with no reduction in growth (Fairchild and Howell 2001). However, winter flounder juveniles held at high densities, even for short periods of time, display elevated levels of cortisol (Sulikowski *et al.* 2006), which suggests that stocking density can act as an environmental stressor rendering the fish more vulnerable to disease.

Both fin erosion and fin rot can occur in wild winter flounder (Bodammer 2000; Ziskowski *et al.* 2008), and fin erosion has been observed in cultured winter flounder (Fairchild and Howell 2001; de Montgolfier *et al.* 2005). Possible causes of fin erosion in cultured winter flounder may include increased aggressive behaviors due to fish size hierarchies (Fairchild and Howell 2001), high stocking density (de Montgolfier *et al.* 2005), unnatural photoperiod (Sakakura and Tsukamoto 2002), unnatural bottom surfaces, and limited food (Latremouille 2003). Fin erosion in winter flounder can be exacerbated by opportunistic bacteria such as *Vibrio anguillarum* (Levin *et al.* 1972) and *Trypanosoma* (Khan 1985); additive effects of the two may cause death (Khan 1985). Treatments for fin rot are reviewed in Latremouille (2003).

Fairchild and Howell (2001) explored the possibility that high stocking densities (up to 300% fish area to tank bottom area) caused an increase in fin nipping, and thus erosion of the caudal fin area. Although there were no differences between treatments, ranging from 50 to 300% stocking density, it was clear that the smallest fish in all treatments had badly damaged caudal fins. They concluded that a size hierarchy had been established in all tanks, and that the smallest fish had suffered from the aggressive behavior (nipping) of the larger fish. One method which may reduce aggressive behavior, even at high stocking densities is manipulating photoperiod. Studies with Japanese flounder have shown that fin nipping and other signs of aggressive behavior only occur during the day and not at night (Sakakura and Tsukamoto 2002). In preliminary photoperiod manipulation trials, survival of juvenile winter flounder was on average 10% higher each week in the 12L:12D photoperiod treatments than in the 24L:0D treatments, suggesting that juvenile winter flounder exposed to a continuous light cycle may be more stressed than those under a more natural lighting regime (Fairchild, unpublished data). While a 24L:0D photoperiod may promote growth (Litvak 1999), this constant light photoperiod regime also may increase aggressive behavior resulting in stressed and fin damaged fish, which may in turn, lead to fish with lower resistance to bacterial infection.

6.4.2 Juvenile diet and nutrition

Juvenile winter flounder are omnivores (Stehlik and Meise 2000) and will readily consume a variety of items in hatcheries including frozen mysids (Stierhoff *et al.* 2006), copepods (Meise *et al.* 2003), blood worms (chironomid larvae; C. Chambers, unpublished data), amphipods, and white worms (*Enchytraeus albidus*; M. Walsh, unpublished data). To date, there is no commercially available feed specifically manufactured for winter flounder, and amino acid requirements for winter flounder have not been determined yet. Researchers either use a coldwater marine species commercial diet or manufacture diets in-house, and feed to satiation (1–7% body weight/day; Howell and Litvak 2000; Ramsay *et al.* 2000; Fairchild and Howell 2001; Plante *et al.* 2005). At UNBSJ, an inexpensive salmonid pellet (Hi-Pro™, Corey Feed Mills, Fredericton, NB, Canada) performed as well as the more costly, specialty pellets (Nippai SF1-3, CATVIS, Hertogenbosch, The Netherlands); juvenile winter flounder specific growth rates

Table 6.2 List of ingredients and their percentage by weight of diets manufactured for juvenile winter flounder by the Canadian Department of Fisheries and Oceans in Halifax, NS.

Ingredient	Percentage of diet	
	Fredette <i>et al.</i> (2000)	Ramsay <i>et al.</i> (2000)
Fish meal (hering 70% CP) ^a	49	45
Wheat middlings	12	12
Dextrin	10	10
Herring oil	8	8
Soybean meal (48% CP)	6	6
Wheat gluten	5	5
Mineral mix	2	2
Soy lecithin	2	2
Vitamin mix ^b	2	2
Sodium alginate	1.3	1.3
Choline chloride	0.7	0.7
CMC (carboxymethylcellulose)	1	1
FinnStim ^c	1	0
Crab meal	0	5

^aCP, crude protein.^bModified Bernhardt-Tomarelli salt mix.^c2-trimethylammonioacetate.

(wet weight) were 3.11% per day and 2.65% per day for the respective diets (Lee and Litvak 1996b). At ISMER, a mixed diet of 40% salmonid starter feed, 10% spirulina, 25% freeze-dried amphipods, and 25% tubifex worms is used for newly-weaned juvenile fish (de Montgolfier *et al.* 2005). At the Canadian Department of Fisheries and Oceans in Halifax, NS, a base diet high in protein is manufactured in-house (Fredette *et al.* 2000; Ramsay *et al.* 2000; Table 6.2). While Ramsay *et al.* (2000) add a freeze-dried *Cancer irroratus* crab meal, Fredette *et al.* (2000) found that the commercial feeding stimulant FinnStim (2-trimethylammonioacetate) supplemented at 1% to a fish meal based diet is an effective feeding stimulant for age 1 winter flounder. Fish fed the diet with stimulant had higher daily feed consumption and growth, and slightly higher feeding efficiency compared to fish fed a control diet.

A crude protein level of 50% with a digestible protein to digestible energy ratio of 26 mg/kJ is optimal for growth of juvenile (0.9–5 g) winter flounder (Hebb *et al.* 2003). Fish fed a 50:10% protein-to-lipid level diet had both a higher specific growth rate and a better feed efficiency ratio compared to fish fed a 40:20% diet, but not significantly different from fish fed a 45:15% diet (Hebb *et al.* 2003). In addition, the protein efficiency of the higher protein level fed fish tended to be higher than in the other fish. With the rising costs of fish meal protein and oil, and the push to make aquaculture more sustainable, there is an increased demand to find alternative proteins in fish feeds. Preliminary studies indicate that a 15% fish meal replacement with either soybean meal or canola protein concentrate is plausible for juvenile winter flounder. At this replacement level, the digestibility of gross energy or crude protein in 159 g, juvenile winter flounder was not different than in fish fed a control fish meal diet; gross energy and crude protein digestibilities of the diets ranged from 91

to 95% and 88 to 92%, respectively (Ramsay *et al.* 2000). Though promising, more research is needed to determine fish growth, feed conversion, and protein retention to understand more completely the ramifications of using a fish meal replacement diet for juvenile winter flounder. Using an RNA:DNA based growth model (Mercaldo-Allen *et al.* 2008) will prove useful for assessing the nutritional condition and evaluating rearing protocols of juvenile (27–52 mm SL) winter flounder.

Like most cultured flatfishes (Venizelos and Benetti 1999), unexplained abnormal pigmentation occurs in a proportion of hatchery-reared winter flounder. de Montgolfier *et al.* (2005) reported that 55% of juveniles in an experiment ranked <3 on a scale of 0–5 with 0 representing total lack of pigmentation and 5 equaling normal pigmentation. At UNH, the frequency of abnormal pigmentation varies annually and is likely attributed to both genetics and nutrition (Fairchild, unpublished data). While these pigment abnormalities do not affect the quality of the fish, they are considered commercially inferior (Venizelos and Benetti 1999). Additionally, fish with hypomelanosis (lack of pigmentation on the ocular side) should not be used for enhancement as they are more susceptible to predation (Fairchild and Howell 2004).

6.4.3 Behavioral conditioning for stock enhancement

Fish for release should be conditioned in the weeks prior to their release to increase their chances of survival in the wild. Typically, culture tanks are devoid of sand yet sediment-naïve cultured winter flounder require at least 48 hours to hone their burial skills (Fairchild and Howell 2004). Providing these fish with a “natural” (both in color and grain size) substrate typical of that in the release site will allow them to adjust pigmentation, may increase site fidelity, and reduce vulnerability to visually-hunting predators (Fairchild and Howell 2004). Alternatively, this conditioning can be done *in situ* at the release site, but care must be taken not to attract predators (Fairchild *et al.* 2008). Feed conditioning also merits consideration. Fish may take 3–4 days before they begin feeding on live prey, and even then, non-traditional food sources may be selected due to size, shape, and color resemblances to pellets (Fairchild, unpublished data). The traditional, formulated diets fed to cultured fish for stock enhancement are unnatural; they are immobile and are often shaped differently than wild diets. Live diets, such as white worms, may enhance the intrinsic tendencies of reared winter flounder to forage for food as they would in their natural environment. Preliminary studies at UNH show that juvenile winter flounder fed white worms prior to release, have higher growth rates and similar RNA/DNA values compared to fish fed pellets in the hatchery (M. Walsh, unpublished data). Of course, tagging of juveniles is necessary to evaluate the contribution of stocked fish to natural populations. To limit handling time and, therefore, stress, air-injected tags are recommended prior to stocking (Sulikowski, Fairchild *et al.* 2005). Though these elicit a stress response, the fish recover within 2 days (Sulikowski, Fairchild *et al.* 2005).

6.5 Growout

While many of the critical steps to develop a winter flounder aquaculture industry have been established, growout protocol is still developing. Growout systems, either land-based or cage culture, likely will be similar to ones used for other flatfish species. There is the potential for cage culture of winter flounder. Though transporting juveniles elicits a stress response, fish stocked as high as 400% during transport and in cages recovered to baseline levels within 48 hours (Sulikowski *et al.* 2005, 2006). Litvak (1999) has had promising results growing winter flounder out in octagonal cages with rigid bottoms. These 6 m deep cages are suspended at the surface. Fish grown out in cages gained 40% in weight in 1 year and were significantly heavier than wild caught fish of the same length.

One potential problem of cage culture is the risk of escapees; sterile, hybrid fish are attractive for cage culture since any potential escapees would be sterile and thus incapable of either interbreeding with wild populations, or establishing an unnatural population in the wild. Winter flounder sperm have been used to fertilize yellowtail flounder, *Pleuronectes ferrugineus*, eggs, however, the early life history characteristics of the offspring were not promising (Park *et al.* 2003). Hybrid fertilization rate, hatching success, and larval survival were significantly lower than in pure parental crosses.

Though winter flounder are a slow-growing fish reaching market size in 2–3 years, production time can be decreased by rearing the fish at warmer temperatures. The market for winter flounder varies seasonally with the availability of catch, size, catch location, and market name. Though all the same species, the fish is marketed dressed or filleted as winter flounder, blackback flounder, and lemon sole. Due to the hardness of this species, winter flounder has great potential for the live market too.

6.6 Summary

Winter flounder is a hardy, eurythermal, euryhaline, and freeze-resistant flatfish, which has great promise in the aquaculture industry. The thick, white fillets have a high market price compared to most New England flatfish. Because winter flounder has been used as a model for laboratory experiments for 100+ years, much of the rearing protocol has been developed. To date, there has been great success in controlled spawning of adult winter flounder, rearing larvae, and producing large numbers of juvenile fish. Certain areas, however, still require research, and winter flounder aquaculture has not expanded to commercial-scale operation. Methods to reduce broodstock mortality are necessary to the success of a commercial-scale operation, as is broodstock domestication; multiple generations are needed for the selection of desirable characteristics. In addition, winter flounder specific diets for both juvenile and adult fish should be developed. The encouraging results of partial replacement of fish meal and oil in the diets with other proteins warrant more research. Further research to reduce abnormal pigmentation would increase the success of aquaculture operations both for market and enhancement. Growout techniques should be developed further

with comparisons between land-based and cage culture systems. Commercial-scale demonstration projects would allow for insightful economic analyses and transfer from research to industry.

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A Responsible Approach to Marine Stock Enhancement

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Abstract.—Declining marine fish populations worldwide have rekindled an interest in marine fish enhancement. Recent technological advances in fish tagging and marine fish culture provide a basis for successful hatchery-based marine enhancement. To ensure success and avoid repeating mistakes, we must take a responsible approach to developing, evaluating, and managing marine stock enhancement programs. A responsible-approach concept with several key components is described. Each component is considered essential to control and optimize enhancement. The components include the need to (1) prioritize and select target species for enhancement; (2) develop a species management plan that identifies harvest opportunity, stock rebuilding goals, and genetic objectives; (3) define quantitative measures of success; (4) use genetic resource management to avoid deleterious genetic effects; (5) use disease and health management; (6) consider ecological, biological, and life-history patterns when forming enhancement objectives and tactics; (7) identify released hatchery fish and assess stocking effects; (8) use an empirical process for defining optimum release strategies; (9) identify economic and policy guidelines; and (10) use adaptive management. Developing case studies with Atlantic cod *Gadus morhua*, red drum *Sciaenops ocellatus*, striped mullet *Mugil cephalus*, and white seabass *Atractoscion nobilis* are used to verify that the responsible approach to marine stock enhancement is practical and can work.

Marine fish populations are declining worldwide. In the United States, current abundance trends are known for only 15 of the most important marine stocks; about half of them are declining (NOAA 1991, 1992). Current harvest rates on most declining stocks are far in excess of exploitation levels needed to maintain the high long-term average yields that could be achieved through contemporary fishery management practices. Projected increases in human population size worldwide suggest this trend will continue into the future (FAO 1991).

Three principal tactics are available to fishery managers to replenish depleted stocks and manage fishery yields: regulating fishing effort; restoring degraded nursery and spawning habitats; and increasing recruitment through propagation and release (stock enhancement). The first two methods form the basis for the current federal approach to managing marine fisheries in the United States. The potential of the third method has not been convincingly documented with marine fishes.

Marine stock enhancement is not a new concept. In fact, hatchery-based stock enhancement was the principal technique used in an attempt to restore marine fisheries during the last part of the nineteenth century and early decades of the twentieth century. However, stock enhancement fell out of favor among fishery

biologists after a half century of hatchery releases produced no evidence of an increased yield. Atlantic cod *Gadus morhua*, haddock *Melanogrammus aeglefinus*, pollock *Pollachius virens*, winter flounder *Pleuronectes americanus*, and Atlantic mackerel *Scomber scombrus* were stocked. Regrettably, when the last of the early marine hatcheries in the United States closed in 1948, after 50 years of stocking marine fishes, the technology had progressed no further than the stocking of unmarked, newly hatched fry. This was partly a result of the early approach to assessment, in which the success of hatchery programs was judged by numbers of fry stocked rather than by numbers of adults surviving to enter the fishery (Richards and Edwards 1986).

A New and Responsible Approach

Two general problems have restricted development of marine stock enhancement technology this century. Lack of an evaluation capability to determine whether hatchery releases were successful has been a major obstacle. Before the development of modern marking methods, fish-tagging systems were not applicable to the small, early life history stages released by hatcheries. The other impediment to development of marine enhancement has been the inability to culture marine fishes beyond

early larval stages to the juvenile stage (fingerlings and larger sizes).

A new approach to marine stock enhancement is long overdue. Faced with declining stocks and an expanding world population, managers around the globe are looking at marine enhancement with renewed interest. To develop and evaluate stock enhancement's full potential, a process is needed for designing and refining stock enhancement tactics based on the combined effects of managing the resource (i.e., the interactive effects of hatchery practices, release strategies, harvest regulations, and habitat restoration on the condition of the managed stock).

Recent advances in both tagging technology and marine fish culture provide basic tools for a new approach to marine enhancement. We now have the technology for benign tagging of fish from juvenile through adult life stages (Bergman et al. 1992). Such tagging provides the basis for a quantitative assessment of stock enhancement success. Several marine fishes can be cultured to provide a wide range of life stages for release (e.g., McVey 1991; Honma 1993). Together, these tools allow an empirical evaluation of survival of cultured fish in the wild, and feedback on hatchery-release effects can be used to refine enhancement strategies. Release effects on wild stocks, and the fisheries based on them, can be quantified and evaluated. Survival can be examined over a range of hatchery practices and release variables (such as culture practices, fish size at release, release magnitude, release site, and season) to identify optimum combinations of hatchery and release strategies.

These new tools provide the basis for significantly increasing wild stock abundances. To ensure their successful use and avoid repeating past mistakes experienced in both marine and freshwater enhancement, we must use a careful approach in developing marine stock enhancement programs. The expression "a responsible approach to marine stock enhancement" embraces a logical and conscientious strategy for applying aquaculture technology to help conserve and expand natural resources. This approach prescribes several key components as integral parts in developing, evaluating, and managing marine stock enhancement programs. Each component is considered essential to control and to optimize the results of enhancement. The components include the need to (1) prioritize and select target species for enhancement; (2) develop a species management plan that identifies harvest opportunity, stock rebuilding goals, and genetic objectives; (3) define quantitative measures of success; (4) use

genetic resource management to avoid deleterious genetic effects; (5) use disease and health management; (6) consider ecological, biological, and life-history patterns when forming enhancement objectives and tactics; (7) identify released hatchery fish and assess stocking effects; (8) use an empirical process for defining optimum release strategies; (9) identify economic and policy guidelines; and (10) use adaptive management. Combining new marine fish culture and tagging technologies with these ten principles is gaining support as a responsible approach to marine stock enhancement.

Empirical data suitable for accurately assessing the effect of hatchery releases on wild populations are often lacking. Partly because of this uncertainty, there is an increasing division of conservationists into two camps—one adamantly favoring increased fishing regulations and habitat protection and restoration in preference to hatchery releases, the other supporting propagation and release as an additional tool to manage fisheries and restore declining stocks. This split must be reconciled. Is stock enhancement of marine fishes a powerful, yet undeveloped technology for rebuilding depleted wild stocks and increasing fishery yields? Or are emerging marine enhancement programs merely futile attempts at recovering precious resources, thus diverting money and attention away from habitat restoration and the regulations needed to control overfishing? To realize the full potential of marine enhancement for the conservation and rapid replenishment of declining marine stocks, we must develop the technology to supplement and replenish marine stocks responsibly and quickly.

We must act now to assess the potential of marine stock enhancement through carefully planned research programs. Using strong inference (Platt 1964), which is essentially the scientific method, and addressing all of the components of the responsible approach concept, research programs will either document the value of marine enhancement or reveal that enhancement is not a useful concept. Without determined and careful attention to the 10 points listed above, marine hatchery releases in the 1990s may serve only to fuel divisiveness between the two conservationist camps, with little or no positive effect on natural resources.

Applying the responsible approach concept to new stock enhancement initiatives is straightforward. Existing enhancement programs may find it useful to review the 10 components discussed here. Incorporating those components expanded upon below, that are not already part of ongoing enhancement programs should provide a measurable

increase in the realized effectiveness of replenishment efforts.

Prioritize and Select Target Species for Enhancement

In the absence of a candid and straightforward method, targeting species for stock enhancement can become a difficult and biased process. Unless attention is focused on the full spectrum of criteria that can be used to prioritize species, consideration of an immediate need by an advocacy group or simply the availability of aquaculture technology can become the driving factors in species selection. Commercial and recreational demand are obviously important criteria, but should they take precedence over other factors?

To reduce the bias inherent in selecting species, a semiquantitative approach was developed in Hawaii to identify selection criteria and prioritize species for stock enhancement research (Leber 1994). This approach involved four phases: (1) an initial workshop, where selection criteria were defined and ranked in order of importance; (2) a community survey, which was used to solicit opinions on the selection criteria and generate a list of possible species for stock enhancement research; (3) interviews with local experts to rank each candidate species with regard to each selection criterion; and (4) a second workshop, in which the results of the quantitative species selection process were discussed and a consensus was sought. This decision-making process focused discussions, stimulated questions, and quantified participants' responses. Panelists' strong endorsement of the ranking results and selection process used in Hawaii demonstrate the potential for applying formal decision making to species selection in other regions.

A critical step in removing bias from the species selection process lies in the type of numerical analysis used. The relative importance of the various criteria can be used in the analysis by factoring the degree to which each fish meets each criterion by the criterion weight. This produces a score for each species. This same concept is used to determine dominance in ecological studies of species assemblages (i.e., relative abundance times frequency of occurrence in samples). Using a trained facilitator to conduct the workshops also reduces bias by focusing activities on achieving results and by encouraging participation by all present.

Formal decision-making tools have been used effectively to prepare comprehensive plans for fisheries research (Bain 1987). Mackett et al. (1983)

discuss the interactive management system for the Southwest Fisheries Center of the National Marine Fisheries Service. Similar processes have been used for research on North Pacific pelagic fisheries, in strategic planning for Hawaii's commercial fishery for skipjack tuna *Katsuwonus pelamis* (Boggs and Pooley 1987), and for a 5-year scientific investigation of marine resources of the main Hawaiian Islands (Pooley 1988).

Develop a Species Management Plan

A management plan identifies the context into which enhancement fits into the total strategy for managing stocks. The goals and objectives of stock enhancement programs should be clearly defined and understood prior to implementation. The genetic structure of wild stocks targeted for enhancement should be identified and managed according to objectives of the enhancement program. What is the population being enhanced? Can it be geographically defined? Clearly, in the interest of both production aquaculture and conservation, effort must be made to maintain genetic diversity (Kapusinski and Jacobson 1987; Shaklee et al. 1993a, 1993b).

Assumptions and expectations about the performance and operation of the enhancement program necessary to make it successful should be identified (such as postrelease survival, interactions with wild stocks, long-term fitness, and disease). Critical uncertainties about basic assumptions that would affect the choice of production and management strategies should likewise be identified and prioritized. Evaluation of these uncertainties should be an integral part of the species management plan, and a feedback loop to evaluate and change production and management objectives should be included.

Define Quantitative Measures of Success

Without a definition of success, how do you know if or when you have it? Explicit indicators of success are clearly needed to evaluate stock enhancement programs. The objectives of enhancement programs need to be stated in terms of testable hypotheses. To be testable, a hypothesis must be falsifiable (Popper 1965). Depending on enhancement objectives, multiple indicators of success may be needed. These could include statements such as

Hatchery releases will provide at least a 20% increase in annual landings of *Polydactylus sexfilis* in the Kahana Bay recreational fishery by the third year of the project.

Monitoring will show less than 3% change in the frequency of rare alleles (frequency less than 0.05) after 5 years of hatchery releases (this assumes that a control for the effects of environmentally induced change in allele frequencies is possible).

Numerous indicators should be identified to track progress over time. Although simplistic, indicators like the two examples above could be linked to success and would provide a basis for evaluating enhancement efforts during the initial period of full-scale releases. Clearly, to examine such indicators requires a reliable, quantitative marking and assessment system for tracking hatchery fish.

Use Genetic Resource Management

The need for genetic resource management in stock enhancement programs is currently the subject of intense public debate, and its importance cannot be over-rated. Responsible guidelines are now becoming available to aid resource managers in revitalizing stocks without loss of genetic fitness that could follow from inbreeding in the hatchery and subsequent outbreeding depression in the wild (Kapuscinski and Jacobson 1987; Shaklee et al. 1993a, 1993b). Once the genetic status of the target stock and the genetic goals of the enhancement program are identified, the approach for managing genetic resources is similar to the approach for managing other enhancement objectives (e.g., controlling the level of impact of stocked fish on abundances of the target population). This approach includes (1) identifying the genetic risks and consequences of enhancement; (2) defining an enhancement strategy; (3) implementing genetic controls in the hatchery and a monitoring and evaluation program for wild stocks; (4) outlining research needs and objectives; and (5) developing a feedback mechanism. These points are discussed in detail by Kapuscinski and Jacobson (1987) and Shaklee et al. (1993a, 1993b).

A genetic resource management plan should encompass genetic monitoring prior to, during, and after enhancement, as well as proper use of a sufficiently large and representative broodstock population and spawning protocols, to maintain adequate effective broodstock population size. Prior to enhancement, a comprehensive genetic baseline evaluation of the wild population should be developed to describe the level and distribution of genetic diversity. This baseline evaluation should at least include the geographical range of the particular stock targeted for enhancement. The monitoring should take place over a long enough period to

observe possible short-term fluctuation or long-term change. The baseline can be used as a basis to determine an effective population or broodstock size to minimize the undesirable genetic effects of inbreeding, changes in allele frequencies, and loss of alleles. Genetic monitoring of the broodstock and its released progeny should be undertaken to measure success. Long-term genetic monitoring of the wild stock after enhancement should also occur to measure possible loss of genetic diversity, which might be attributed to enhancement efforts.

Maintenance and proper use of a sufficient broodstock population may be one of the toughest and most expensive components of marine stock enhancement. It is also one of the most important. The typically high fecundity rate of marine fish provides the opportunity for a greatly reduced effective population size in a hatchery environment because relatively few adults could potentially contribute a large number of eggs. Fortunately however, marine fish are genetically more homogeneous than freshwater and anadromous species on a relative scale, and genetic studies show relatively little stock separation due to geographic, clinal, or temporal factors (Gyllenstein 1985; Waples 1987; Bartley and Kent 1990; King et al. 1995, this volume). In vagile marine species gene flow is often sufficient to homogenize the genetic structures over broad areas. Regardless, sufficient numbers of broodstock must be used so that the genetic diversity (including rare alleles) of the fish being released is the same over time as their wild counterparts.

Hubbs-Sea World Research Institute (Hubbs) has been an early promoter of a responsible genetic management plan. Hubbs leads a consortium of California researchers who are evaluating the feasibility of enhancement of white seabass *Atractoscion nobilis*. Although the genetic profile of progeny from an individual spawn may differ from wild spawns, use of multiple hatchery spawns can approximate the genetic variability observed in the wild. Bartley and Kent (1990) successfully used this concept with white seabass and showed that over 98% of the genetic variability observed in the wild could be maintained with an effective population of 100 broodfish.

Texas Parks and Wildlife Department's (Texas) enhancement program for red drum *Sciaenops ocellatus* provides a good example of maintaining a large broodstock with yearly replenishment (McEachron et al. 1995, this volume). Texas has 140–170 adult broodstock for its program, with an annual replacement of at least 25%. In Norway, studies of allele frequencies are being used to com-

pare broodstock and their progeny with wild populations of Atlantic cod (Svasand et al. 1990).

Use Disease and Health Management

Disease and health guidelines are important to both the survival of the fish being released and the wild populations of the same species or other species with which they interact. Florida Department of Environmental Protection (Florida) has developed an aggressive and responsible approach in this area in association with their red drum enhancement project (Landsberg et al. 1991). Florida's policy requires that all groups of fish pass a certified inspection for bacterial and viral infections and parasites prior to release. Maximum acceptable levels of infection and parasites in the hatchery populations are established based on the results of screening healthy wild populations.

Form Enhancement Objectives and Tactics

During the design phase of enhancement programs ecological factors that can contribute to the success or failure of hatchery releases should be considered. Predators, food availability, accessibility of critical habitat, competition over food and space, environmental carrying capacity, and abiotic factors, such as temperature and salinity, are all key variables that can affect survival, growth, dispersal, and reproduction of cultured fish in the wild. Predatory losses and food availability have long been thought to be among the principal variables that mediate recruitment success in wild populations (Lasker 1987; Houde 1987).

Habitat degradation in marine environments can also affect recruitment success. For example, seagrass meadows are important nursery habitats for fishes and crustaceans (see Kikuchi 1974). In vegetated aquatic environments, habitat availability and habitat quality (e.g., structural complexity) have been shown to mediate survival from predators (Crowder and Cooper 1982; Stoner 1982; Main 1987). In some cases, habitat degradation in marine environments may be so complete that certain habitats are unsuitable for stock enhancement (Stoner 1994). To enhance fisheries in some locales, restoration of coastal habitat may be the first priority.

The authors feel strongly that marine stock enhancement should never be used as mitigation to justify loss of habitat. However, we also feel that enhancement efforts with cultured fishes can fill a void where critically important habitats such as coastal wetlands and estuaries, which provide nurs-

eries for early life stages, are irretrievably lost or degraded.

In addition to ecological factors, there may be physiological and behavioral deficits in hatchery-reared fish that strongly reduce survival in the wild (e.g., swimming ability, feeding behavior, predator avoidance, agonism, schooling, and habitat selection). In Japan, Tsukamoto (1993) has evaluated the effect of behavior on survival of cultured madai *Pagrus major* (called red sea bream by Tsukamoto) released into the sea. Tsukamoto's results indicate that a predator-avoidance behavior (tilting), in which wild fish lay flat against the substratum, may be reduced or absent in cultured fish during the first few days after release into the sea. Abnormal tilting behavior was directly correlated with mortality rate. For certain learned behaviors, exposure to behavioral cues and responses by wild fish in hatchery microcosms may be needed to overcome behavioral deficits (Olla and Davis 1988).

A solid understanding of the ecological and biological mechanisms mediating target species abundances can require exhaustive field studies for each species considered for enhancement. Whole careers have been dedicated to understanding mechanisms behind animal distributions and abundance; it does not seem practical to hold off on stock enhancement research until the ecological mechanisms are completely understood. However, failure to consider such factors can result in poor performance of released fish at best and at worst have negative impacts on natural stocks (Murphy and Kelso 1986).

Our viewpoint is that preliminary, pilot-scale experimental releases with subsequent monitoring of cultured fish afford a direct method for evaluating assumptions about the effects of uncontrolled environmental factors. For example, assumptions about carrying capacity in particular release habitats can and should be evaluated through pilot releases conducted prior to full-scale enhancement at those sites (Leber et al. 1995, this volume). This approach is elaborated below.

Identify Released Hatchery Fish and Assess Stocking Effects

One of the most critical components of any enhancement effort is the ability to quantify success or failure. Without some form of assessment, one has no idea to what degree the enhancement was effective or, more critically, which approaches were totally successful, partially successful, or a downright failure. Natural fluctuations in marine stock abundance can mask successes and failures. Maximiza-

tion of benefits cannot be realized without the proper monitoring and evaluation system.

Tagging or marking systems that are benign and satisfy the basic assumption that identified fish are representative of untagged counterparts are essential, but weren't available until relatively recently. The detrimental effects of external tags are well documented (Isaksson and Bergman 1978; Hansen 1988; McFarlane and Beamish 1990), and few fishery managers or researchers defend their use today, especially with juvenile fish. Useful information retrieved from external tags is usually restricted to migration and growth rates of relatively large fish (Scott et al. 1990; Trumble et al. 1990).

In recent years, a few identification systems (e.g., coded wire tags, passive integrated transponder tags, genetic markers, and otolith marks) have been developed that meet the requirements that identified fish are representative of the species with regard to behavior, biological functions, and mortality factors, and thus provide unbiased data (Buckley and Blankenship 1990). The story of the development and now widespread use of the coded wire tag (Jefferts et al. 1963) is well known, and it is fair to say that it has revolutionized the approach to stock enhancement (Soloman 1990).

With an unbiased tag or mark, quantitative assessment of the effects of release is possible. In developing enhancement programs, evaluation of hatchery contributions can be partitioned into at least four distinct stages: initial survival, survival through the nursery stage, survival to adulthood (entry into the fishery), and successful contribution to the breeding pool. In Hawaii, the percent of hatchery fish in field samples taken after pilot releases of striped mullet *Mugil cephalus* has been as high as 80% in initial collections, 50% in some nursery habitats through the tenth month after release, and (in a recreational fishery in Hilo, Hawaii) as high as 20% of the catch (Leber, in press; Leber et al. 1995; Leber et al., in press). In Norway, genetic markers are beginning to show that released Atlantic cod produce viable offspring in the wild (Jorstad 1994).

Assessment of the effects of release should go further than evaluation of survival and contribution rates of hatchery fish. Evaluation of hatchery fish interactions with wild stocks is also critical. Clearly, evaluation of genetic impact is important. It is equally important to understand whether hatchery releases increase abundances in the wild or simply displace the wild stocks targeted for enhancement. At least one experimental study in Hawaii has documented that released hatchery fish can indeed in-

crease abundances in a principal nursery habitat, without displacing wild individuals (Leber et al. 1995).

Use an Empirical Process to Define Optimum Release Strategies

Just as preliminary releases can be used to evaluate ecological assumptions, pilot release experiments afford a means of quantifying and controlling the effects of release variables and their influence on the performance of cultured fish in coastal environments (Tsukamoto et al. 1989; Svasand and Kristiansen 1990; Leber, in press; Willis et al. 1995, this volume).

Experiments to evaluate fish size at release, release season, release habitat, and release magnitude should always be conducted prior to launching full-scale enhancement programs. These experiments are a critical step in identifying enhancement capabilities and limitations and in determining release strategy. They also provide the empirical data needed to plan enhancement objectives, test assumptions about survival and cost effectiveness, and model enhancement potential. The lack of monitoring to assess survival of the fish released by marine enhancement programs early in this century (through the 1940s) was the single greatest reason for the failure of those programs to increase stock abundances and fishery yields (Richards and Edwards 1986).

Based on the results of pilot experiments by The Oceanic Institute in Hawaii, hatchery-release variables were steadily refined to maximize striped mullet enhancement potential. This resulted in an increase in recapture rates by at least 400% over a 3-year period (Leber et al. 1995; Leber et al., in press.) During the third year of pilot studies in Kaneohe Bay, hatchery fish provided at least 50% of the striped mullet in net samples during the entire 10-month collection period after releases. An understanding of how fish size at release and release habitat affected survival were the primary factors needed to increase recapture rates. However, understanding the interaction of release season with size at release and release habitat also had significant effect on refinement of release strategies. The apparent doubling effect on abundances in the third year was achieved with a release of only 80,000 juveniles into the principal striped mullet nursery habitat in Kaneohe Bay, the largest estuary in Hawaii. A subsequent study documented that mullet releases did not displace wild juveniles from that nursery habitat (Leber et al. 1995). Thus, hatchery

releases in Kaneohe Bay appear to be increasing population size in the primary nursery habitat. Clearly, these pilot experiments are crucial for managing enhancement impact.

Identify Economic and Policy Objectives

Initially, costs and benefits can be estimated and economic models developed to predict the value of enhancement. This information can be used to generate funding support through reprioritization, legislation, or user fees. The information can contribute to an explicit understanding with policy makers and the general public on the time frame that is needed for components such as adaptation of culture technology and pilot-release experiments before full-scale releases can begin. The education of the public and policy makers on the need and benefits of a responsible approach is also important. In Florida, pressure is mounting to drop the responsible approach concept involving pilot-scale releases and instead plant millions of red drum fry as a neighboring state has done (Wickstrom 1993). Advocates of the latter approach simply assume that the bigger the numbers planted, the better.

Use Adaptive Management

Adaptive management is a continuing assessment process that allows improvement over time. The key to this improvement lies in having a process for changing both production and management objectives (and strategies) to control the effects of enhancement. Essentially, adaptive management is the continued use of the nine key components above to ensure an efficient and wise use of a natural resource. The use of adaptive management is central to the successful application of the approach outlined above. Some minimum level of ongoing assessment is needed, superimposed over a moderate research framework that provides a constant source of new information. New ideas for refining enhancement are thus constantly considered and integrated into the management process.

Summary

The need for marine stock enhancement has been identified, and we must learn from mistakes made in the past. The necessity and benefit of following a responsible approach in implementing enhancement cannot be overemphasized. Several organizations have started to subscribe to this new approach to marine stock enhancement. The juveniles from their pilot releases are just starting to enter the fisheries, so the results are not known.

The exception is the striped mullet enhancement program in Hawaii at The Oceanic Institute. This program has shown the benefits that can be gained from closely following the approach outlined in this paper. In addition to The Oceanic Institute's decision to develop a proper genetic management plan and to make quantitative assessments of the effects of hatchery releases on wild populations, it performed numerous pilot studies to optimize release strategies.

Without these pilot experiments, Hawaii researchers would not have increased survival rate by over 400% in Kaneohe Bay nor provided a 20% contribution to the catch in the recreational fishery in Hilo Bay. We predict that identifiable fish from each of the other programs referenced will also have a substantial effect on the catch and validate our suggested approach. What is needed now is a concerted effort by the managers of new and existing enhancement programs to use, evaluate, and refine the approach described here.

Given the worldwide decline in fisheries catch rates, bold new initiatives are needed to revitalize fisheries. We need to take care, though, to preserve existing stocks as we work to restore and increase the harvest levels of those stocks using cultured fishes.

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Responsible Approach to Marine Stock Enhancement: An Update

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Marine stock enhancement is a set of management approaches involving the release of cultured organisms to enhance or restore fisheries. Such practices, including sea ranching, stock enhancement, and restocking, are widespread, of variable success, and often controversial. A set of principles aimed at promoting responsible development of restocking, stock enhancement, and sea ranching has been proposed by Blankenship and Leber [American Fisheries Society Symposia 15: 167–175 (1995)], and has gained widespread acceptance as the 'Responsible Approach'. Fisheries science and management, in general, and many aspects of fisheries enhancement have developed rapidly since the responsible approach was first formulated. Here we provide an update to the Responsible Approach in light of these developments. The updated approach emphasizes the need for taking a broad and integrated view of the role of enhancements within fisheries management systems; using a stakeholder participatory and scientifically informed, accountable planning process; and assessing the potential contribution of enhancement and alternative or additional measures to fisheries management goals early on in the development or reform process. Progress in fisheries assessment methods applicable to enhancements and in fisheries governance provides the means for practical implementation of the updated approach.

Keywords fisheries enhancement, stock enhancement, sea ranching, restocking, responsible approach, planning, assessment, population dynamics, models

INTRODUCTION

Many of the world's fisheries are fully exploited or overexploited, as well as suffering from the effects of aquatic habitat degradation. Global capture fisheries production is stagnant, while seafood demand is steadily increasing (FAO, 2009); a number of formerly productive stocks have collapsed with only limited evidence of recovery, and ecosystem-level impacts of biomass removal and fishing gear disturbance have become increasingly evident (Hutchings, 2000; Pauly et al., 2002; Hilborn et al., 2003; Hilborn, 2007b).

Besides control of fishing effort and habitat protection or restoration, aquaculture-based enhancement is a third principal means by which fisheries can be sustained and improved

(Munro and Bell, 1997; Welcomme and Bartley, 1998; Blaxter, 2000; Bell et al., 2005). Aquaculture-based fisheries enhancement is a set of management approaches involving the release of cultured organisms to enhance, conserve, or restore fisheries. This definition covers a great diversity of enhancement fisheries systems including 'Sea ranching', 'Stock enhancement', and 'Restocking' (Bell et al., 2006; Lorenzen, 2008). Here we focus on fundamental attributes shared by most enhancement systems but also emphasize how different objectives and situations give rise to different system designs. For simplicity, we refer to all forms of aquaculture-based fisheries enhancements as 'enhancements' and to the target organisms as 'fish'.

Aquaculture-based enhancements can, at least in principle, generate a range of benefits (Howell et al., 1999; Leber et al., 2004; Bell et al., 2008; Lorenzen, 2008). In biological terms, enhancement can (1) increase yield through manipulation of population and/or food web structure, thus raising fisheries production at low external inputs and degree of habitat modification;

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(2) aid the conservation and rebuilding of depleted, threatened, and endangered populations; and (3) provide partial mitigation for habitat loss and ecosystem effects of fishing. This may give rise to economic and social benefits, including new opportunities for fisheries-related livelihoods (Smith et al., 2005; Garaway, 2006). Enhancements can also provide incentives for active management and better governance of common pool resources (Arbuckle, 2000; Garaway et al., 2006; Tomiyama et al., 2008). However, many enhancements have failed to deliver significant increases in yield or economic benefits, and/or have had deleterious effects on the naturally recruited components of the target stocks (Hilborn, 1998; Levin et al., 2001; Arnason, 2001; Naish et al., 2007). Sometimes enhancements have contributed to management failure by encouraging or compensating for counterproductive changes in fishing patterns or for habitat degradation (Meffe, 1992; Taylor, 1999).

While some enhancement initiatives have increased yields, generated economic and social benefits, and helped create better fisheries management institutions, only a few such 'success stories' have been documented in the scientific literature (Pinkerton, 1994; Lorenzen et al., 1998; Drummond, 2004; Uki, 2006; Garaway, 2006; Becker et al., 2008). Overall, the contribution of enhancements to global fisheries production has remained small, at below 2% of the global total (Lorenzen et al., 2001). It is, thus, pertinent to ask why enhancements have not made a greater contribution to fisheries. We believe there are several contributing factors. Success in fisheries management is measured against an increasingly broad set of criteria: biological (yield, ecosystem indicators), economic, social, and institutional attributes (Charles, 2001; Garcia and Charles, 2007). Enhancements can score well on a range of criteria, but only under certain conditions. These include existing ecological, economic, and social conditions; and technologies and institutional arrangements that are well adapted to those conditions. Moreover, enhancements need to add value to, or outperform alternative management measures, such as fisheries regulation or habitat restoration, which may be either cheaper or provide a wider range of benefits. These considerations suggest that enhancement initiatives need to be assessed, if not positively driven, from a fisheries management perspective rather than the aquaculture production perspective that has been traditionally dominant.

The effectiveness of stocking cultured organisms, though, has been hampered by lack of a scientific, institutional, and fisheries-management perspective in planning, design, implementation, and evaluation of enhancement programs (Cowx, 1994; Blankenship and Leber, 1995; Munro and Bell, 1997; Leber, 1999; Hilborn, 1998, 1999; Lorenzen et al., 2001; Bell et al., 2005, 2006, 2008; Bartley and Bell, 2008; Lorenzen, 2008). Although fishery managers began to stock cultured fishes into the sea in the 1880's, no scientific publications appeared about effectiveness of releases until empirical studies of anadromous salmonids began to be published in the 1970's (Hager and Noble, 1976) followed by the first studies of marine fishes in 1989 and 1990 (Tsukamoto et al., 1989; Svåsand and Kristiansen, 1990a, 1990b; Svåsand et al., 1990; Kristiansen and Svåsand, 1990). Thus, lacking a foundation of quantitative information for evaluating stocking's real potential as a tool in the fishery management toolbox, by the 1990's the marine enhancement field had largely floundered for over a century (Leber, 1999).

In response to a clear need for change, Cowx (1994, for enhancements in freshwater systems) and Blankenship and Leber (1995, for marine systems) published early platform papers calling for a responsible approach to stock enhancement. Those early papers presented a set of principles aimed at promoting the responsible development of culture-based fisheries/ranching, stock enhancement and restocking. Since then, there have been concerted efforts to apply responsible approaches to the development of new enhancements and to reform existing, operational enhancements in this light (e.g., Mobrand et al., 2005; Zohar et al., 2008).

The ten principles for developing, evaluating, and managing marine stock enhancement programs set out in Blankenship and Leber (1995) have gained widespread acceptance as the 'Responsible Approach' to stocking (Table 1). The 'Responsible Approach' has been widely cited and has provided a key conceptual framework for several subsequent publications (e.g., Munro and Bell, 1997; Hilborn, 1999; Bell et al., 2005, 2006, 2008; Taylor et al., 2005; Zohar et al., 2008). More importantly, it has been used to guide hatchery development and reform processes in Australia (Taylor et al., 2005; Gardner and Van Putten, 2008; Potter et al., 2008; Taylor and Suthers, 2008), Denmark (Støttrup et al., 2008), Japan (Kitada, 1999; Kuwada et al.,

Table 1 Elements of a responsible approach outlined in Blankenship and Leber (1995)

1. Prioritize and select target species for enhancement by ranking and applying criteria for species selection; once selected, assess reasons for decline of the wild population.
2. Develop a management plan that identifies how stock enhancement fits with the regional plan for managing stocks.
3. Define quantitative measures of success.
4. Use genetic resource management to avoid deleterious genetic effects on wild stocks.
5. Implement a disease and health management plan.
6. Consider ecological, biological, and life-history patterns in forming enhancement objectives and tactics; seek to understand behavioral, biological, and ecological requirements of released and wild fish.
7. Identify released hatchery fish and assess stocking effects on fishery and on wild stock abundance.
8. Use an empirical process for defining optimal release strategies.
9. Identify economic objectives and policy guidelines, and educate stakeholders about the need for a responsible approach and the time frame required to develop a successful enhancement program.
10. Use adaptive management to refine production and stocking plans and to control the effectiveness of stocking.

2000, 2004), New Caledonia (Purcell and Simutoga, 2008), Phillipines (Le Vay et al., 2008), and the USA (e.g., Leber, 2004), particularly in California (Bartley et al., 1995), Florida (Willis et al., 1995; Leber, 2004; Tringali et al., 2008), Georgia (Woodward, 2003), Hawaii (Leber et al., 1996, 1997, 1998; Friedlander and Ziemann, 2003), Maryland (Zohar et al., 2008), Mississippi (Blaylock et al., 2000), New Hampshire (Fairchild et al., 2005), North Carolina (Eggleston et al., 2008), South Carolina (Smith et al., 2003; Jenkins et al., 2004), Texas (Karls-son et al., 2008), and Washington (Mobrand et al., 2005). At the same time, there has been a rapid increase in peer-reviewed literature on effects and effectiveness of stocking.

KEY DEVELOPMENTS SINCE THE RESPONSIBLE APPROACH WAS FIRST FORMULATED

Fisheries science and management in general, and many aspects of fisheries enhancement have developed rapidly since the responsible approach was first formulated. Governance in many fisheries has changed from open access and/or government-regulation to alternative, market- and community-based approaches (Hilborn et al., 2005). This has created stronger and more effective governance, creating conditions that may also be conducive to developing and sustaining enhancements. In some cases, enhancement initiatives have been instrumental in bringing about change in governance with wider benefits (Drummond, 2004; Lorenzen, 2008). At the same time, ecological and social impacts of fisheries and their management have come to the forefront of management decision making and public debate. Management goals have become increasingly multi-dimensional (Hilborn, 2007a). Spatial heterogeneity and dynamics in marine ecosystems and social systems, scientific recognition of smaller-scale connectivity, and movements to set aside marine areas for conservation have given rise to marine spatial planning (Lorenzen et al., 2010).

In parallel, and often in interaction with the aforementioned developments in fisheries, significant changes have occurred in the science and practice of fisheries enhancement. Perhaps the most significant of these has been a drive towards fully integrating enhancements into fisheries management frameworks and decision making. This has progressed furthest in the Japanese and New Zealand scallop enhancements and in US Pacific salmon hatchery programs (Drummond, 2004; Mobrand et al., 2005; Uki, 2006; HSRG, 2009). The shift towards looking at enhancements from a fisheries management perspective is facilitated by the emergence of stock assessment models and tools that allow evaluation of the contribution of enhancements to management goals and tradeoffs with other harvest and habitat management (Walters and Martell, 2004; Lorenzen, 2005; Mobrand, Jones, and Stokes Associates, 2006). A broader view of the role of enhancement in fishery systems has also emerged (Lorenzen, 2008).

Many other areas of enhancement science and practice have seen substantial, incremental development. Domestication pro-

cesses and their management are increasingly well understood (Gross, 1998; Thorpe, 2004; Araki et al., 2008; Frankham, 2008). Many studies have been conducted to evaluate ecological differences between wild and released hatchery fish and their implications for population dynamics (Fleming and Petersson, 2001; Lorenzen, 2005). Rapid methodological and conceptual development has occurred in population genetics. This has shown widespread occurrence of adaptive genetic variation at relatively small spatial scales and fitness effects of hybridization between wild and hatchery fish (Conover et al., 2006; Araki et al., 2008; Fraser, 2008). Methodological advances now also allow marking of fish at any life stage (e.g., Tringali, 2006).

These developments make it necessary to revise the 'responsible approach' to take into account, in particular, the paradigm shift towards analyzing and managing enhancements from a fisheries management perspective. The developments also provide the tools for implementing the shift.

Most enhancements remain weak in four particular areas: (1) fishery stock assessments and modelling are integral to exploring the potential contribution of stocking to fisheries management goals; yet both are found lacking in most stock enhancement efforts in coastal systems; (2) establishing a governance framework for enhancements is largely ignored in stocking programs, thus, diminishing opportunities for integrating enhancement into fishery management; (3) involvement of stakeholders in planning and execution of stocking programs is key from the start, but they are rarely made an integral part of program development; and (4) adaptive management of stocking is not well integrated into enhancement plans, yet is critical to achieving goals, improving efficiencies, and understanding and controlling the effects of stocking on fisheries and on wild stocks. We expand on these points here and emphasize the importance of their inclusion in the responsible approach.

OUTLINE OF AN UPDATED RESPONSIBLE APPROACH TO DEVELOPMENT AND REFORM OF ENHANCEMENTS

We propose an updated Responsible Approach to developing and reforming enhancements, comprising 15 elements (Table 2) arranged in three stages as follows:

- Stage I: Initial appraisal and goal setting
- Stage II: Research and technology development including pilot studies
- Stage III: Operational implementation and adaptive management

Our updated approach is staged in order to ensure that key elements are implemented in the appropriate phases of development or reform processes. In particular, it is important to conduct broad-based and rigorous appraisal of enhancement contributions to fisheries management goals prior to more

Table 2 The updated responsible approach

Stage I: Initial appraisal and goal setting
(1) Understand the role of enhancement within the fishery system.
(2) Engage stakeholders and develop a rigorous and accountable decision making process.
(3) Quantitatively assess contributions of enhancement to fisheries management goals.
(4) Prioritize and select target species and stocks for enhancement.
(5) Assess economic and social benefits and costs of enhancement.
Stage II: Research and technology development including pilot studies
(6) Define enhancement system designs suitable for the fishery and management objectives.
(7) Develop appropriate aquaculture systems and rearing practices.
(8) Use genetic resource management to maximize effectiveness of enhancement and avoid deleterious effects on wild populations.
(9) Use disease and health management.
(10) Ensure that released hatchery fish can be identified.
(11) Use an empirical process for defining optimal release strategies.
Stage III: Operational implementation and adaptive management
(12) Devise effective governance arrangements.
(13) Define a fisheries management plan with clear goals, measures of success, and decision rules.
(14) Assess and manage ecological impacts.
(15) Use adaptive management.

detailed research and technology development and operational implementation. This basic requirement applies to both development of new and/or reform of existing enhancements.

Stage I: Initial Appraisal and Goal Setting

Stage I is focused on a broad-based appraisal of potential enhancement contributions to fisheries management goals and the design of a planning process. Elements of Stage I may be re-iterated in later stages as appropriate.

(1) Understand the role of enhancement within the fishery system

Premise: Enhancements enter into complex fishery systems. It is crucial to consider the fishery system, broad objectives for management, and the full range of management options when assessing potential for, developing, and using enhancements.

Enhancements enter into complex fisheries and their role within them must be considered clearly from the outset. Outcomes of enhancement are determined by interacting, biological, and human dimensions of the fishery system into which they enter and are evaluated against a broad set of criteria. Enhancement 'success stories' often show how positive outcomes are dependent on matching enhancements to fisheries characteristics, transforming governance arrangements, and seeking ways in which enhancement can add value to other management approaches (Pinkerton, 1994; Lorenzen et al., 1998; Drummond, 2004; Uki, 2006; Garaway, 2006; Becker et al., 2008). Con-

versely, many enhancements have failed not or not only for technical reasons, but because they did not address management issues in the fishery (e.g., sometimes enhancements are developed for lightly exploited stocks or stocks of little fisheries interest), or inadvertently caused problematic responses (such as an increase in fishing effort on already overfished stocks).

Therefore, it is of utmost importance to gain a good understanding of the fisheries system at the start of any initiative aimed at developing or reforming fisheries enhancements. The initial fisheries system analysis has the multiple aims of summarizing relevant characteristics of the fishery system, understanding current management outcomes and their causes, and exploring ways in which outcomes may be improved. A broad framework for analyzing enhancement fishery systems is given in Lorenzen (2008). The framework sets out how in operational interactions, situational variables (attributes of the resource, fishing, aquaculture production, habitat and environment, stakeholders, markets and governance arrangements) influence outcomes of enhancement initiatives through physical-biological pathways and through those mediated by stakeholder action (Figure 1). It may also help in understanding how in longer-term, dynamic interactions, situational variables are modified in response to the outcomes of operational interactions. While not a fully specified model, the framework provides an aid for thinking through the logic of enhancement fisheries systems, and exploring options for their development or reform. This is best done in three steps: (1) establishing how situational variables affect current outcomes of the fishery or enhancement; (2) setting explicit goals for development or reform; and (3) assessing how goals might be achieved through modifications in situational variables. Criteria that should be used to evaluate outcomes include biological production, resource conservation, economic benefits and costs, contribution to livelihoods, and sustainability of governance arrangements. The analysis thus involves a preliminary appraisal of virtually all elements of the Responsible Approach. The analysis is best carried out by a multi-disciplinary team and in cooperation with stakeholders. This may require an iterative process, with an initial analysis identifying relevant team members and stakeholders and a subsequent, more in-depth analysis once these have been brought on board.

Setting or clarifying goals of fisheries management in general and of the enhancement initiative in particular is an important aspect of analysis. Only if wider management goals are defined and appreciated is it possible to set specific and appropriate goals for the enhancement. Many fundamental arguments about the role of enhancements concern congruence (or lack thereof) between wider, particularly conservation-oriented goals and more immediate, often fisheries production-centered goals (e.g., Meffe, 1992; Taylor, 1999). Goals at different levels must be made explicit and areas of congruence or conflict identified and addressed constructively in the decision process (Element (2)).

The enhancement fishery system analysis should inform implementation of subsequent steps and other elements of the Responsible Approach, in particular those of Stage I. By the

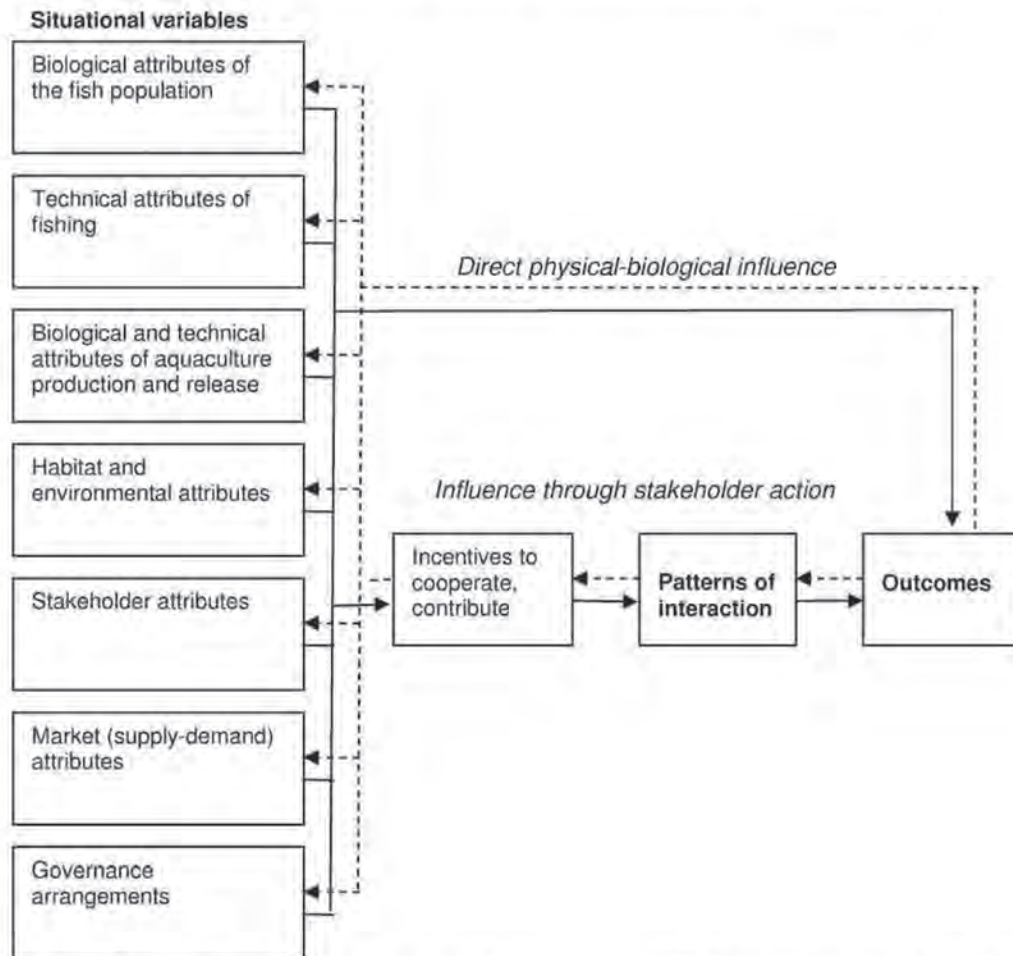


Figure 1 Framework for analyzing enhancement fisheries systems (modified from Lorenzen, 2008). Operational interactions between elements are shown as solid lines and determine outcomes in the short term when the situational variables are fixed. In dynamic interactions, shown as dashed lines, situational variables are modified in response to the outcomes of operational interactions.

end of the analysis, the basic features of the fishery and its management outcomes should be well documented, stakeholders identified, and the ground prepared for establishing a decision making process for development or reform.

(2) *Engage stakeholders and develop a rigorous and accountable decision-making process*

Premise: Constructive engagement with stakeholders through a decision making process that is participatory, structured, and makes good use of science is crucial to the successful development or reform of enhancements.

Many successful enhancements owe much to decision making arrangements that involved both stakeholder participation and rigorous use of science (Lorenzen, 2008). Stakeholder engagement is crucial because it brings stakeholder's intimate knowledge of the fishery system into the decision process, builds trust, and encourages commitment to decision outcomes. Rigor-

ous use of science promotes effectiveness and accountability. At the same time, it must be accepted that real decision processes are always a compromise and that the goal of reaching a balanced decision acceptable to stakeholders may take precedence over adherence to the letter of process.

Good examples of decision processes that have the desired features can be found in particular in the hatchery reform movement in the Pacific Northwest of North America (Blankenship and Daniels, 2004; Mobrand et al., 2005; HSRG, 2009). On a smaller scale and in a developing country context, such a process has been documented by Garaway et al. (2006) and Lorenzen (2008).

Key principles for designing such processes include the following. All relevant and interested stakeholders should be engaged: both primary (those, like fishers and aquaculture producers, whose actions directly impact on the enhancement outcomes) and secondary (those who have a legitimate interest but no direct impact). Usually this will involve, at a minimum, individuals or organizations involved in fisheries, aquaculture,

and conservation, as well as regulatory agencies and scientists. Stakeholder analysis may be used to identify stakeholders and establish the nature and strength of their interests and interactions (Grimble and Chan, 1995). Scientific expertise will be required from multiple disciplines including fisheries science, aquaculture, genetics, ecology, and economics. For larger initiatives, a multi-disciplinary scientific advisory team should be constituted to include broadly experienced 'integrators' as well as disciplinary experts. Appraisal of enhancements involves considerations that 'general' scientific experts may not be familiar with. Hence, at least some of the scientists involved should have specialist knowledge and experience of enhancements. For smaller initiatives, advice may be provided by broadly trained and experienced professionals (sometimes referred to as 'barefoot scientists', Prince, 2010).

There are three core functions in the process: facilitation of the process itself, stakeholder input, and scientific assessment. Organization of these functions and emphasis may vary, from a stakeholder-driven process with access to scientific advice to a science-driven process with stakeholder consultation (Blankenship and Daniels, 2004; Garaway et al., 2006; Tringali et al., 2008). The facilitation role may be taken on by government, a stakeholder group, scientists, or a separate organization, such as a time-limited initiative. Ground rules for engagement in the process should be established jointly and typically will include trust building, respect for divergent views, developing a shared understanding, and collaborative problem solving (Ansell and Gash, 2008).

The process itself should also be agreed upon collaboratively. Typically, it will include a collaborative fisheries systems analysis (cf. Element 1 above), goal setting, and identification of management options including enhancement, fishing regulations, and habitat restoration. This would be followed by quantitative assessment of potential contributions of options to the management goals identified (Element 3), prioritization of species or stocks where relevant (Element 4), and assessment of economic and social benefits and costs (Element 5). Outputs from these analyses provide the basis for collaborative decision making. A structured decision process using some form of tradeoff or decision analysis is important to make assumptions and values explicit, to promote a focus on key issues, and to address potential inequalities in stakeholder influence (e.g., Janssen, 1994). The initiative may develop into a longer-term, iterative process and possibly lead to the establishment of more permanent governance arrangements (see also Element 12).

(3) Quantitatively assess contributions of enhancement to fisheries management goals

Premise: The ultimate aim of enhancements is to contribute to the achievement of fisheries management goals. This is possible only where enhancements are technically effective and outperform or add value to alternative management measures, such as fishing regulations or habitat management. The potential contribution of enhancement to man-

agement goals can and should be assessed early on in the development or reform process.

Quantitative assessment of enhancement contributions to fisheries management goals is important for several reasons (Lorenzen, 2005). First, quantitative benefits, such as increased target population abundance, yield, or economic rent, are often the motivation for enhancements, and thus crucial indicators of success. Secondly, quantitative tradeoffs between enhancement, harvest, and habitat management determine whether enhancements add value to other forms of management. Thirdly, quantitative analysis, even if carried out under conditions of large uncertainty, provides a 'reality check' for often exaggerated expectations by, or promises to, stakeholders. Quantitative assessment early on in the development or reform process is crucial to preventing large investment in, and subsequent maintenance of ill-conceived enhancement programs.

Quantitative assessment should explore the likely outcomes of fishing regulations, releases of cultured fish and, where relevant, habitat restoration on fisheries catches and the population abundance and structure of cultured, wild, and hybrid (cultured-wild) fish. Then assessment should include quantification of uncertainties in the form of a risk assessment. Due to recent progress in the development of population dynamics models and assessment methods for enhancements, such assessments can now be carried out (Walters and Martell, 2004; Lorenzen, 2005; Sharma et al., 2005). An assessment tool based on a general population model for enhancements (Lorenzen, 2005) is now available in the freeware package *EnhanceFish* (Medley and Lorenzen, 2006). There are also a number of more fishery-specific models, such as the AHA model now used to assess many Pacific salmon hatchery operations in USA (Möbrand, Jones, and Stokes Associates, 2006). Such models provide powerful and general tools for evaluation of enhancement programs, from early planning to full-scale operation. Comparative empirical studies and meta-analyses now provide prior information on virtually all required parameters, so that it is possible to conduct exploratory analyses even when there are virtually no stock specific data (see Lorenzen, 2005, 2006 for references). Where available, model parameters may be estimated from quantitative assessments of the wild stock and from release experiments with marked hatchery fish. The most comprehensive applications of quantitative assessment in improving enhancement programs can be found in Hatchery Reform processes (Möbrand et al., 2005; HSRG, 2009). Examples of prognostic evaluations include Lorenzen (2005), Loneragan et al. (2006), and Rogers et al. (2010).

(4) Prioritize and select target species and stocks for enhancement

Premise: When multiple species or stocks are being targeted for hatchery releases, criteria need to be developed to remove bias from the selection process so that species

and stocks can be prioritized based on an array of decisive factors.

Sometimes enhancements are being considered for a particular stock only, but often, initiatives have a broader remit and involve choice of species or stocks. Species and stocks considered for enhancement should be subjected to a suitability analysis that can help filter poor candidates from good ones. Just as stakeholder demand, alone, for more fish is not adequate justification for conducting enhancements, model prediction that enhancement would be useful in restoring a fishery, albeit the most significant, is only one of several primary factors that need to be considered before implementing hatchery production for releases.

Selection should be based on a process that scores species on enhancement potential, based on criteria, such as stock assessments and fishery management needs; preliminary enhancement modeling results; extent of habitat and recruitment limitations; likely impact on resident biota; aquaculture capability, or potential, for mass production of juveniles; cost-benefit considerations; life-history and dispersal patterns, etc. Unless attention is focused on the full spectrum of criteria used to prioritize species and stocks, consideration of an immediate need by an advocacy group and simply the availability of aquaculture technology have throughout much of the history of enhancements become the driving factors in species selection.

To reduce the bias inherent in selecting species, a semi-quantitative approach was developed in Hawaii to identify selection criteria and prioritize species for stock enhancement research (Leber, 1994). This approach involved four phases: (1) an initial workshop, where selection criteria were defined and ranked in order of importance; (2) a community survey, which was used to solicit opinions on the selection criteria and generate a list of possible species for stock enhancement research; (3) interviews with local experts to rank each candidate species with regard to each selection criterion; and (4) a second workshop, in which the results of the quantitative species selection process were discussed and a consensus was sought. This decision-making process focused discussions, stimulated questions, and quantified participants' responses. Panelists' strong endorsement of the ranking results and selection process used in Hawaii demonstrate the potential for applying formal decision-making to species selection in other regions.

(5) *Assess economic and social benefits and costs of enhancement*

Premise: Economic and social benefits and costs of enhancement and of alternatives should be assessed at all stages of program development.

Consideration of economic and social benefits and costs is critical to decision making on whether enhancement initiatives should proceed or continue, and how they should be operated. The first step should be a bio-economic analysis of the fishery,

considering situations with and without enhancement (Arnason, 2001; Whitmarsh, 2001; Lorenzen, 2005). This analysis can build on the quantitative biological assessment (Element 3) and is fairly straightforward in commercial fisheries where market values for inputs and outputs are readily determined. Both, equilibrium analyses and non-equilibrium analyses considering the discounted flow of costs and benefits when enhancements are started up or modified should be conducted (see e.g., Lorenzen, 2005). The *EnhanceFish* package includes such bio-economic modelling capabilities (Medley and Lorenzen, 2006). In principle, economic analyses should account for externalities and non-market costs and benefits but in practice these are often omitted at least initially (Whitmarsh, 2001). We strongly recommend conducting at least a basic bio-economic analysis to assess whether an enhancement initiative is at all likely to be economically beneficial, given results from the quantitative biological assessment and approximate monetary values.

Recreational fisheries produce an unpriced product (the recreation experience), which can be valued by contingent valuation. Abundance of catchable fish is only one of many factors that affect the demand for, and value of, the recreational experience. Hence, the relationship between fish abundance and recreational demand may be weak and it should not be assumed that an increase in fish abundance due to stocking will create a proportionate increase in demand or value (Loomis and Fix, 1998).

Wider social benefits and costs of enhancements may be analyzed using the sustainable livelihoods framework (Allison and Ellis, 2001; Smith et al., 2005). This framework is particularly useful where livelihoods involve large elements of subsistence activities or non-market exchanges, for example in coastal areas of the developing world. Enhancement initiatives can bring about far-reaching changes in key assets, such as human capital (new knowledge and skills that may also be transferred to other activities), financial capital (individual, corporate or group income), and social capital (new opportunities to engage in networks and exchanges) (Garaway, 2006). The distribution of enhancement costs and benefits is sometimes inequitable among stakeholders, potentially leading to conflict. This may be the case, for example, where access arrangements to resources change (Garaway, 2006; Garaway et al., 2006). The social distribution of benefits and potential for conflict should also be considered and assessed in detail where concerns emerge. Finally, impacts of enhancements on wider ecosystem services may be considered (see Holmlund and Hammer (2004) for a very broad assessment framework and case study).

Stage II: Research and Technology Development Including Pilot Studies

Stage II is focused on elements of research and technology development that can be conducted at experimental or pilot scale, prior to or in parallel with operational-scale enhancements.

- (6) Define enhancement system designs suitable for the fishery and management objectives

Premise: Enhancements may be developed in a variety of fisheries situations with a view to achieving different goals. Different situations and goals give rise to very different biological-technical enhancement system designs (combinations of hatchery practices, release, and fishing regimes). Objectives and potential system designs should be clarified early on in the development process because different designs may require very different technologies and governance arrangements.

Enhancement approaches can be used in different situations and for different purposes, which in turn lead to very different design criteria for the biological-technical components of enhancement systems: aquaculture production, release strategies, fishing practices, and auxiliary (e.g., habitat) manipulations (Cowx, 1994; Utter and Epifanio, 2002; Bell et al., 2006; Lorenzen, 2008). Designs for the alternative systems are at least partially incompatible; hence, it is important to clarify the situation and goals of the enhancement program, and decide on appropriate system design criteria before embarking on detailed technology development for components. The most fundamentally important question that must be answered in system design is whether the purpose of the program is primarily production or conservation-oriented.

Five main types of marine fisheries enhancement systems may be distinguished, in a sequence ranging from the most production-oriented to the most conservation-oriented type: sea ranching, stock enhancement, restocking, supplementation, and re-introduction (Utter and Epifanio, 2002; Lorenzen, 2008). Outline design criteria for the different system types are given in Table 3.

Sea Ranching

Ranching systems operate for species that do not recruit naturally or for which natural recruitment is considered unimportant. Ranching systems are stocked and harvested to maximize somatic production (commercial fisheries) or the abundance of catchable-sized fish (recreational fisheries), often manipulating populations in ways that could not be achieved in naturally recruiting populations (Lorenzen, 1995). Because direct genetic interactions with wild stocks are absent, post-release fitness of cultured fish is primarily an economic rather than a conservation issue. Selective breeding may be used to improve performance (Jonasson et al., 1997). Sterile fish may be used where reproduction in the natural ecosystem is possible but undesirable.

Stock Enhancement

Stock enhancement involves the continued release of hatchery fish into a self-recruiting wild population, with the aim of

sustaining and improving fisheries in the face of intensive exploitation and/or habitat degradation. Stock enhancements can increase overall abundance of catchable fish and fisheries yield, while allowing for higher exploitation rates than could be sustained by the natural stock alone (Lorenzen, 2005). Aquaculture practices and genetic management are focused on maintaining wild population characteristics in cultured fish. Stocking and harvesting rates are strongly constrained by stock conservation considerations where stocked and wild population components interact ecologically and genetically and are harvested jointly (integrated enhancement programs). Impacts on the wild population component can be reduced by separating the cultured/stocked and wild population components as far as possible (Utter, 2004; Lorenzen, 2005). Where both components can be fully separated, management considerations for the enhanced fishery are similar to those of sea ranching. However, full separation is difficult to achieve in practice and, in general, wild stock conservation will remain an important consideration in enhancement programs (Lorenzen, 2005).

Re-Stocking

Re-stocking involves time-limited releases of hatchery fish, aimed at rebuilding depleted populations more quickly than would be achieved by natural recovery. In re-stocking, the release number must be substantial relative to the abundance of the remaining wild stock if rebuilding is to be significantly accelerated. Fishing intensity should be low in order to maximize the contribution of wild and released cultured fish to population growth (Lorenzen, 2005). Re-stocking calls for close ecological and genetic integration of wild and cultured stocks, combined with very restricted harvesting. Genetic management is clearly focused on maintaining the characteristics of the wild population.

Supplementation

Supplementation is defined here as the release of cultured fish into very small and declining populations, with the aim of reducing extinction risk and conserving genetic diversity (Hedrick et al., 2000; Hildebrand, 2002). Supplementation serves primarily conservation aims, and specifically addresses threat processes in small and declining populations: demographic stochasticity, loss of genetic diversity, and Allee effects (Caughley, 1994). Supplementation typically involves only moderate releases in order not to depress the wild population component further and stringent restrictions on harvesting. Genetic management is clearly focused on maintaining the structure and adaptations of the wild stock, with particular attention being paid to maximizing effective population size in the hatchery through full factorial or minimum kinship mating designs.

Table 3 Design criteria for biological-technical components of enhancement systems serving different objectives (modified and expanded from Lorenzen, 2008)

	Sea ranching	Stock enhancement	Re-stocking	Supplementation	Re-introduction
Aim of enhancement	Increase fisheries catch	Increase fisheries catch while conserving or increasing naturally recruiting stock	Rebuild depleted wild stock to higher abundance	Reduce extinction risk and conserve genetic diversity in small populations	Re-establish populations in historical range
Wild population status	Absent or insignificant	Numerically large Possibly depleted relative to carrying capacity	Numerically large or small Depleted relative to carrying capacity	Numerically small Possibly declining At risk of extinction	Locally extinct
Aquaculture management	Production-oriented Partial domestication Conditioning for release Possibly induced sterility	Integrated programs: as for re-stocking Separated programs: as for sea ranching	Conservation-oriented Minimize domestication Conditioning for release	Conservation-oriented Minimize domestication Conditioning for release	Conservation-oriented Minimize domestication Conditioning for release
Genetic management	Maintain genetic diversity Selection for high return	Integrated programs: as for re-stocking Separated programs: as for sea ranching; also selection to promote separation	Preserve all wild population genetic characteristics	Preserve all wild population genetic characteristics, Maximize effective population size in hatchery	Assemble diversity of adaptations or use stocks adapted to similar habitats
Population management	Stocking and harvesting to create desired population structure	Integrated programs: Restricted stocking and harvesting to increase catch while conserving naturally recruiting stock Separated programs: as for sea ranching; also measures to promote separation	High stocking density over short period; temporarily restricted harvesting or moratorium	Moderate stocking density relative to wild population, no or very restricted harvesting	Low stocking density but sufficient for establishment, minimal harvesting

Re-Introduction

Re-introduction and translocation involve temporary releases of cultured or captured fish with the aim of re-establishing a locally extinct population (Reisenbichler et al., 2003). A founding population may be assembled from multiple locations to maximize genetic diversity and potential for rapid adaptation. Continued releases should be avoided so as not to interfere with evolutionary processes in the newly established population. Fishing, likewise, should be restricted to allow rapid build-up of the population.

(7) *Develop appropriate aquaculture systems and rearing practices*

Premise: The design of aquaculture systems and rearing practices influences production efficiency and the fitness of released fish. Typically there is a tradeoff between these aspects that must be considered in system design and rearing protocols.

Aquaculture systems and rearing practices greatly influence the success of any enhancement through their impacts on both, production efficiency in the culture system and stocking effectiveness in the natural system. Considerable efforts may be required just to develop the basics of culture systems and rearing practices suitable for maintaining new species and closing their life cycle. Once these basics are known, attention must be paid to culture efficiency, post-release performance, and tradeoffs between the two. Culture systems that are efficient at producing juveniles also tend to subject fish to an inadvertent or intentional process of domestication, promoting traits that are associated with low post-release fitness (Fleming and Petersson, 2001; Thorpe, 2004; Araki et al., 2008). The optimal balance between culture efficiency and post-release fitness may vary between enhancement system designs (Element 6): efficient mass-production of moderately fit juveniles may well be economically optimal in marine ranching, while fitness is at a premium in conservation-oriented programs.

Domestication involves plastic developmental responses to the culture environment and an altered selection regime and has strong, almost always negative impacts on the capacity of fish to survive, grow, and reproduce in the wild (Olla et al., 1998; Fleming and Petersson, 2001). While it is virtually impossible to completely avert responses to captivity without also losing the advantage of culture in terms of survival and reproductive success, management approaches have been developed to produce wild-like types that maintain or re-establish certain characteristics of the wild types.

The production of wild-like types in culture requires attention to both the sampling of fish for the founder population and its subsequent management in captivity. Founders should be representative of the wild population, and encompass sufficient diversity of genotypes and life history phenotypes to allow re-establishment of viable populations in the wild (Miller and Kapuscinski, 2003). Once the captive population is established, both genetic and environmental management are important to

promote maintenance of wild characteristics. The holistic solution of providing a near-natural environment for fish to live in and possibly reproduce can maintain natural selection and developmental cues. However, this approach is often impractical and, in addition, may negate the survival advantage of culture, which after all is the rationale for instituting enhancement programs in the first place. Far more interventionist genetic resource management and developmental manipulations are usually required.

Developmental manipulations to promote wild traits are important to raise performance after release, and some such manipulations may also reduce selection for culture traits. Typical manipulations include physical environmental features (e.g., temperature, water currents), nutrition, and feeding practices (Tanaka et al., 1998). Environmental enrichment (Berejikian et al. 1999), life skills training (Brown and Laland, 2001) and soft release strategies (Brown and Day, 2002) can successfully promote behavioral skills that may increase survival of released fish. The fact that cultured fish respond readily to habitat enrichment and life skills training by displaying "wild" behavioral patterns (Brown and Laland, 2001) attests to the maintenance of their enormous developmental plasticity. Exposure to variable spatial and foraging cues in the hatchery environment provides fish with enhanced behavioral traits that may be associated with improved survival in the wild (Braithwaite and Salvanes, 2005). While many such manipulations have been shown to promote wild-like traits in laboratory tests, their effectiveness at achieving the ultimate goal, increased lifetime fitness in the wild, has not been widely tested. Results so far have not shown very large effects on long-term survival (e.g., Fuss and Byrne, 2002). Evaluation of impacts of culture practices on survival in the wild (rather than on proxy indicators) should receive a high priority in technology development, despite the associated costs and timescales.

(8) *Use genetic resource management to maximize effectiveness of enhancement and avoid deleterious effects on wild populations*

Premise: Genetic resource management is important to both enhancement effectiveness and conservation of wild population genetic structure, fitness, and evolutionary potential. Attention to genetic resource management is required both in the hatchery operation and in managing the mixed wild/hatchery stock.

Genetic attributes affect fitness and evolutionary potential of stocked and wild fish. There are three areas of direct genetic impacts to consider: (1) potential disruption of neutral and adaptive spatial population structure; (2) effects of hatchery spawning and rearing on genetic diversity and fitness of stocked fish; and (3) genetic consequences for wild stocks of interactions with released hatchery fish. In addition, there may be indirect genetic effects of enhancement on wild stocks (Utter and Epifanio, 2002). Genetic issues and management approaches vary considerably between enhancement system designs (see Element 6).

Wild fish populations show spatial structure in selectively neutral markers where isolation has been sufficiently strong and long-term, and adaptive genetic variation that may be maintained by natural selection even at more moderate levels of isolation (Utter, 2004; Conover et al., 2006). Where no specific studies have been conducted, the default assumption should be that local adaption exists at scales of tens of kilometers in marine systems, and possibly at smaller scales in estuarine and freshwater systems (Reisenbichler, 1988; Palumbi, 2004; Conover et al., 2006). Hatchery practices should reflect and maintain this structure by using brood stock of local origin where possible. Not doing so is likely to carry substantial penalties in terms of post-release fitness, with implications for both enhancement effectiveness and risks to the wild population (Reisenbichler, 1988; Araki et al., 2008). In the case of re-introduction where the local population has been lost, it may be best to assemble a founder population from diverse locations and let natural selection take its course.

Hatchery populations often experience loss of genetic diversity (heterozygosity, allelic diversity) due to low effective population size and consequent genetic drift and inbreeding, though this can be averted relatively easily through appropriate brood stock management (Verspoor, 1988; Kincaid, 1995; Norris et al., 1999). Certain breeding schemes, such as full factorial or minimum kinship designs, allow maintaining a very high effective population size relative to census size in hatchery populations (Miller and Kapuscinski 2003; Fraser, 2008).

Hatchery populations also tend to show rapid loss of fitness in the wild due to genetic adaptation to the hatchery environment, which may be further exacerbated by artificial selection (Berejikian and Ford, 2004; Araki et al., 2008). Loss of fitness is more difficult to avert than loss of diversity. Measures aimed at minimizing fitness loss include rearing in near-natural environments, minimizing time in captivity, partially replenishing brood stock with wild fish in regular intervals, equalizing family size, or fragmentation of brood stock to reduce potential for adaptation (Araki et al., 2008; Frankham, 2008).

Genetic mixing of released hatchery with wild fish can have consequences for diversity and fitness of wild stocks. Consequences depend on the genetic characteristics of both stock segments and their admixture proportions and are managed by implementing sound genetic management of hatchery stocks (see above) and controlling admixture proportions through stocking and fishing practices. Provided that care is taken to maintain spatial genetic variation, the main risks to genetic diversity arise when wild populations of large effective population size are 'swamped' by hatchery fish derived from comparatively small numbers of breeders (Ryman and Laikre, 1991; Duchesne and Bernatchez, 2002). This situation can arise relatively easily in stock enhancement and restocking programs because high fecundity of fish combined with high survival of early life stages in culture makes it possible to produce very large numbers of offspring from very few parents. Where the effective population size of hatchery fish is much lower than that of wild fish, the admixture proportion of hatchery fish needs to be limited by

restricting the magnitude of releases or selective harvesting of hatchery fish. Relatively high admixture proportions, however, may be acceptable in time-limited releases, such as those carried out for restocking (Duchesne and Bernatchez, 2002). Of course, the reverse effect (the hatchery population having a higher effective population size than the wild population) can occur, and is desired in supplementation programs (Hedrick et al., 2000).

Reduced fitness of cultured fish can depress the productivity of mixed, naturally recruiting populations while at the same time, reducing the risk of displacement of wild by cultured fish compared to a situation where both components are of equal fitness. The effect of reduced fitness on the productivity of a mixed wild and cultured population, however, is greatest at intermediate levels of maladaptation: well adapted cultured populations have a greater impact on wild fish but do not affect mixed population productivity, while poorly adapted cultured populations contribute little to the mixed population and have little impact on its wild component (Lorenzen, 2005). The magnitude of impacts on wild population abundance and productivity of course also depends on the relative abundance of the populations: even poorly adapted fish can have large impacts if released in great numbers and over long periods of time (Ford, 2002; McGinnity et al., 2003). Empirical studies have found evidence for reduced productivity in some mixed wild-cultured salmonid populations, but not in others (Chilcote, 2003; Sharma et al., 2006; Araki et al., 2007a, 2007b).

Where direct genetic interactions between stocked hatchery and wild fish are absent, i.e., in sea ranching or in stock enhancement programs where both populations components are separated, selective breeding may be used to improve the post-release performance of hatchery fish (Jonasson, 1997). Selection may also be used to promote separation of the population components, for example by selecting for differences in spawning seasonality (Mackey et al., 2001).

Several guidelines and policies have been developed for genetic management of enhancements, including Miller and Kapuscinski (2003) and Tringali et al. (2007). When setting up a genetic management plan, it is crucially important to consider the particular situation, management goals and system design of the enhancement in question.

(9) Use disease and health management

Premise: Releases of hatchery fish can transfer disease or parasites from the hatchery stock to wild stocks and may also affect epidemiological dynamics through addition of susceptible hosts. Thus a health management process is required that includes at minimum health screening of fish prior to release, but may require further measures where enhancements are carried out on a large enough scale to affect host dynamics significantly.

Disease transfer is a major risk associated with fisheries enhancement programs. Impacts of enhancements on disease status of wild or mixed stocks may occur due to two mechanisms:

(1) introductions of alien pathogens, and (2) changes in host population density and structure (in terms of age, size, and immune status) that affect the dynamics of established pathogens. The most dramatic disease impacts of cultured fish so far documented have been caused by introductions of alien pathogens (Johnsen and Jensen, 1991; Wagner, 2002). Such introductions may result from movements of cultured stocks even within their natural range, because the ranges of hosts and parasites are not necessarily identical (Johnsen and Jensen, 1991). Cultured fish released into the wild can increase the reservoir of susceptible hosts substantially. Because transmission of infections is usually density-dependent, most pathogens can only sustain populations when hosts occur above a certain density, and prevalence may rise with increasing density (Anderson, 1981). Release of cultured fish could, therefore, foster establishment of pathogens where they can not be supported by natural populations, or increases in the prevalence or intensity of infection.

Disease and health management concerns need to be considered from the inception of building a hatchery to the time fish are released into the natural environment. Bartley et al. (2006) describe a comprehensive disease and health risk analysis that encompasses a risk identification, assessment, and management framework to allow wise decisions for release of hatchery-reared juveniles, including species selection, hatchery site selection, hatchery protocols, culture conditions, and monitoring and surveillance. Controlling infectious diseases in culture is crucial to minimizing disease interactions with wild fish, but not always sufficient (Bartley et al., 2006). Changes in disease ecology brought about by releases of cultured fish need to be considered where releases are numerically large. Vaccination of fish for diseases where this option is available has the dual benefit of controlling disease in culture and mitigating against the introduction of a large number of susceptible hosts into natural environments through culture in open systems or release.

Several US states and organizations (e.g., California Department of Fish and Game, Florida Fish and Wildlife Conservation Commission) have developed aggressive and responsible approaches in association with their enhancement projects. Their policies require that all groups of fish cultured for stocking pass a certified inspection for specific bacterial and viral infections and parasites prior to release. The certification must be made by a certified aquatic veterinarian. Maximum acceptable levels of parasites, etc., in the hatchery populations are established based on the results of screening healthy wild populations.

(10) *Ensure that released hatchery fish can be identified*

Premise: Virtually all aspects of enhancement research and management require an ability to identify released hatchery fish. Various traditional and innovative tagging methods are available.

One of the most critical components of any enhancement effort is the ability to quantify success or failure. Without some form of assessment, one has no idea to what degree the enhance-

ment was effective or, more critically, which approaches were totally successful, partially successful, or a downright failure (Blankenship and Leber, 1995). Natural fluctuations in marine stock abundance can mask successes and failures. Maximization of benefits cannot be realized without the proper monitoring and evaluation system. Thus, it is crucial to use a reliable marking technique to identify released hatchery fish and distinguish them from wild fish.

Without an unbiased tag or mark, quantitative assessment of release impact is impossible. All, or a high and known proportion of fish released from hatcheries should be marked in order to allow assessment of hatchery fish performance and contribution to population abundance and catch. In order to assess interactions with wild conspecifics, it is also recommended to mark a sufficient number of wild juveniles in the same sizes/locations as hatchery fish are being released (e.g., Leber et al., 1995). Marking wild fish is critical to determine whether wild survival to recruitment is depressed as hatchery production increases (Walters and Martell, 2004; Brennan et al., 2008).

Today's fishery scientists have tagging tools in their arsenal that now enable research not even feasible in 1995. Tagging systems innovations have resulted in technologies that are smaller, 'smarter', more automated, more reliable, and longer-lasting than ever before. Revolutionary advances in fish tagging and marking technology have been made. With each new innovation, seemingly another monitoring breakthrough or logistical constraint in release-recapture studies is solved.

Most enhancements release small juveniles or fry, which are difficult to mark with many conventional tags. Tagging or marking systems that are benign and satisfy the basic assumption that identified fish are representative of untagged counterparts are essential. Although electronic tags and external tags can be used to mark larger individuals, there remains a limited number of marking systems for small fingerlings and fry. Three marking systems that have proven to be most effective for tracking the small fishes released by hatcheries (typically < 100 mm) are the coded-wire tag (high information content; see applications by Hager and Noble, 1976; Leber et al., 1998; Johnson et al., 2008), genetic fingerprinting (micro-satellite DNA, intermediate information content; e.g., Tringali, 2006; Tringali et al., 2008), and otolith marks (low information content; e.g., Tsukamoto et al., 1989). Although electronic tags are highly desirable for several reasons (remote sensing, high information content, easy information recovery, some last the life of the fish), they have not yet been reduced enough in size for use with small fish.

(11) *Use an empirical process for defining optimal release strategies*

Premise: Enhancement cannot be conducted effectively without pilot release experiments to identify optimal release strategies. The effects that release tactics have on hatchery organisms and on hatchery and wild stock interactions must be taken into account. Interactions of release tactics strongly influence survival of hatchery organisms

and can be identified with pilot releases and accounted for in designing viable release strategies.

Survival of hatchery fish in the wild is highly dependent upon release strategies (Hager and Noble, 1976; Bilton et al., 1982; Tsukamoto et al., 1989; Svåsand et al., 1990). Choices made about release parameters can have dramatic effects on post-release survival—to such a degree that they can account for total mortality of released fish if the wrong choices are made (e.g., Leber and Arce, 1996). Pilot release experiments afford a means of quantifying and controlling the effects of release tactics and their influence on the performance of cultured fish in coastal environments (Blankenship and Leber, 1995; Munro and Bell, 1997; Leber, 1999, 2002).

Key release parameters that affect survival of hatchery fish released into the wild include fish size-at-release (e.g., Hager and Noble, 1976; Tsukamoto et al., 1989; Svåsand and Kristiansen, 1990b; Leber et al., 1996, 1997, 1998, 2005; Lorenzen, 2000), release season (e.g., Bilton et al., 1982; Leber et al., 1997, 1998; Hines et al., 2008), release habitat and microhabitat (e.g., Leber and Arce, 1996; Leber et al., 1998; Gardner and Van Putten, 2008; Hines et al., 2008), release magnitude (Brennan et al., 2008; Hines et al., 2008), transport and release associated stress (Sulikowski et al., 2005; Fairchild et al., 2009), and acclimatization at release sites (Brennan et al., 2006; Fairchild et al., 2008; Hervas et al., in press). These and similar experimental studies have shown that choices made about release parameters can result in significantly different mortality rates of released hatchery fish. In most cases, the greater mortality rates associated with 'poor' choices relative to 'good' ones was evident shortly after release. In many cases, differences in mortality rates were associated with interactive effects of release tactics (for example, differential effects of size-at-release varied with release season or with release habitat, and sometimes with both (e.g., Leber et al., 1998; Tringali et al., 2008). Some differences were counter intuitive and habitat specific, precluding generalities about, say, size-at-release effects upon survival. Confronting models with data about the short term effects of release tactics based on empirical field comparisons is important for developing successful enhancement release strategies and improving predictive value of the models. Clearly, release strategies cannot be developed successfully without understanding the interactive effects of release tactics.

Density dependent mortality should be a key consideration in enhancements (Hilborn, 1999; Levin et al., 2001; Lorenzen, 2005), but accounting for it in choices about release strategies can be difficult and expensive. Understanding interactions among release magnitude, size at release, release habitat, and the timing of releases can be accomplished via pilot release experiments designed to evaluate release-tactic interactions and surplus productive potential in specific habitats. Such data are then valuable for choosing release tactics that help avoid competitive displacement of hatchery or wild fish (Brennan et al., 2008; Berejikian et al., 2008). Effects of density dependence can be explored in enhancement models (Lorenzen, 2005; Medley

and Lorenzen, 2006), and then coupled with field experimentation to optimize release strategy. Brennan et al. (2008), in attempting to double juvenile recruitment in nursery habitats of common snook, showed competitive displacement could be avoided in some habitats, but resulted in loss of a significant portion of hatchery fish at another. Berejikian et al. (2008) showed steelhead releases could increase salmon spawning nests (redds) without interfering with wild redds by controlling size at release and seasonal timing of releases.

Our viewpoint is that quantitative field experiments to develop optimal release strategies should always be conducted in pilot-scale releases prior to launching large enhancement programs (Leber, 1999, 2002). Empirical field experiments are a critical intermediate step in identifying enhancement capabilities and limitations and in determining release strategy. They also provide empirical data needed to plan enhancement objectives, test model assumptions about survival and cost-effectiveness, and refine enhancement models.

Stage III: Operational Implementation and Adaptive Management

Elements in Stage III are those that require attention when an enhancement is implemented at fully operational scale.

(12) Devise effective governance arrangements

Premise: Effective governance arrangements are essential for sustaining operational enhancements and minimizing any adverse impacts. Governance arrangements should facilitate effective coordination of the enhancement fishery system and operation of its components. Development or reform of enhancements often requires changes in governance, from small adjustments to radical transformations.

Governance failures are at the heart of many fisheries problems (Hilborn, 2007b). This is perhaps even more so in fisheries enhancements, which entail investments in the resource that can be sustained only under good governance, as well as technical interventions that can cause substantial damage if used inappropriately. Governance failures are evident where beneficial enhancements are not sustained or ineffective or damaging enhancements are not reformed or discontinued.

Effective governance requires a good understanding of stakeholder interests and behavior and of the institutional arrangements that are in place (or could be put into place) to govern stakeholder action. Stakeholder attributes including interests and behavior should already be well understood from Elements (1) and (2) in Stage I. The most critical institutions to understand and manage usually are those that relate to fishing (including access and ownership issues); but those governing aquaculture production, release, and environmental impacts can also have important implications (Pickering, 1999; Hilborn et al., 2005). Institutional arrangements should also, critically, provide a means for coordinating the different parts of the enhancement

fisheries system such that each part operates in a way that contributes to a positive net outcome (Lorenzen, 2008). Governance arrangements can be structured into three levels: (1) governmental, (2) collective choice rules which determine how operational rules can be made by stakeholders, and (3) operational rules.

Initial appraisal (Stage I) and technology development (Stage II) in the development or reform of enhancements can usually be conducted within existing governance arrangements, with additional but flexible and temporary consultation processes. Starting new or reforming existing enhancements at operational scale often requires changes in governance. The extent to which governance arrangements pertaining to enhancements can be changed, of course, depends on the wider governance framework. In some jurisdictions, major changes can be made quite easily while in others, even minor adjustments require major efforts.

Governance systems for fisheries typically fall into one of four broad categories: open access, government regulation, community management, or private use rights. Open access typically results in dissipation of economic rent and resource degradation and provides little incentive for enhancement. Enhancements are particularly vulnerable to unsustainable patterns of behavior because they require investment into the resource. Government regulation through total harvest limits and input controls can solve these problems in principle but evidence suggests that community-based or individual use rights combined with some government oversight perform best because they give fishers a stake in management and provide incentives for sustainable behavior. Community-based systems can perform well where boundaries of the resource and those who can use it are clearly defined, fishers are involved in designing rules and in monitoring, and low-cost mechanisms exist for setting sanctions and resolving disputes—attributes most commonly found in local, small-scale fisheries (Ostrom, 1990, 2008). Individual rights-based systems can perform well in situations less suited for community-based approaches, including large-scale commercial fisheries. There are good examples of sustainable, self-governing enhancement fisheries involving both community-based approaches (Pinkerton, 1994; Garaway, 2006) and private use rights (Drummond, 2004).

Rules and regulations regarding aquaculture production may be extensive and cover *inter alia* facility design and operation, stock management and movement, disease control, and welfare. In some cases there are specific rules for enhancements, such as the genetics and health policies implemented in Florida (Tringali et al., 2007, 2008). Often, however, rules have been designed primarily with aquaculture in mind and may conflict with practices that are deemed desirable in enhancements. For example, the practice of replenishing captive broodstock with wild fish on a regular basis to minimize domestication effects may conflict with biosecurity protocols aimed at establishing disease-free broodstock. Rule compliance with respect to aquaculture production and release is variable and, as in the case of the fisheries component, is usually best where stakeholders have been involved in making the rules.

Arrangements for coordination among the various components or enhancement fisheries systems are often inadequate. Such arrangements must integrate multiple organizations, rules and regulations; a feat that may be best achieved through polycentric or network governance (Gibbs, 2008; Lorenzen et al., 2010).

Implementation of enhancements can be greatly facilitated by good governance arrangements that are increasingly adopted in capture fisheries and may also, in turn, facilitate the emergence of such arrangements (Anderson, 2002; Hilborn et al., 2005; Lorenzen, 2008; Lorenzen et al., 2010). Availability of enhancement technologies and investment in the resource can provide the impetus for governance change. The transformed institutional arrangements can be far more effective at regulating resource use than those previously in place. This can be a major, if somewhat indirect, benefit from enhancements (Garaway et al., 2006; Drummond, 2004; Tomiyama et al., 2008). Enhancements also expand the tactical management tool box and provide opportunities for trading off different management interventions. For example, in spatially zoned management systems, enhancement in one zone may be traded against closing the fishery in another etc. (Lorenzen et al., 2010).

(13) *Define a fisheries management plan with clear goals, measures of success and decision rules*

Premise: To increase the likelihood of success and avoid long-term maintenance of enhancements that are unsuccessful, management plans should be devised for enhanced stocks with clear goals, measures of success and decision rules. Stock management goals should reflect wider fisheries management and conservation goals and be associated with specific measures of success (measurable indicators and reference points). Decision rules should set out what actions are to be taken in the light of realized measures of success.

Operational enhancements require the coordinated management of stocking and fishing operations in order to achieve management goals for the enhanced stock. Management plans with clear goals, measures of success, and decision rules are crucially important to the development of successful enhancements where potential exists and (just as important), to ensuring that ineffective or damaging enhancements are phased out. Unfortunately, many enhancement initiatives are marred by unclear or inappropriate goals, lack of evaluation and lack of decision making.

Goals defined for the enhanced stock should reflect wider fisheries management and conservation goals. Goals should be outcome- rather than input-oriented (specifying, for example, a net increase in fisheries catches rather than a number of fish released). Because enhancements tend to involve partial replacement of wild with stocked fish, it is important to specify explicit abundance or catch goals for both segments rather than a relative contribution goal (which could be met without any net increase

in abundance or catch if replacement is complete. For each explicit goal, measures of success should be defined. These will involve specific indicators and ways of measuring them as well as reference points. Reference points are values of the indicator that management should achieve (target reference points) or not exceed (limit reference points). Decision rules specify what action should be taken if a target reference point is not achieved or a limit reference point exceeded.

Management goals in most fisheries are multi-dimensional and this should be reflected in the definition of measures of success and decision rules. A set of measures of success might include, for example: 'fisheries catches increased by 20%; any concomitant reduction in the wild population component not to exceed 10%, no reduction in wild population genetic diversity measures, costs per recaptured fish not exceeding fisher's willingness to pay, no persistent conflicts generated between fishing stakeholders'. Corresponding indicators would include catch data, abundance estimates, measures of genetic diversity, estimates of enhancement costs and fisher's willingness to pay, and monitoring of conflicts. The required information would be obtained by a combination of fisheries monitoring, research surveys and possibly stakeholder consultation (about conflicts). Decision rules might specify for example increases in release numbers if catch increases are below 20% but all other criteria are met, reduction in release numbers if the wild population component is reduced by more than 10% or impacts on genetic diversity are apparent, or phasing out the enhancement if costs exceed willingness to pay. Setting appropriate and realistic reference points will always require consultation and judgement, but may be helped greatly by quantitative assessment and modeling. There are well-established reference points for capture fisheries, but enhanced fisheries involve additional and different considerations particularly with respect to cultured-wild fish interactions. The proportionate natural influence (PNI) factor defined in the hatchery reform process is an example of a reference point that has emerged to address such issues (Moberg et al., 2005; HSRG, 2009). Management strategy evaluation—modeling of the full fisheries management system including assessment and decision making—may be used to test the performance of alternative indicators and reference points, as is commonly done in capture fisheries (Butterworth and Punt, 1999).

Needless to say, the management plan and, in particular, the goals and decision rules should be defined through a participatory and accountable process. This maximizes buy-in and the likelihood that constructive decisions will actually be made and implemented.

(14) *Assess and manage ecological impacts*

Premise: Impacts of operational enhancements on wild populations and ecosystems can be significant, either positive or negative. Such impacts arise from intra- and inter-specific, biological and technical interactions. Ecological impact assessment and management needs to form part of any development or reform process.

Potential ecological impacts should be appraised early on in the development or reform of enhancements (Stage I). However, because impacts may become apparent only once enhancements are scaled up to fully operational scale, empirical assessments and where required, remedial management should be conducted in Stage III.

Ecological impacts of enhancements can arise from intra- and interspecific, biological and technical interactions. Intraspecific interactions should be assessed comprehensively through quantitative assessment, genetic resource management and the stock management plan (Elements 3, 8, and 13). Some additional research studies may be required, for example to assess the fitness in the wild of stocked and hybrid stocked-wild fish.

Interspecific biological interactions can arise where cultured fish increase the abundance of existing wild populations or establish new populations where the species was previously absent. In either case, the strongest impacts on other fish species are likely to arise due to predation from stocked fish, or due to biogenic habitat modification by stocked species that may, for example, reduce macrophyte abundance or reduce or increase turbidity (Caddy and Defeo, 2003; Eby et al., 2006). This could lead to trophic cascades, particularly in simple food webs, but such dramatic effects of enhancements appear to be rare. Interspecific competitive interactions tend to be weaker, but may also be significant (e.g., Levin and Williams, 2002).

Technical interactions, which may be intra- or interspecific, arise when the aquaculture or harvesting operations for cultured fish affect wild populations. This may occur through broodstock capture, changes in fishing pressure, or habitat modifications resulting from harvesting or culture operations. Broodstock capture can have significant impacts when wild populations are small, which is typically the case in captive breeding programs for conservation. Broodstock capture for the Mekong giant catfish breeding program, for example, has contributed to a dramatic decline in wild population abundance (Lorenzen et al., 2007). In larger populations, changes in fishing pressure associated with large-scale release programs are the most common technical interactions. Pacific salmon enhancements may have increased fishing pressure on mixed wild-hatchery stocks, though the impact of this on wild stocks remains controversial (Hilborn and Eggers, 2000). Non-target species may be harvested inadvertently or deliberately (e.g., in the case of predator control programs sometimes implemented with enhancements). Conversely, harvest restrictions brought in to protect stocked fish may also reduce pressure on wild stocks (Lorenzen et al., 1998; Lorenzen, 2008). Significant habitat disturbance may also result from harvesting operations, particularly for benthic species. Finally, aquaculture facilities supplying operational-scale enhancements can be large enough to entail significant habitat modifications. These examples, while not exhaustive, show that technical interactions can be significant and should be assessed.

Assessment of ecological impacts is best started by identifying the set of possible impacts (for example, on the basis of the overview provided here) and then scoring the risk of occurrence and potential significance of each. Frameworks developed for

ecological risk assessment of fisheries may be adapted for this purpose (e.g., Fletcher, 2005; Hobday et al., 2007). Interspecific biological impacts may be further quantified by predatory impact or ecosystem modelling (Taylor and Suthers, 2008).

Monitoring and management requirements for environmental impacts are broadly related to the magnitude of the enhancement program. A special case is where there are many similar programs that are individually small, but collectively substantial. In this case, rather than conducting cursory assessments on individual programs, it is preferable to conduct an in-depth assessment on a representative sub-set of systems.

(15) Use adaptive management

Premise: 'Actively adaptive' management needs to be firmly established as part of the operational plan for enhancements. Adaptive management enables evaluation of the performance of hatchery releases and provides the means to resolve critical uncertainties, improve the efficiency of release strategies, refine operational plans and achieve the goals of enhancement.

Development and management of enhancements often proceeds under conditions of uncertainty and may also be affected by change in environmental conditions, fishing pressure, management goals, etc. Management must deal constructively with such challenges, resolving uncertainties and adapting to change.

Assessment of impact is an integral part of 'actively adaptive' management, which entails posing and answering questions about enhancement effectiveness that allow steady adaptation and improvements to be made in operational plans (Walters and Hilborn, 1978; Walters and Martell, 2004). Adaptive management has associated costs beyond those to produce and release fish, and those costs may preclude this essential element of enhancements from being incorporated into operational plans. Without adaptive management, enhancements cannot operate efficiently and potential changes or improvements may not be recognized, which could have been used to meet goals. In the worst case, where no assessment is conducted and are operating blindly with no sense of what is being achieved, negative impacts may be occurring.

The key to effective management of enhancement impact lies in having a process for changing both production and management plans to control enhancement performance and effectiveness. To use adaptive management, a moderate level of ongoing assessment is needed, superimposed over a modest research framework that provides the new information needed to understand the effects of the enhancement system, refine enhancement strategies and tactics and achieve goals and manage uncertainty. New opportunities for refining enhancement are thus constantly generated and integrated into the management process. An important corollary of adaptive management is that change should be anticipated. This implies that aquaculture facilities, for example, should be designed in such a way that operation can be

adapted relatively easily when modifications are deemed necessary (Blankenship and Daniels, 2004).

If uncertainties in the outcome of alternative management options are low and courses of action can be identified that will almost certainly lead to the achievement of objectives, these courses of action may be implemented. If uncertainties are high, it is important to assess whether a reduction in these uncertainties is likely to allow substantially improved management regimes to be developed. When this is the case, the reduction of uncertainties becomes an important objective in its own right, and courses of action should be evaluated for their potential to yield the necessary information. Experimental management may be passive, i.e., rely on 'natural' variation in management regimes to generate information, or active when variation is introduced deliberately. Which of the two adaptive strategies is implemented will depend on specific circumstances, including the degree of control that can be exercised over management actions.

Explicitly experimental management actions may be implemented and their outcomes monitored to gain crucial information (McAllister and Peterman, 1992; Walters, 1997; Garaway and Arthur, 2002). Experimental approaches may be the only way of resolving certain fundamental uncertainties. For example, varying stocking numbers over a wide range temporally and spatially may be the only way of disentangling enhancements and environmental change impacts on wild populations (Walters and Martell, 2004). It must be appreciated, however, that the costs of setting up, monitoring, and evaluating management experiments can be considerable. Therefore, experimental management should be implemented only where anticipated benefits warrant this expenditure.

When designing management experiments, careful consideration should be given to experimental design (McAllister and Peterman, 1992; Walters, 1997). Key issues are: (1) replication—ideally, this should be temporal (before and after intervention) as well as spatial (parallel measurements at similar sites where no intervention has been carried out); (2) contrast—the intervention should be substantial in order to have a measurable effect; (3) sampling effort—each replicate unit must be sampled with sufficient intensity to allow detection of an impact of the expected magnitude (MacGregor et al., 2002).

IMPLEMENTING A RESPONSIBLE APPROACH

The responsible approach sets out broad principles that may be implemented under a wide range of different circumstances and in different management settings. Not all elements are relevant under all circumstances, but most will be. No element should be discounted simply because its implementation is difficult. In our experience, integrative elements that require constructive engagement between the fisheries and hatchery constituencies and between science, management and stakeholders are most often ignored or postponed. For example, the

elements 'develop a species management plan' and 'identify economic and policy objectives' of the 1995 Responsible Approach have often received only cursory attention compared to elements that are readily addressed by science alone. We urge colleagues and stakeholders to tackle all elements of the new approach and where necessary, to seek new institutional structures and processes for doing so.

The Responsible Approach can be implemented at different levels: individual enhancements, sets of similar enhancements, or at state or national level. A balance must be struck between program size and assessment/management effort. However, where many small enhancements are being developed or operated, cumulative impacts may become an issue. We suggest conducting strategic assessments on a representative sub-set of such enhancements where relevant.

We have provided a set of principles but resisted the temptation to set out a fully specified framework or process, for several reasons. First, any framework or process of sufficient generality to be useful in the diverse situations the principles apply to would be prohibitively complex and quite likely, muddle rather than clarify the key issues. Secondly, designing a locally appropriate framework and process is in itself a key element of implementing a Responsible Approach, promoting interaction among stakeholders and scientists and buy-in to the planning outcomes. Thirdly, relevant assessment and planning frameworks are likely to be in place in many locations and integrating key principles into such existing frameworks is likely to be more effective than bringing in a new framework. Finally, stakeholders and, in particular, decision makers often resist being constrained by overly prescriptive frameworks—we recognize this and encourage them to make inspired and responsible choices by drawing on the principles set out here.

DISCUSSION

We have provided a set of issues that need to be addressed if enhancements are to be developed or reformed responsibly. For each point, we provide a rationale (why the point is important) and refer readers to key publications and tools that will allow them to address the issue raised. Many issues require specialist knowledge and skills and we encourage practitioners to assemble interdisciplinary teams for development or reform processes.

The updated Responsible Approach differs from its predecessor in that it takes a broad systems view of enhancements and accords equal weight to the dynamics of their biological and human components. It requires an integrated, quantitative, and participatory analysis of the contribution enhancement could make to fisheries management goals and should be conducted at the very beginning of any enhancement initiative. Many elements of the updated Responsible Approach have direct equivalents in the earlier version and have simply been updated in the light of new knowledge. Exceptions are Elements 1, 2, and 3 of

the updated approach, which provide more explicit and detailed guidance on Element 2 of the old approach. Also, Elements 7 and 14 of the updated approach expand on and clarify issues covered in Element 6 of the old approach. An explicit focus on governance arrangements has been added in the new approach (Element 12).

The updated responsible approach takes account of and can guide implementation of several related policy instruments and guidelines, including the Code of Conduct for Responsible Fisheries (FAO, 1995, 1997) and the IUCN Guidelines for Re-introductions (IUCN, 1998).

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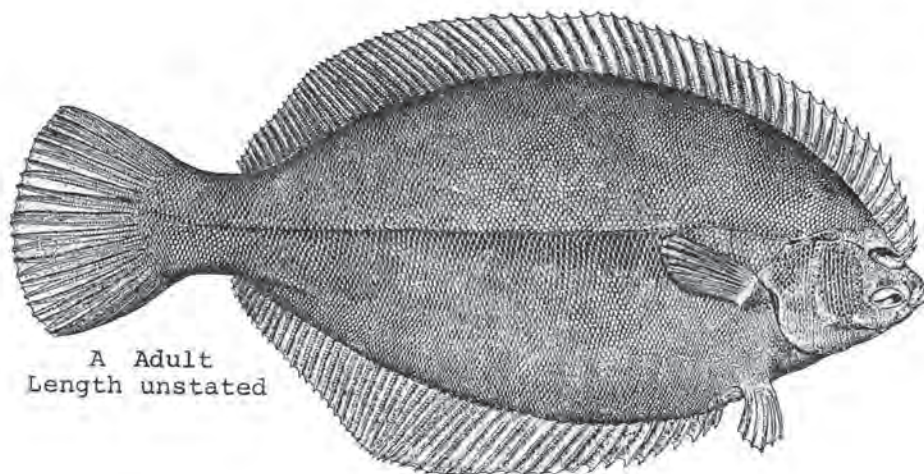
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Winter Flounder Diagrams: Martin and Drewry 1978



A Adult
Length unstated

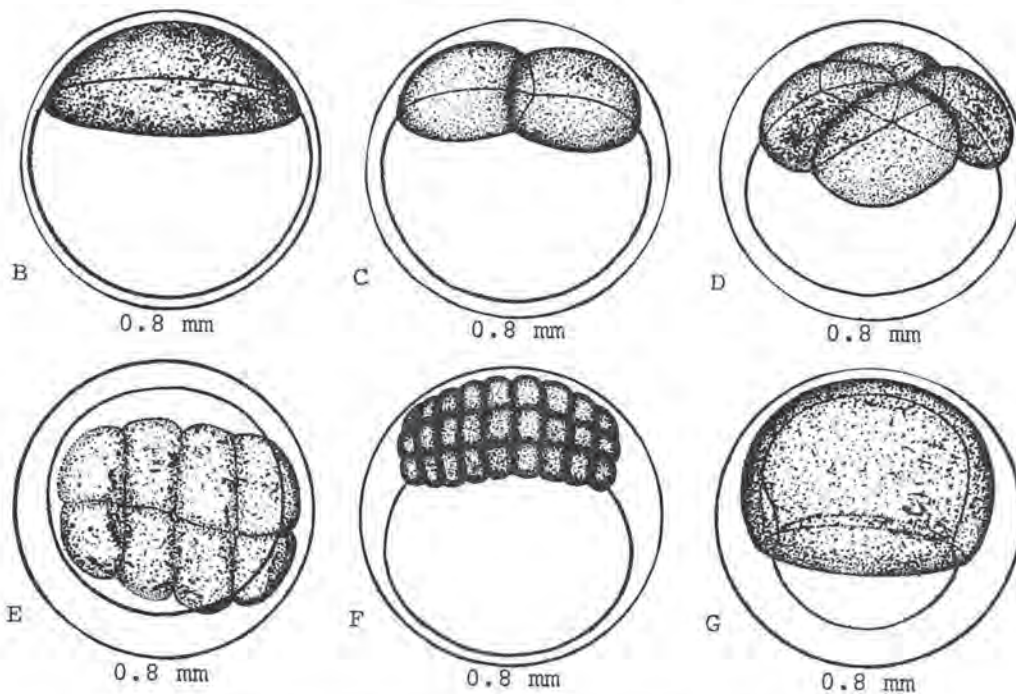


Fig. 104. *Pseudopleuronectes americanus*, Winter flounder. A. Adult, length unstated. B. Unfertilized egg, 0.8 mm. C. Egg, 0.8 mm, two-cell stage. D. Egg, 0.8, four-cell stage. E. Egg, 0.8 mm, eight-cell stage. F. Egg, 0.8 mm, morula. G. Egg, 0.8 mm, embryo differentiating, embryonic shield overgrowing yolk. (A, Tracy, H. C., 1908: pl. 6. B-G, Breder, C. M., Jr., 1923: fig. 274.)

EGGS

Location: Eggs have a specific gravity of 1.085–1.095 compared to 1.010–1.024 for the water,^{2,20} therefore lie on bottom;^{5,14,17,19,32} found in about 30 cm water over mud flats;²² eggs discharged by groups of circling fish so that they are spread over an area;²⁵ occur singly and in aggregates of 2, 3 and 4.¹³

Unfertilized eggs: .33–.93 mm.²⁷

Fertilized eggs: Spherical;⁵ sizes from .71⁴⁰ to .96 mm,²⁶ average 0.80 mm;^{2,47} membrane surface "resembled fine grained leather in texture";⁴⁰ adhesive;^{2,12,37} yolk colorless, with finely tuberculate surface.⁴⁰

EGG DEVELOPMENT

At 20.6 C, first cleavage at 2 1/4 hours after fertilization. At 24 hours, blastoderm broken up into a high number of cells; at third day, neural tube formed; by the end of the sixth day, somites forming at mid-body, Kupffer's vesicle formed, optic anlagen visible; by the end of the ninth day, optic cup complete, xanthophores sprinkled over body, these punctulate; on the fifteenth day, concentrations of chromatophores in a vertical band in the caudal region, heart beating, cephalic region finely tuberculate, hatching beginning.³⁵

Eggs develop to hatching over the range -1.8 to 18 C, but with lower hatching success above 10 C,⁴⁷ in another

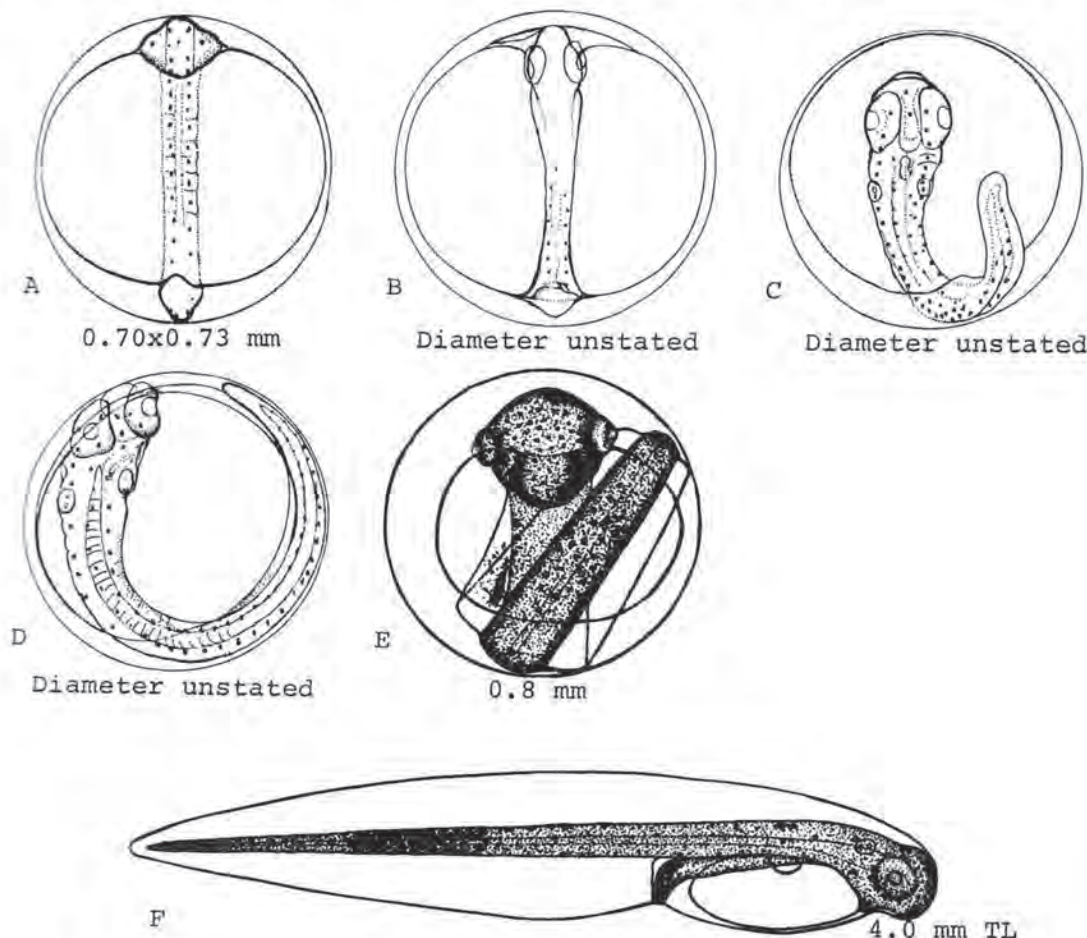


Fig. 105. *Pseudopleuronectes americanus*, Winter flounder. A. Egg, 0.70 mm x 0.73 mm, ventral view, eye cup visible, pigment present, somites visible. B. Egg, diameter unstated, dorsal view, about the same stage as fig. A. C. Egg, diameter unstated, advanced embryo. D. Egg, diameter unstated, near hatching. E. Egg, 0.8 mm, ventral view, near hatching. F. Yolk-sac larva, 4.0 mm TL, newly hatched. (A-D, Agassiz, A., and C. O. Whitman, 1885: pl. 16. E-F, Breder, C. M., Jr., 1923: fig. 274.)

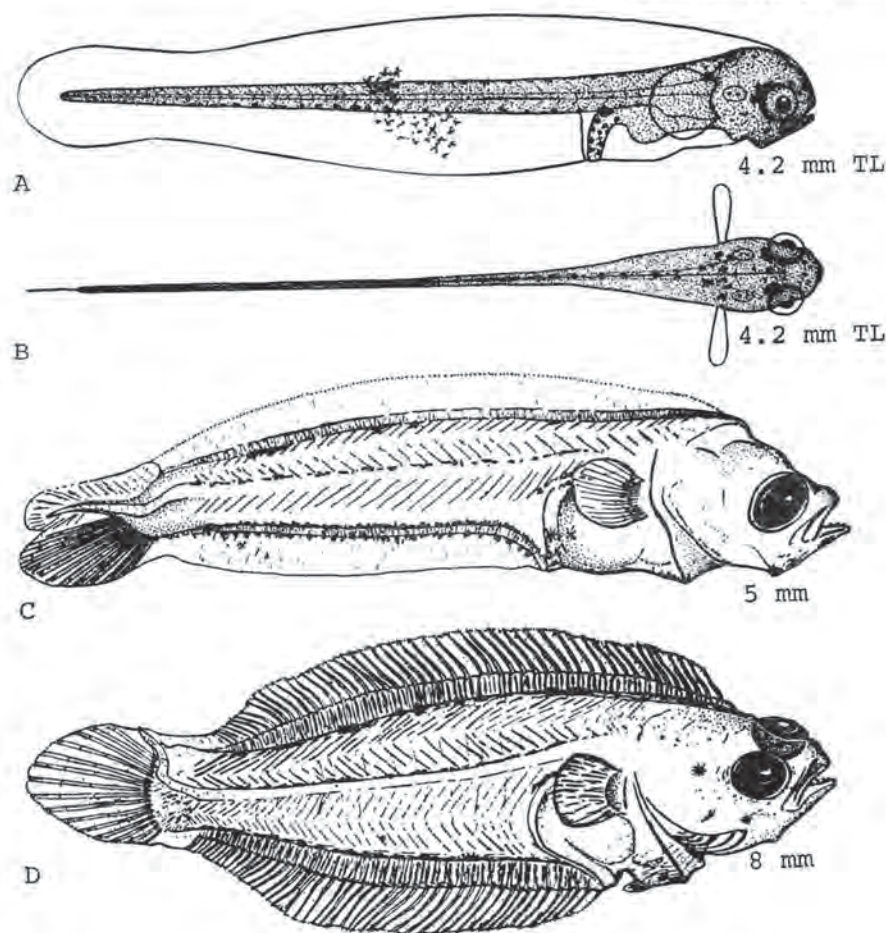


Fig. 106. *Pseudopleuronectes americanus*, Winter flounder. A. Larva, 4.2 mm TL, 19 days old. B. Larva, 4.2 mm TL, dorsal view, 19 days old. C. Larva, 5 mm, fin rays differentiating, flexion occurring. D. Juvenile, 8 mm. (A-B, Breder, C. M., Jr., 1923: fig. 274. C-D, Bigelow, H. B., and W. W. Welsh, 1925: fig. 260.)

set of experiments decreasing survival as temperature increases from 3 C; ⁴⁸ hatching occurs over the range 4.5 ¹³ ppt to 40.0 ppt salinity, ⁴⁸ hatching takes place at 35 ppt, but rate of development retarded; ⁴⁷ optimum survival and developmental rates dependent on temperature-salinity combination but this optimum lies somewhere in the range between 15 and 35 ppt salinity. ⁴⁸

Incubation period: Either die or hatch by 16-24 days at 0 C, average 21 days; ¹³ 25 days at 3 C; ⁴⁸ 24-28 days at 4-5 C, average 26; 17-19 days at 12-17 C, average 18; ¹³ 15-18 days at 2.7-3.3 C ¹⁴ or 17-18 days. ^{20, 41}

YOLK-SAC LARVAE

Hatch at 2.3 mm ²⁶ to 3.5 mm, ¹⁰ yolk absorbed by 5 mm. ^{14, 16}

At hatching almost transparent except for eyes which may or may not be chrome yellow and a vertical pigment band on tail, also pigmentation around vent. ¹⁶

LARVAE

From 5 mm ^{14, 16, 20} until eye migration complete at 6.5 mm, ¹⁸ 8 mm ¹⁴ or 9 mm.

Dorsal fin rays 60-76 at metamorphosis, anal 45-58; ²⁰ eye migration completed over a 3 day period; ⁷ otoliths not ossified before left eye reaches median position (about 7 mm); ⁴⁹ at 5 mm, position of finfold similar to that of adult fins, notched between incipient dorsal and caudal by 5.8 mm; notochord flexes by 5.8 mm. ¹⁶

Pigmentation: Patch of pigment at angle of lower jaw; 4

Using the YSI DO200 Meter

The DO200 is a relatively simple instrument that should be accurate enough for fish culture. It does not have a barometer on it, so for calibration, the atmospheric pressure for the location sampled will be needed. This value can be obtained from various weather stations and/or internet sites, or by using a simple wall-mounted weather station. These DO meters are surprisingly expensive, so it's worth it to take good care of both the instrument itself and the probe that does the actual measuring. Avoid getting the meter itself wet (though it can deal with being splashed), as well as any sharp shocks to either the meter or the probe. After use, the probe **MUST** be thoroughly rinsed, as material build-up on the membrane can cause erratic and inaccurate readings.

These meters use screw-on type membrane caps, which are relatively easy to replace. The necessary parts are included in the membrane kit. It is recommended that membranes are replaced every four to eight weeks, but at the CML, careful rinsing after each use can extend this interval, and the membranes are replaced whenever the readings become erratic and suspect.

The following directions are presented here for convenience. For more detailed instructions, consult the manual that came with the meter. Read the manual in full before using the meter. When using the meter in the hatchery, it should be sanitized before beginning the measuring process and between individual tanks. The probes can be sanitized by dipping the probe and the first 12" or so of cable into either a mild bleach solution (about a tablespoon of bleach in about 2L of water), isopropyl alcohol or hydrogen peroxide. At the CML, the bleach solution method is preferred.

Calibration — The accuracy of the dissolved oxygen measurements are only as good as the calibration of the meter. At the CML, meters are calibrated daily. These meters will maintain their calibration when the power is turned off, but in general (especially if there is rapidly changing weather) the barometric pressure changes enough day-to-day to warrant daily calibration.

1. Acquire values for barometric pressure (in mBars) and the salinity of the water to be measured.
2. If the sponge inside the probe storage bottle is dry, add five to six drops of clean tap water to the sponge and turn the bottle over to allow excess water to drain away.
3. Slide the probe into the storage bottle.
4. Turn on the meter by pressing the power button. Wait 10-15 minutes for the meter to warm up and the readings to stabilize.
5. Press the "CAL" button.
6. Use the arrow keys to enter the barometric pressure. Remember to enter in mBars.
7. Press the "return arrow" key to view the calibration value in the lower right of the display. Once this value is stable, press the "return arrow" key again.
8. Use the arrow keys to enter the correct salinity of the water to be measured. Press the "return arrow" key again. The unit is now calibrated.

Taking Measurements

1. If the unit is not already powered on, turn it on by pressing the power button. The meter will perform a quick diagnostic test and display "over." When the test is done, the message will disappear and the temperature will appear in the lower right of the display.
2. Sanitize the probe and the first 12" of the cable if working in the hatchery.
3. Immerse the probe in the water to be sampled, shake it a bit to dislodge any air bubbles, and move it up and down at a rate of 6" per second.
4. Record the readings. Record the dissolved oxygen in ppm (mg/L). If desired, the meter can be switched to measure in percent saturation by pressing the "mode" button.
5. When all measurements have been completed, rinse the probe and cable very well and return the probe to the storage bottle. Power off the unit.

List of Supply Companies

Aquatic Eco Systems, Inc.
www.aquaticeco.com
Ordering: 877-347-4788
Free Tech Support: 407-598-101

Aquatic Research Instruments
www.aquaticresearch.com
800-320-9482

Argent
<http://www.argent-labs.com/argentweb-site/indexold.html>
800-426-6258

Ben Meadows
www.benmeadows.com
800-241-6401

Cole Palmer Instrument Co.
www.colepalmer.com
800-323-4340
Forestry Suppliers, Inc.
www.forestry-suppliers.com
800-647-5368

Hallprint Fish Tagging Solutions
www.hallprint.com
800-537-1614

Legend Inc.
www.lmine.com
775-786-3003

Memphis Net & Twine Co
www.memphisnet.net
888-674-7638

Northwest Marine Technology
www.nmt-inc.com
360-468-3375

Onset Corporation
www.onsetcomp.com
800-564-4377

Reed Mariculture
www.reedmariculture.com
408-377-1065

Sigma-Aldrich
www.sigmaaldrich.com/united-states.html

Skretting
<http://www.skretting.ca>
Spectrum products: <http://www.skretting.com/spectrum>
Bio-Oregon (Regional Contact): <http://www.bio-oregon.com/>
877-221-2429

YSI Instruments, Inc.
www.ysi.com
800-659-8895

