Lumpfish Hatchery Handbook

Elizabeth A. Fairchild, Michael R. Pietrak, and Gary S. Burr

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Introduction

Lumpfish (*Cyclopterus lumpus*), a species native to the Gulf of Maine, is a proven "biological delouser," consuming sea lice when reared in salmonid net pens (Imsland et al. 2014a, 2014b, 2014c, 2015b, 2018; Powell et al. 2018b). Because of this capacity as a natural biocontrol (aka cleanerfish), lumpfish hatcheries and grow-out facilities to support Atlantic salmon (*Salmo salar*) and rainbow/steelhead trout (*Oncorhynchus mykiss*) farms have been multiplying in Europe. In Norway alone, lumpfish production has grown exponentially; from 2012 to 2016, lumpfish hatcheries increased from five to 24 (Sveier and Breck 2018) and are now producing upwards of 34+ million lumpfish annually (Directorate of Fisheries 2021). To meet global demands, it is estimated that lumpfish production needs to exceed 50 million fish annually to keep pace with salmon farm production and development (Powell et al. 2018b). Atlantic salmon and steelhead trout farms in Maine and New Hampshire want to incorporate lumpfish into their operations too, however, currently, there is not a US lumpfish source, though plans are in place for the first US commercial lumpfish hatchery in Maine.

Due to this industry-driven need, researchers at the University of New Hampshire (UNH) and the US Department of Agriculture's National Cold Water Marine Aquaculture Center (NCWMAC) have begun working towards the goal of domestic production of lumpfish. Further, we have formed the US Lumpfish Consortium, an informal group consisting of researchers from universities and federal laboratories plus aquaculture industry partners. The group is open to any persons or groups interested in promoting research on the culture and use of lumpfish in US aquaculture.

With commercialization in Europe and Canada, basic lumpfish culture methods (e.g., strip spawning, egg incubation, first feeding, larval and juvenile culture) have been determined (reviewed in Powell et al. 2018b and Treasurer 2018). Yet despite the numerous organizations developing lumpfish culture techniques globally and the promise of lumpfish as an environmentally sustainable strategy for parasite control, very little information is available in either the peer reviewed literature or the gray literature on husbandry practices; most of the literature focuses on lumpfish use in salmonid farms. Informal networks of research institutions working on lumpfish are the only open dialogue of current and critical lumpfish culture issues; the Global Cleanerfish Group (facilitated by Danny Boyce, Memorial University of Newfoundland) and the US Lumpfish Consortium (led by Elizabeth Fairchild and Mike Pietrak) are the two such groups. Due to the limited information available, we recognize the need for accessible lumpfish hatchery protocols, especially for emergent commercial facilities in the US.

Lumpfish aquaculture research has been ongoing since the 1980s (Davenport 1983; Benfey and Methven 1986; Brown 1986), and since the 1990s, some of this research, emphasizing the egg through juvenile stages, has occurred at UNH (Irons 1999; Rackovan 2016; Spada 2021). During 1993-94, lumpfish culture techniques from wild captured broodstock were developed for the roe market, providing approximately 5,000 lumpfish to a private Maine business to grow out the fish

in cages (Irons 1999). Wild-collected egg batches also have been reared successfully providing juveniles for salinity preference studies (Rackovan 2016) and age 3 fish to the NCWMAC for experimental studies. Furthermore, realizing the US industry interest in lumpfish, scientists at UNH, NCWMAC, and the University of Maine have been collaborating via the US Lumpfish Consortium to utilize our institutional knowledge and history of lumpfish and other cold water marine finfish culture research to catalyze lumpfish commercialization and use in the US. Since 2019, the Fairchild Lab has produced on average ~15,000 juveniles/year at the UNH Coastal Marine Laboratory (CML) for a series of hatchery (Spada 2021) and steelhead trout-lumpfish caging studies (Doherty 2021). In addition, the NCWMAC has reared fish for complimentary hatchery studies.

At UNH and the NCWMAC, we continue to utilize our institutional knowledge and history of lumpfish culture research to address some of these existing culture hurdles and provide the greater aquaculture industry with techniques to increase hatchery production so that sea lice mitigation is less costly and more sustainable, which may make new lumpfish business ventures less financially risky. We offer this lumpfish hatchery manual as a start, realizing that rearing cleanerfish is a relatively new and rapidly evolving aquaculture sector and, as such, we do not know all. We include anecdotal observations, tricks of the trade we have found useful, and results of recent studies. We hope that this manual will be helpful.

Chapter 1 Lumpfish Biology

Lumpfish (*Cyclopterus lumpus*) are a cold-water, marine finfish in the order Scorpaeniformes, family Cyclopteridae. They are identified easily by their grayish coloration, globiform body lined with three rows of tubercules on each side, and, most notably, the presence of a ventral suction disk, formed from their modified pelvic fins, which they use to adhere to rocks, algae, and other marine structures (Collette and Klein-MacPhee 2002; Figure 1.1).

Figure 1.1: Adult lumpfish A) Adult female lumpfish at the UNH Coastal Marine Laboratory. Photo: S. Schaier/UNH. B) Ventral side with suction disk visible. Photo: M. K. Munley/UNH.

Distribution

Lumpfish are semi-pelagic (Blacker 1983) and inhabit cold waters ranging from the intertidal to depths of 868 m (Parin, et al. 2002), though most commonly found in 50-150 m waters (Stein 1986) and distributed across the North Atlantic Ocean (Davenport, 1985; Figure 1.2). In the western Atlantic, lumpfish range from Nunavut, Hudson Bay to James Bay and Labrador in Canada and southwards to New Jersey, USA (Collins 1979; Blacker 1983; Collette and Klein-MacPhee 2002; Kennedy et al. 2016; FishBase 2021). In the eastern Atlantic Ocean, lumpfish are found from the Barents Sea, west to Iceland and Greenland, and south to Spain (Bañón et al. 2008; FishBase 2021). Five distinct genetic groups of lumpfish occur in the following regions: West Atlantic (USA-Canada), Mid Atlantic (Iceland), East Atlantic (Faroe Islands, Ireland, Scotland, Norway and Denmark), English Channel (England) and Baltic Sea (Sweden; Whittaker et al. 2018).

Reproduction

Lumpfish spawn in the late spring (March through May) in the southwestern Gulf of Maine, and closer to early summer (May to June) along the northeast Maine coast (Cox and Anderson 1922). Lumpfish are sexually dimorphic. Females are larger than males (Cox and Anderson 1922; Goulet 1988) and during the spawning season, male lumpfish turn vivid hues or red, orange, yellow, and fuchsia, or develop hints of yellow or orange along their fin edges, while females remain gray or grayish-teal (Figure 1.3). In early spring (February to March), adults move inshore to spawn along the rocky coasts (Cox and Anderson 1922; Goulet et al. 1986; Collette and Klein-MacPhee 2002). Males migrate first, establish territories, and build nests to attract the females (Davenport 1985; Goulet et al. 1986). Lumpfish display courtship behavior including nest cleaning, fin brushing, and quivering (Goulet et al. 1986). Females spawn 2-3 sticky, demersal egg masses on the surface of the nest which then are fertilized and molded by the males into the nest, forming a funnel shaped depression in the middle of the egg mass (Goulet et al. 1986). Eggs are large, ranging in size from 2.0 to 2.6 mm in diameter (Brown 1992). Female lumpfish then move offshore, while the males stay to guard and tend the eggs until hatching. Male lumpfish will aerate the eggs by “puffing” water on them and fanning them with their fins,

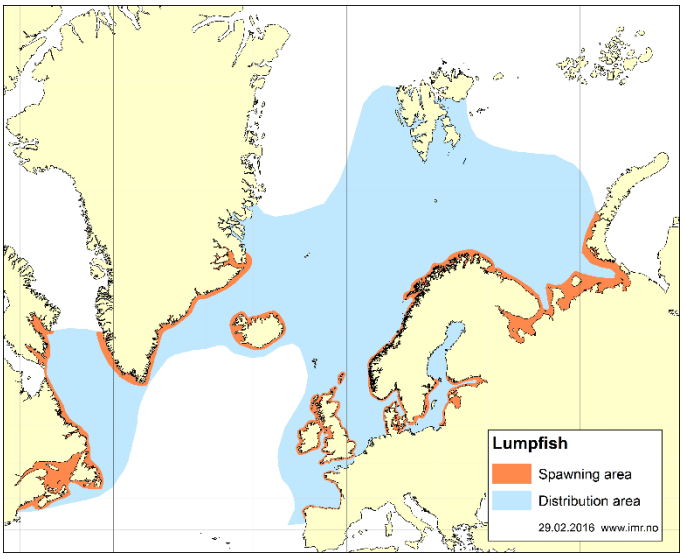

Figure 1.2: *The geographical range of lumpfish, including major spawning areas*
(Image from: Institute of Marine Research; https://www.hi.no/en/hi/temasider/species/lumpfish)

as well as defend the nests from predatory organisms by removing sea urchins and periwinkles and chasing away other fishes (Goulet et al. 1986). After approximately 6-8 weeks, depending on water temperatures, the eggs hatch (Cox and Anderson 1922; Collins 1976; Goulet et al. 1986; Martin-Robichaud 1991) and presumably the males return to offshore areas soon thereafter.

Fecundity estimates for Canadian and Icelandic lumpfish generally range from 50,000 to 200,000 eggs (Gregory and Daborn 1982; Kennedy and Ólafasson 2020). Relative fecundity ranges from 34,700 to 40,400 ooyctes/kg body weight (Kennedy and Ólafsson 2020; Poutney et al. 2020).

Lumpfish exhibit spawning site fidelity as has been observed through mark-recapture studies in Canada (DFO 2011) and Iceland (Kennedy et al. 2015). Despite the lengthy spawning season, individual lumpfish appear to exhibit temporal fidelity too and return to spawn in the same locations during the same time periods in subsequent years (Kennedy and Ólafsson 2019).

Early Life History and Juvenile Ecology

Once the eggs hatch in early summer, the larvae are dispersed by currents and adhere to benthic or pelagic macroalgae. Newly-hatched lumpfish range from 4.0 to 7.4 mm TL (Benfey and Methven 1986; Brown 1986; Collette and Klein-MacPhee 2002) and are shaped much like a tadpole with a large head and body and a long, slender tail. By approximately 34 mm TL, tubercules start to form and the hatchlings begin to look like miniature adult lumpfish (Collette and Klein-MacPhee 2002).

Juvenile lumpfish are highly associated with macroalgae, either in tidepools or in the upper 0.5 m of the water column (Daborn and Gregory 1983; Moring 1989). In Maine, juveniles < 26 mm TL live among *Zostera* (sea grass) beds or *Laminaria* (kelp), and then move to *Ascophyllum nodosum* (rockweed) in the inner bays (Moring 1989), preying on small invertebrates associated with floating seaweeds or found in the plankton including amphipods, copepods, isopods, cumaceans, and even small fish larvae, including lumpfish (Moring 1989; Tully and O'Ceidigh 1989; Davenport and Rees 1993; Ingólfsson and Kristjánsson 2002). They depend on the seaweed for transportation as it passively drifts, protection from predators, and an increase in food sources (Vandendriessche et al. 2007). Lumpfish grow relatively quickly in their first year of life, reaching approximately 35 to 70 mm TL (Martin-Robichaud 1991). During this time, they are able to divert the majority of their energy towards growth since they cling to algae and wait for prey to pass by (Brown 1986; Killen et al. 2007). In New Hampshire waters, juvenile

lumpfish are found over a wide array of temperatures (8.6 to 22.3 ℃) and salinities (21.9 to 34.0 ppt; Rackovan 2016). At approximately one year of age, the juveniles become mostly pelagic and begin to move offshore (Moring 2001).

Adults

Figure 1.3: Mature lumpfish
Mature male (left) and female (right) lumpfish at the UNH Coastal Marine Laboratory.

Lumpfish can live up to approximately 10 to 15 years and grow to 38-40 cm, however, the largest known lumpfish recorded was a 58 cm female (Bigelow and Schroeder 2002). In the wild, males reach sexual maturity in two to three years while it takes females three to four years (Albert et al. 2002; Hedeholm et al. 2014). In captivity, sexual maturity can occur much quicker with many females reaching maturity in under two years (Boyce et al. 2018; Powell et al. 2018a; Wittwer and Treasurer 2018). Adult diet consists of crustaceans, polychaetes, and jellyfish (Cox and Anderson 1922). Natural predators include harbor seals (de la Vega et al. 2016), hooded seals (Haug et al. 2007), and Greenland sharks (Nielsen et al. 2014). Though not exploited in the US at the time of this publication, lumpfish are harvested in other countries for their roe (caviar), meat, and, more recently, to supply broodstock to lumpfish hatcheries that supply cleanerfish to the salmon industry.

Chapter 2 Spawning

Lumpfish spawn in the late spring (March-May) in the southwestern Gulf of Maine (GoM), and closer to early summer (May-June) along the northeast Maine coast. Males and females differentiate during the spawning season by color: males become red while females remain gray (see Figure 1.3). In Feb-March, adults move inshore to spawn along the rocky coasts (Cox and Anderson 1922; Goulet et al. 1986; Collette and Klein-MacPhee 2002). Females spawn 2-3 sticky, demersal egg masses and then move offshore, while the males stay and guard and tend the eggs until hatching, approximately 6-8 weeks (~300 degree days, DD) depending on water temperatures (Cox and Anderson 1922; Collins 1976; Goulet et al. 1986; Martin-Robichaud 1991).

Broodstock

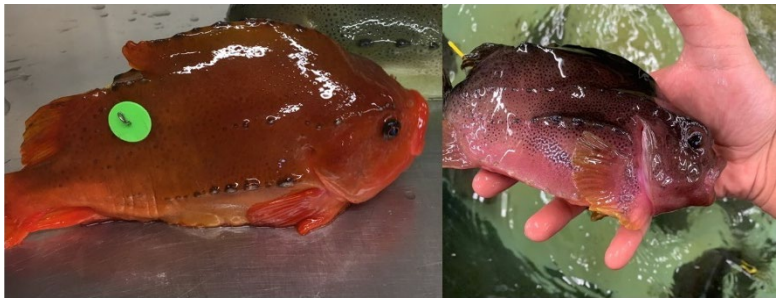

Figure 2.1: Male lumpfish
Mature males can display a range of bright colors includes oranges, pinks, and purples. In addition to the colors, they often develop silvery side patches like the male in the righthand photo.

Mature lumpfish are easy to recognize visually. As the males mature, they adopt bright colorations, typically hues of oranges, pinks, and purples (Figure 2.1). Males generally are smaller than the females. Males can mature anywhere from 200 g in size and larger although they are typically in the 800-1,200 g size range. Females can mature as early as 800 g but are normally 1 kg in size or larger at sexual maturity. Females can easily reach sizes of 2.5 kg and larger. The females will generally remain a dark blue gray to lighter shade of blue green depending on the color of the tank they are in. As the females begin to mature, they start to swell around the urogenital opening (Figure 2.2)

Efforts to allow fish to spawn naturally in tanks almost always ends in masses of unfertilized eggs. If you would like to try to spawn them naturally you need to construct nests for the males to guard. These can be horseshoe shaped piles of beach cobble, or things such as bucket or flowerpots turned on their sides. The females will then lay egg masses at one or more males nests allowing the males to care for the eggs. Again, it must be stressed that this technique has rarely resulted in fertilized eggs in captivity.

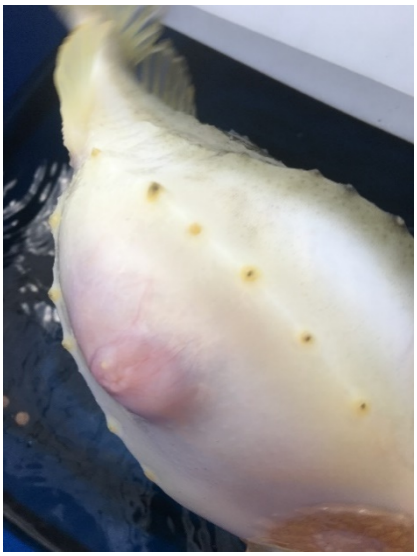

Figure 2.2: Maturing female
Swelling of the female's urogenital region indicating she is maturing for spawning.

Fortunately, lumpfish can be strip spawned easily which is the preferred method for spawning. Typically, the males are lethally spawned with the gonads surgically extracted, while the females are (nonlethally) manually strip spawned. Then the eggs can be artificially fertilized allowing for intentional brood stock selection for desired traits.

Collecting Milt and Storage

It is possible to collect milt via hand stripping and this should be tried on males to see if they are flowing or not. First select the male to be spawned, then dry off the fish around the urogenital pore area to prevent sea water from mixing with the milt. Stripping the milt requires very firm pressure and can often lead to getting urine being expelled too. It is good practice to strip into some sort of smaller cup such as a small paper cup and if the collection from the squeeze has urine mixed in, it can be discarded. If the collection is good and clean, you can set it aside and continue to add any additional clean collections to it from the same fish. It is not necessary to anesthetize the animals as they can be safely handled, however it may assist in extracting milt.

Our experience in captivity, is that the males are not freely flowing when the females are ready. As a result, most facilities lethally spawn the males. Once the selected male has been euthanized, the gonads are surgically extracted (Figure 2.3). They are then macerated to release the milt. This can be done using a commercial device designed for this purpose, a repurposed commercial device such as a garlic press, or simply by repeated chopping in a beaker with a scalpel. Once the gonad has been macerated, the milt and tissue need to be strained either through a cheese cloth lined fine mesh strainer like a chinois. We have utilized sterile partitioned filter sample bags (Whirl-Pak SKU: B01385) for this purpose very effectively. Once the milt is separated from the tissue, it can be pipetted out for evaluation and use.

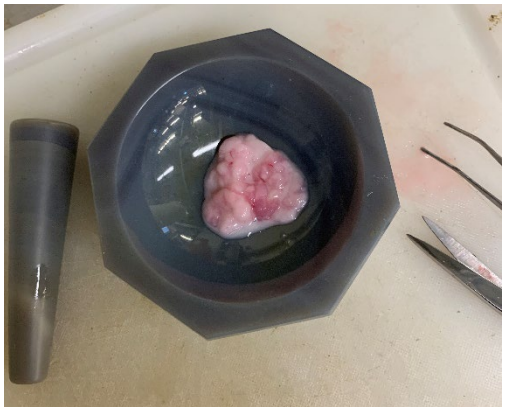

Figure 2.3: Lumpfish testes
Gonads dissected from a euthanized male lumpfish prior to being macerated to release the milt.

There are several ways that milt quality can be evaluated. First, motility can be checked by placing a small sample on a slide, mixed with a drop of sea water to observe motility. Disposable urinalysis slides work well for this. Pountney et al. (2020) report that other common quality measurements such as optical density and spermatocrit measurements can be used as well.

Since most milt is collected using lethal collection techniques, the ability to store milt both short term and long term is important. Pountney et al. (2020) evaluated five different sperm extenders and determined that Modified Turbot Extender (see Resources for formula), Herring Ringer's (see Resources for formula), and Spermcoat (Cryogenetics) all are effective for storing milt for at least 14 days at 4 °C. Sperm should be diluted at 1:5 or at manufacturer's recommendations and then stored in vented cell culture flasks in to optimize the oxygen availability. Milt also can be cryopreserved for long-term storage and then thawed for use (Norðberg et al. 2015).

Collecting Eggs

Females will ripen over a period of several weeks to a month or more. During this time it is possible to distinguish females preparing to lay eggs as their urogenital opening or vent will begin swelling. Females should be monitored on a regular basis. It can be helpful if they can either be housed in small individual tanks or marked with easily identifiable external tags. This will allow each individual's reproductive status to be tracked and allow for a more accurate estimate of when they might be ready to spawn. We utilized a subjective 0-4 scale (Figure 2.4) based on the amount of

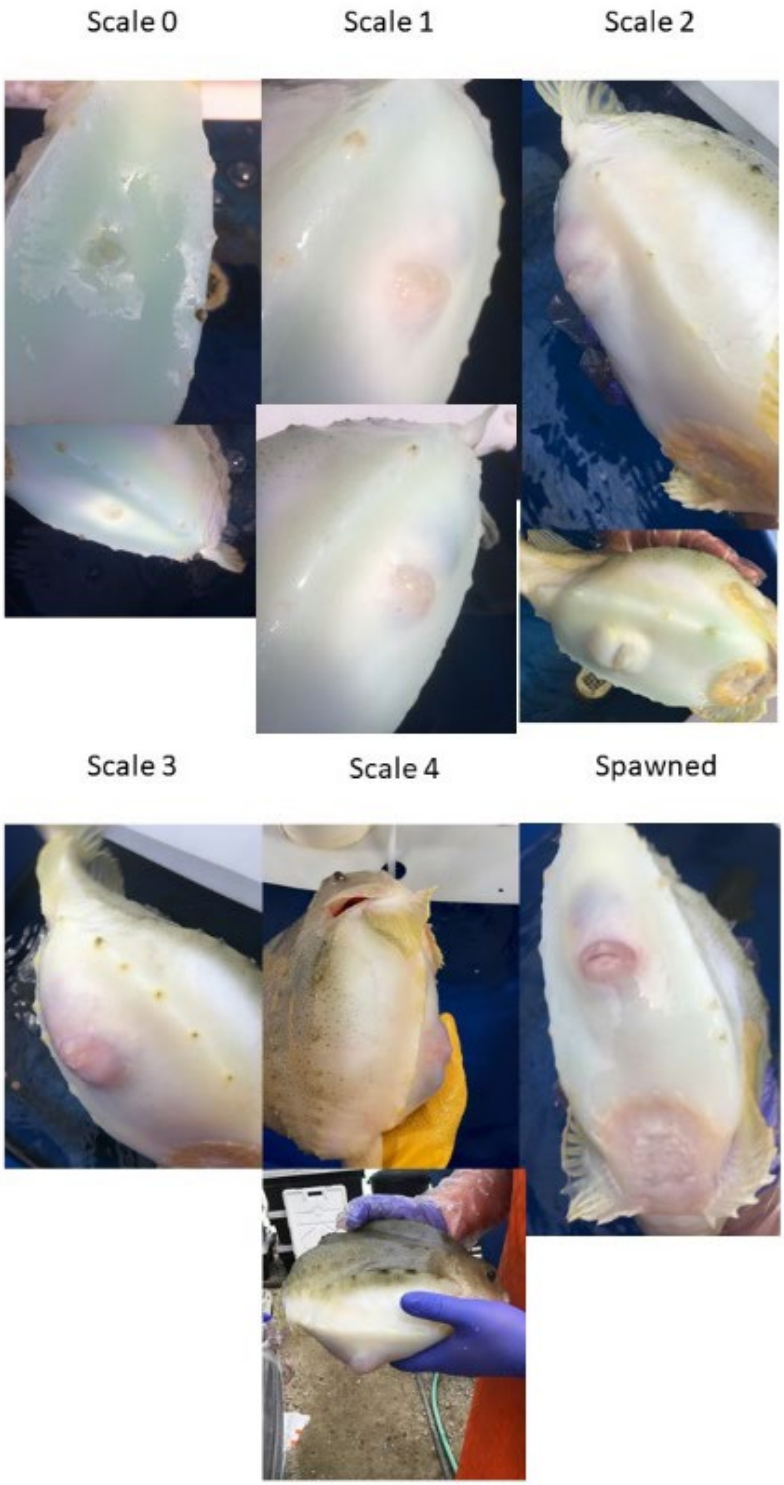

Figure 2.4: Female lumpfish spawning readiness scale
Examples of each score for the spawning readiness scale developed at NCWMAC. The scale ranges from 0 – not maturing to 4 – imminently spawning. The final image illustrates a female that has previously spawned.

swelling of the vent to estimate time to potential spawning. This was helpful in determining how frequently to check each female and to minimize handling.

Females should be checked each morning when close to spawning. They tend to spawn late in the day or during the night, so it is important to identify females that are likely to spawn in the morning just before they spawn naturally. Females can be checked by patting them dry around the vent area to prevent seawater from getting into the eggs. Then they can be gently squeezed over a plastic beaker or other dry container to collect the eggs (Figure 2.5). They should release eggs easily when light pressure is applied. In some instances, the membrane sealing the vent can be punctured by gently inserting a gloved finger, however this is not recommended. The broken membrane will allow sea water in and increase the likelihood of the female becoming bound up with eggs. Waiting several days longer for the female to release with gentle pressure will be more successful than attempting to rush the egg collection.

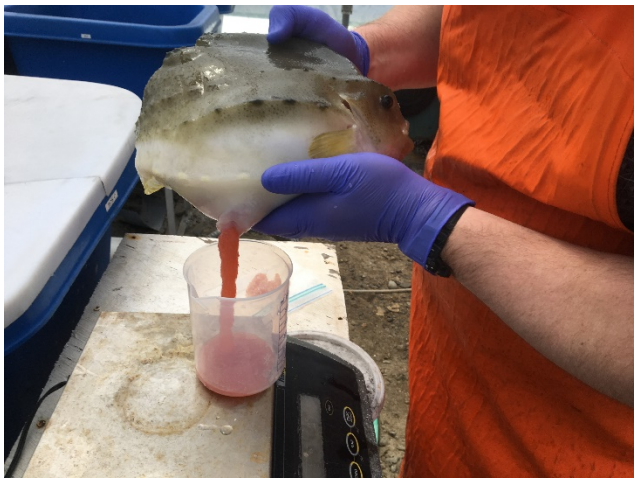

Figure 2.5: Strip spawning a female lumpfish
Ripe females can be gently squeezed to release their eggs into a container for fertilization.

Fertilization

Once the eggs have been collected, it is critical that no sea water be added to them. It is suggested that the volume of eggs be measured and recorded. The eggs are now ready to be fertilized. Most groups utilize a dry fertilization technique. For this, add approximately 1 mL of the milt to the eggs, stir the gametes, and then place the eggs in a refrigerator for approximately 25 minutes to allow for fertilization. Once the eggs are removed from the refrigerator, sea water is added to the eggs to allow them to water harden for 15-30 minutes. They will become sticky at this point and can be placed into a container or mold to achieve a desired egg mass shape. Alternatively, we use a wet or semi-wet fertilization process. Once the eggs are ready to fertilize a small amount of sea water is added to the milt prior to the addition of the milt to the eggs. This activates the milts. A commercial milt activator could be utilized instead. The activated milt is added to the eggs and gently stirred. The eggs are allowed to sit for approximately 5 minutes and then sea water is added to water harden. As before, the eggs can then be placed into rearing containers or molds to achieve the desired egg mass shape and thickness.

Chapter 3 Egg Incubation

In the wild, lumpfish eggs are sticky and demersal, and tended by males until hatching, approximately 6-8 weeks (~300 degree days, DD) depending on water temperature (Cox and Anderson 1922; Collins 1976; Goulet et al. 1986; Martin-Robichaud 1991). In hatcheries, egg incubation time is 250-275 DD at 10 °C or about 27-30 days at 9-10 °C (Boyce 2018).

Incubator Systems

Incubator Types and Flow Rates: Lumpfish eggs can be incubated successfully in many different shaped and sized containers, but most hatcheries use upwelling incubators with water flow entering from the base of the incubator and exiting via an outlet near the top (Bolton-Warberg et al., 2018; Johannesen et al. 2018; Treasurer et al. 2018). In the UK, Swansea University's Centre for Sustainable Aquatic Research uses 70 L upwelling incubators stocked at 0.5 kg eggs/incubator (Treasurer et al. 2018). At the National University of Ireland Galway's Carna Research Station, 12 and 70 L incubator cones with a 6 L/min flow rate have been used (Bolton-Warberg et al., 2018). Floating baskets in flow-through seawater systems also are widely used (Boyce et al. 2018; Steinarsson and Árnason 2018), provided water flow and aeration is sufficient to deter fungal growth. At the MRI Aquaculture Research Station in Iceland, flow rate is maintained at 10 L/min through the incubator baskets (Steinarsson and Árnason 2018) whereas at Memorial University of Newfoundland in Canada, water turnover occurs once per hour in floating baskets (Boyce et al. 2018). Standard salmon egg incubator trays can be adapted to incubate lumpfish eggs as well.

Lighting: Little information exists on best lighting regime during incubation. Some facilities expose the eggs to natural photoperiod (Steinarsson and Árnason 2018) or a 12 light: 12 dark lighting cycle (Boyce et al. 2018), whereas others use opaque incubators so that the eggs are not exposed to light until just prior to hatching.

Water Quality: Water supplied to the incubators should be high quality, filtered to at least 100 µm, and UV sterilized to 30-45 mw/cm^2.

Temperature: Egg development is temperature dependent (Table 3.1) with optimal incubation temperature at 8-11 °C (Boyce, 2018).

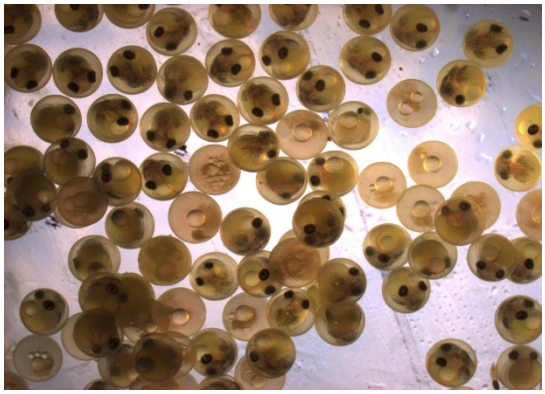

Figure 3.1: Developing lumpfish eggs
As the lumpfish embryos develop, it is obvious to distinguish living from dead eggs. Living eggs clearly have an embryo developing within them whereas the dead eggs are clear to white with no distinct cellular differentiation.

Table 3.1: Egg development time.
Documented effect of temperature on incubation duration (degree days) of lumpfish eggs.

Incubation Temperature (°C)	Degree Days to Hatch	Reference
7	280	Steinarsson and Árnason 2018
8-9	270-350	Treasurer 2018
9.5	270-350	Boyce et al. 2018
10	250-275	Boyce 2018
10	200-300	Treasurer et al. 2018

Egg Disinfection

Using a dissecting microscope, eggs should be inspected regularly to verify fertilization success and normal embryonic development and safeguard against fungal infections (Figure 3.1). Egg disinfection may not necessarily be needed if the eggs appear to be devoid of fungus and "clean," however several disinfection methods can be used.

- Initial disinfection of the eggs can be done in a 4 minute bath of 500 ppm glutaraldehyde in seawater, then the eggs rinsed, and stocked into the incubators as is done in the Faroes (Johannesen et al. 2018).
- In Norway, eggs are disinfected in a 10 minute buffodine (2.5 ml/L water) bath (Treasurer 2018).
- A 500 ppm glutaraldehyde bath for 4 minutes is used in the Faroe Islands (Treasurer 2018).

Verifying Fertilization

Because lumpfish eggs are somewhat opaque, monitoring early embryonic development is tricky. However, at approximately 100 DD, fertilized eggs become 'eyed' if healthy (Boyce et al. 2018). At this point, egg batches should be weighed (g) and can be evaluated for fertilization rate by calculating the percentage of 'eyed' eggs for multiple small, weighed subsamples per egg batch.

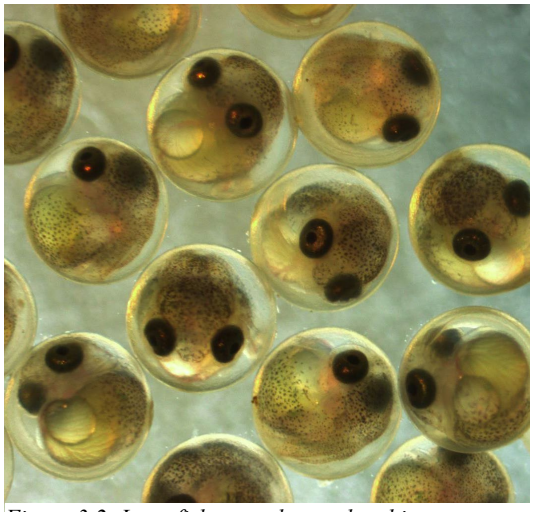

Figure 3.2: Lumpfish eggs close to hatching
Lumpfish embryos at 252 degree days are very advanced developmentally with their bodies curled around the yolk sac. Their eyes are well developed, melanophores (pigment spots) are clearly visible, and their hearts can be observed beating (orange area).

Hatching Systems

Once embryological development is advanced (Figure 3.2; embryos spinning in eggs) and 250 DD have elapsed, egg masses are transferred into hatching buckets suspending into the hatching tanks. Hatching buckets are simple as 5-gallon utility

buckets with a bottom water inlet and hung in and over the hatching tank at an angle so that the water from the bucket gently cascades into the hatching tank (Figure 3.3). As the larvae hatch from the eggs, they swim voluntarily into the hatching tank, but the residual egg cases and unhatched eggs remain in the hatching bucket which is removed once hatching is complete. Hatching can last up to seven to 10 days (Bolton-Warberg et al., 2018; Treasurer et al. 2018) with majority of hatching occurring during the night (Benfey and Methven 1986).

Figure 3.3: Hatching buckets
Lumpfish hatching bucket suspended in larval tank (on left) and close up of hatching bucket (on right).

Development at Set Degree Day Intervals

Lumpfish egg development lasts approximately 280-300 degree days (DD). Figure 3.4 shows a time series of egg development taken at the National Cold Water Marine Aquaculture Center (NCWMAC) in 2018. Eggs were incubated at an average temperature of 12 ℃.

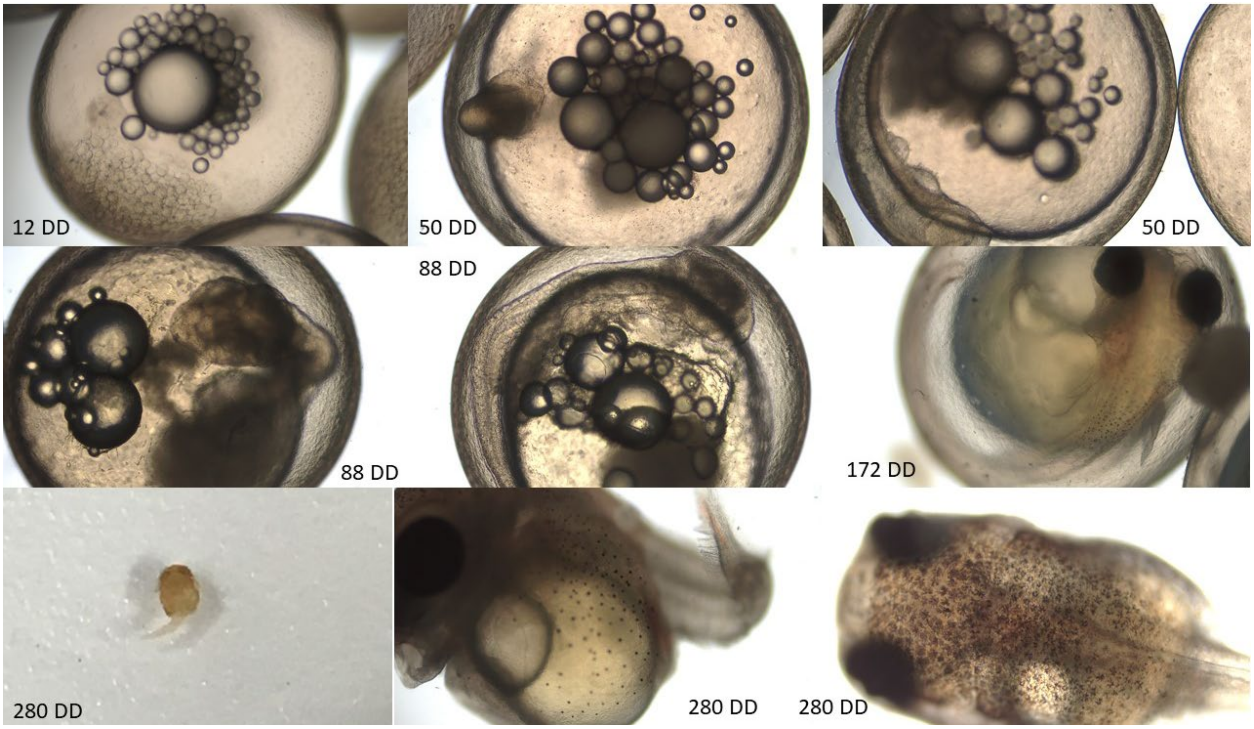

Figure 3.4: Lumpfish egg development time series with degree days (DD) noted

Top Row

Left: Fertilized egg at 12 DD. Note the lipid droplets in the center of the egg and the dividing cells at the 7 o'clock position of the egg.

Center & Right: Embryo development at 50 DD. The center image depicts a head-on view of the developing embryo, while the righthand image is a side view the clearly depicts the development of the notochord.

Middle Row

Left & Center: Embryo at 88 DD. Formation of unpigmented eyes can be seen.

Right: Embryo at 172 DD. The eyes are now pigmented, and the heart can be seen just below the left eye.

Bottom Row

Newly hatched lumpfish at 280 DD.

Chapter 4 First Feeding and Weaning

Newly-hatched lumpfish range from 4.0 to 7.4 mm TL (Benfey and Methven 1986; Brown 1986; Collette and Klein-MacPhee 2002), and feed endogenously for about 4 to 7 days post hatch (dph), depending on temperature, until their yolk sac has been absorbed (Benfey and Methven 1986; Brown et al. 1992). Hatchlings have a well-developed digestive system (Brown et al. 1997), thus enabling hatcheries to start feeding lumpfish larvae directly with a microparticulate diet and altogether avoid live feed culture. However, survival and growth appear higher when larvae are fed enriched *Artemia* prior to formulated feeds (Belova 2015). Further, use of live feed reduces fish feeding intervals and tank cleaning frequencies during the first few weeks as *Artemia* do not settle out of the water column as quickly as formulated feeds do.

Different facilities have developed different strategies for first feeding to balance survival, growth, available resources, and system cleanliness. At the University of New Hampshire's Coastal Marine Laboratory (CML), we feed newly-hatched lumpfish enriched *Artemia* for the first 10 days, then cofeed with a weaning diet for 15 days, followed by gradually weaning the fish off live feed over 10 days so that they are fully weaned onto a formulated diet by 35 dph (Figure 4.1). The National Cold Water Marine Aquaculture Center (NCWMAC) utilizes a strategy of going directly to microparticulate diets. They rear newly hatched fish in 8-inch PVC pipes with a false bottom, allowing uneaten feed to pass through, and utilize automatic feeders.

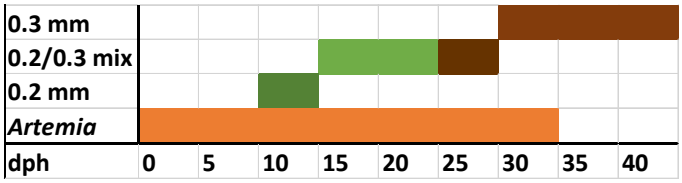

Figure 4.1: Live feed timeline
Overview of the lumpfish live feed and weaning timeline used at the University of New Hampshire's Coastal Marine Lab in 2019-2021. dph: days post hatch.

Live Feed Approach: Enriched *Artemia* Production

Since the complete enriched *Artemia* production cycle takes two full days to hatch, enrich, and feed out, we typically start live feed production about one week prior to expected needs allowing us to work out any unexpected kinks and train new personnel. We culture *Artemia* strictly by a batch procedure, preparing one day's feeding at a time. Our methods are not the state-of-the-art and we are by no means live feed experts, but our system yields reliably good output for research scale *Artemia* production.

Cysts: Dried cysts are available from several suppliers. At the CML, we use a "premium" grade 90% hatchout variety from Brine Shrimp Direct. The amount of *Artemia* cysts needed depends on the quantity of fish larvae requiring feed. At the CML, a 1.8 m diameter tank fully stocked with fish larvae is supplied a minimum of 5.4 million *Artemia* three times per day or 3,000 prey/L. The amount of *Artemia* hatched out simply depends on the number of tanks that need to be fed. Experience suggests that with the premium-grade *Artemia*, 50 g of cysts will yield on average 30 million hatched *Artemia*. However, because *Artemia* cysts are harvested from the wild, there will always be some variability, and counts should be done to quantify the yields and verify consistency.

Decapsulate Cysts or Not?

Artemia cysts in their dried state have a hard, virtually indigestible layer called the chorion that envelops the cyst which can be removed through a decapsulation process. The advantages to doing so not only include the removal of an item that larval fish cannot digest and that potentially could cause gut obstructions and death, but increases cyst hatch rates, and eliminates a potential source for culture contamination. The chorion also causes unhatched cysts to float, as a small amount of air gets trapped underneath it and makes these unhatched cysts somewhat nutritious for the fish, since it becomes completely digestible. The disadvantages to decapsulation are that it adds more steps and time to live feed production, already a laborious process. Further, 100% sodium hydroxide, the chemical required to remove the chorion, is highly caustic and creates an exothermic reaction so careful use is paramount. While at the CML we have decapsulated *Artemia* cysts when culturing other marine finfishes such as winter flounder, we do not do this for lumpfish.

Hatching Setup

a. Tank Requirements:

The ideal hatching container for *Artemia* cysts is one that allows for good circulation throughout the hatching process, as well as providing a convenient method for harvest. At the CML, where production is suited to research scale, we use 17 L clear acrylic hatching cones, with gate valves installed at the bottom of the cones (Figure 4. 2). The cones are mounted in premade PVC stands at a height such that various harvesting containers can fit underneath the valve. During *Artemia* production, at least one hatching cone always is filled with heated, sanitized water and ready for use. The Brine Shrimp Direct cysts used at the CML recommend a density of 1 g/L, however, we usually hatched them at ≤ 3 g/L.

Figure 4.2: Artemia hatching cone set up

b. 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 light source should be provided. If the clip-on lights are utilized, we recommend 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.

c. Aeration Requirements:

During the hatch, the cysts must remain in constant suspension through high 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. This produces an adjustable supply of large bubbles that keep the cysts in suspension and the dissolved oxygen at saturation. The air should be turned up relatively high for hatching, as good oxygen saturation and good circulation are 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.

d. Temperature Requirements:

The ideal temperature for *Artemia* hatching is generally 28-30 °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. Ensure temperature is monitored several times daily, especially at the beginning of the annual production cycle as we have found temperature settings and output can vary from heater to heater.

A supply of warm (30 °C) seawater, which is used mainly for rinsing *Artemia* between the hatching and enriching phases, also is needed. At the CML, we create a warm seawater reservoir by filling a 220 L, plastic tank with filtered, UV sterilized water and heating the water with a single 300 W aquarium heater. A small submersible pump attached to a length of 0.5-inch 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 needed. The reservoir is refilled at the end of each day, bleach added, and then neutralized the following day when needed.

Enrichment Setup

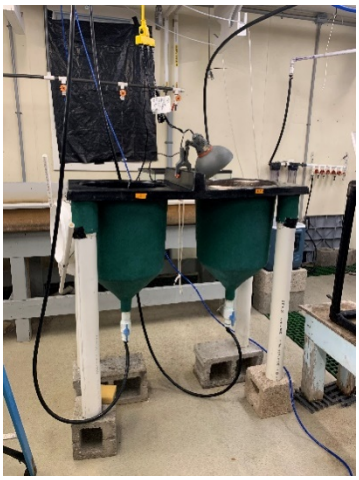

Figure 4.3: Artemia enrichment tank set up

a. Tank Requirements:

After hatching, the *Artemia* need to be enriched so that they are more nutritious for larval fish. The ideal enriching container is one that allows for good circulation as well as providing a convenient method for harvest. At the CML, *Artemia* are enriched in opaque, semi-conical, fiberglass, 40 L tanks with a valve installed at the bottom (Figure 4.3). 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. In addition, because the *Artemia* are hatched and active when they are placed in the enrichment tank, the tank should already be at temperature (i.e., has been filled, sanitized, and heated the prior day).

b. Lighting Requirements:

As with the hatching process, light also is required for the enrichment. At the CML, light is provided by a clip-on spotlight suspended above and pointed down at the tank. The light should be far enough away so that the heat produced will not affect the temperature of the water.

c. Temperature Requirements:

The ideal temperature for enriching *Artemia* is generally 26 °C. At the CML, this is provided by standard 300 W aquarium heaters, one in each enrichment tank. Ensure temperature is monitored several times daily, especially at the beginning of the annual production cycle as we have found temperature settings and output can vary from heater to heater.

d. 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, air is introduced through the bottom valve of the tank. During enrichment, the water in the tank should resemble a rolling boil, however, 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 quickly in the enrichment tank, therefore, supplemental oxygen is required once the enrichment is added. We do this via welding-grade oxygen delivered to an ultra-fine pore diffuser located near the bottom of the tank to maintain dissolved oxygen levels at 100% saturation.

Water Preparation and Sanitation

Culture water for all forms of live feed at the CML is ambient salinity seawater pumped through a filter contained activated glass media, 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. 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.

Although household bleach is readily available and easy to work with, it contains stabilizers that extend the time of potency. Because of these stabilizers, aeration is often unable to neutralize the chlorine. Therefore, we use a sodium thiosulfate solution in combination with aeration to neutralize the bleach. 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 hatching or enrichment tanks for the first time, turn the aeration system down to a minimal level after adding the dose of chlorine. This will provide maximum sanitation in the time allowed and prevent any reduction in potency caused by aerating the water. After the sodium thiosulfate is added (typically 24 hrs later), aeration is increased to aid in neutralization and also for circulation and degassing during the actual culture period.

Counting Artemia

It is important to count hatched *Artemia* to determine the yield of cysts from a particular lot (hatched *Artemia*/gram of cysts). This is accomplished by pipetting a small volume (0.1 - 0.2 mL) of hatched *Artemia* onto a counting slide, adding a few drops of formalin and waiting approximately 30 minutes for all the *Artemia* to die. Then with a counter, number of hatched *Artemia*/sample can be extrapolated to estimate the total quantity. At the CML, *Artemia* are counted each day after the hatching phase using a sample of hatched *Artemia* from the enrichment tank (but prior to the enrichment being added) 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.

Hatching, Enriching, and Harvesting Process

a. Hatching Artemia:

Hatching out *Artemia* cysts essentially consists of weighing out the appropriate aliquot of cysts or decanting the correct volume of decapsulated *Artemia*, adding them to the hatching cone, and waiting 22 hours. We add cysts to hatching cones at 14:00 so they are ready for harvest at 12:00 the following day.

b. Harvesting Hatched Artemia:

We harvest hatched *Artemia* at 12:00 each day. To maintain the warm culture and enriching environment, 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. Ensure that the reservoir has sufficient volume, is the right temperature, and has been properly neutralized with sodium thiosulfate prior to harvesting hatched *Artemia*. Harvest the hatched *Artemia* by removing the heater, turning off the aeration, and angling the light to the bottom of the hatching cone to attract the phototactic *Artemia* to the valve at the bottom of the cone. Wait until the *Artemia* have settled to the bottom creating obvious color delineation in the tank, then harvest them via the valve into a 105 micron sieve suspended in a rinsing bucket supplied with the warm reservoir water and an aeration source (air stone; Figure 4.4). Do not allow the clear water and brown-colored, unhatched cysts to drain into the sieve; dump this residue down the drain. Rinse the harvested *Artemia* for 10-15 minutes or until the rinse water is clear, then wash the *Artemia* from the sieve gently into the enrichment tank that has already been filled, heated, sterilized, and neutralized.

Figure 4.4: Artemia harvesting set up

c. Enriching Artemia:

Although hatched *Artemia* are added to the enrichment tanks by 12:30, we do not add enrichment products until 17:30 since only 16-18 hours are suggested for enrichment and we want to harvest the enriched *Artemia* at 10:00 the following day. Just prior to adding the enrichment product we turn on the supplemental Oxygen. We use Ori-Green (Skretting) as the enrichment, added commensurate with enrichment tank volume and *Artemia* density. For a 40 L tank, 16 g Ori-Green is weighed out, blended with 1.5 L freshwater, and poured into the enrichment tank.

d. Harvesting Enriched Artemia:

Enriched *Artemia* are harvested by 10:00 each day and put into cold storage. Since they will be fed to larval fish, the enriched *Artemia* can be harvested from the tank into a 150 micron sieve,

and rinsed with whatever clean, cold seawater (similar temperature to what the fish are raised in) is available for 10-15 minutes or until the rinse water is clean. The colder the rinse water, the better as it will slow the *Artemia*'s metabolism and prevent them from absorbing the enrichment. With this method, a 24-hr supply of *Artemia* is harvested at once and then fed out or stored for later feedings.

e. Storing Enriched Artemia: To retard absorption of the enrichment and limit *Artemia* growth, harvested enriched *Artemia* should be stored at 4-6 °C ideally until use. This can be something as simple as a cooler, supplied with aeration and frozen ice bottles that get periodically replaced over the cold storage period as the ice thaws (Figure 4.5). At the CML, the enriched *Artemia* harvested in the morning is partitioned out into thirds with feedings to lumpfish occurring around 12:30, 18:00, and the following day at 8:00.

Figure 4.5: Cold storage set up for harvested enriched Artemia

Weaning

Co-feeding: The weaning of live feed begins with a period of co-feeding in which both enriched *Artemia* and a 0.2 mm diet are fed to the lumpfish (Figure 4.6). During the co-feeding period, *Artemia* rations do not change, but the weaning diet is provided to the fish prior to the *Artemia* to introduce the lumpfish to the dry diet. At first the diet is offered three times/morning, then feedings and diet size increase to hourly and 0.2/0.3 mm, respectively until approx. 26 dph.

Weaning: When weaning in earnest begins, the frequency and amount of live feed offered to lumpfish decrease (Figure 4.6). First the morning live feed ration is eliminated, followed five days later by the elimination of the midday live feed ration and the increase of feed size from the 0.2/0.3 mm mix to just 0.3 mm diet. Another five days later, the evening live feed ration ends and lumpfish only are fed 0.3 mm diet.

Feeding provisioning: Due to the nature of the fine weaning diets, they are susceptible to clumping up if exposed to any moisture or high humidity and, thus, using them in automated feeders can be difficult. If automated feeders are used, it is important to ensure they do not get clogged and are tested frequently. Providing several additional hand feedings per day is recommended to observe fish behavior to see if adjustments are needed in the weaning schedule.

Tank maintenance: Once co-feeding starts, the lumpfish tanks will require more frequent cleaning and water quality will need to be monitored more carefully. Lumpfish will attach to all smooth surfaces but seem to be less attracted to light colors (Spada, 2021). At CML, our lumpfish tanks are black-walled, with pale blue bottoms and we suspend dark gray pvc panels in

the tank to increase surface area (Figure 4.7). Still, small lumpfish will adhere to the tank bottoms so careful siphoning into a contained catch basin is needed (see Ch. 8 General Husbandry for further info). In addition, at this time, we add supplemental oxygen to the lumpfish tanks to maintain 100% saturation.

Co-feeding Schedule

Starting	dph		8am	9am	10am	11am	12pm	1pm	2pm	3pm	4pm	5pm	6pm	7pm
9/10	13-16	Artemia												
		0.2 mm												
9/14	17-25	Artemia												
		0.2/0.3 mix												
		hand feed												

Weaning Schedule

Starting	dph		8am	9am	10am	11am	12pm	1pm	2pm	3pm	4pm	5pm	6pm	7pm
9/23	26-30	Artemia												
		0.2/0.3 mix												
		hand feed												
9/28	31-35	Artemia												
		0.3 mm												
10/3	36	Artemia												
		0.3 mm												

Figure 4.6: Weaning schedule
Example of co-feeding and weaning schedules for lumpfish at the University of New Hampshire's Coastal Marine Lab in 2019-2021. Shaded bars indicate one feeding per hour. Schedule modified from Memorial University of Newfoundland's lumpfish co-feeding and weaning schedule.

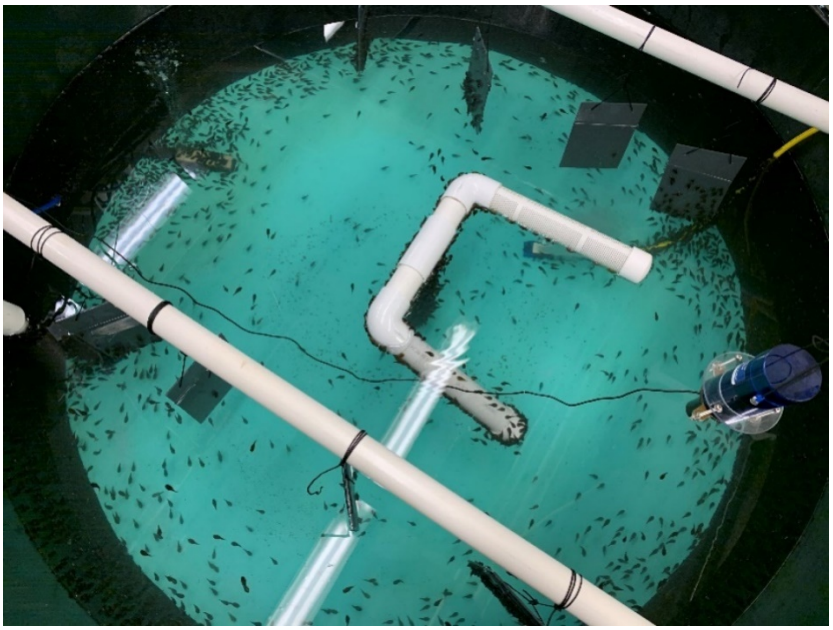

Figure 4.7: Larval rearing tank
Lumpfish rearing tank at the UNH Coastal Marine Laboratory with suspended PVC panels to increase tank surface area.

Microparticulate Diet Approach

Figure 4.8: "Pipe" hatching tank
NCWMAC developed pipe hatching tank containing newly hatched lumpfish.

Prior to hatch, eggs are transferred to the hatching tank. The NCWMAC has developed a hatching tank that consists of 8-inch PVC pipe set into an 8-inch coupling with no-see-um mesh sandwiched in between to create a false bottom to the tank (Figure 4.8). The hatching tank is set within a larger tank and water flow is placed directly into the hatching pipe. To clean the tanks, the pipe can be moved within the larger tank, to another tank, or to a bucket of water and then the original tank can be siphoned easily with no impact to the larval lumpfish. Every several days the bottom of the pipe should be cleaned by using gentle water flow to wash any accumulated feed through the mesh bottom of the pipe. The use of a false bottom tank of some type is important in the success of going directly to microparticulate diets. Solid bottom tanks will need to be siphoned daily to maintain cleanliness, increasing the risk of damaging larval lumpfish and reducing survival. Also, use of the NCWMAC pipe hatching tanks is not recommended if feeding live feed as the live feed can disperse into the larger tank, reducing the value and effectiveness of live feed. However, conical tanks with false bottoms will work with either feeding strategy.

Fish are fed a microparticulate diet with increasing size. They are started on a 150 µm sized diet at 1 day post hatch (dph) and stay on this diet for the first 2 weeks (Figure 4.9). At 15 dph, they are transitioned to a 1:1 mix of 150 and 300 µm sized diets until 30 dph when they are transitioned to 300 µm diets. At the NCWMAC we have utilized several automatic feeders, with limited success, and supplemented with hand feeding. The best combination we have used to date is the Fish Mate F14 feeder set to feed at 9:00, 12:00, 15:00 and 18:00. A minimum of two feeders per tank are needed as humidity will moisten the microparticulate diets creating a gummy paste. The feeders should be rotated such that each morning prior to the first feeding, a new feeder with dry feed is placed in a position to feed the pipe. This schedule can then be supplemented with regular handfeeding in-between regular feedings.

Gemma 300							
Gemma 150/300 mix							
Gemma 150							
dph	0	5	10	15	20	25	30 +

Figure 4.9: Microparticulate diet timeline
Overview of the lumpfish microparticulate size feeding timeline. dph: days post hatch.

Chapter 5 General Juvenile Husbandry

Once lumpfish hatch, they immediately use their ventral suction disc to adhere to any available surface, including the walls and bottoms of the larval and grow out tanks (Figure 5.1), which means care must be taken during routine maintenance to avoid cleaning gear – fish interactions. Adjusting tank design and general rearing protocols to promote fish growth, survival, and welfare while also minimizing issues pertaining to water quality, tank cleaning, and fish aggression is important for any hatchery. Tools that may aid in achieving this for lumpfish production include tank bottom type, tank color, size grading, and stocking densities.

Figure 5.1: Juvenile lumpfish
Juvenile lumpfish adhere to all tank areas, though less so on lighter surfaces than darker surfaces.

Juvenile Rearing Tanks

Tank Bottom Type: Modifying tank bottoms and color can be an effective method to reduce juvenile lumpfish adhesion. Lumpfish prefer smoother surfaces to rougher ones, providing hatcheries with a tool to deter the adhesion of larvae and juveniles to specific tank areas. In a study at the University of New Hampshire's Coastal Marine Laboratory (CML) with small, juvenile lumpfish (mean initial size: 0.35 g; mean final size: 1.6 g) we evaluated a series of different tank bottoms including smooth vs rough, light vs dark, and false bottom tanks (Spada 2021). All tanks provided a good rearing environment for the juvenile lumpfish but some resulted in fewer lumpfish utilizing the bottom and thus making tank cleaning easier and faster. We found that the false bottom tanks were the most efficient in terms of reduced cleaning time needed and promoted the highest fish growth and survival. Significantly fewer juvenile lumpfish adhered to rough bottomed tanks compared to smooth bottom tanks, but while these tanks proved to be effective at deterring lumpfish from sticking, this was at the cost of cleaning efficiency. Rough bottomed tanks were more difficult to clean and we speculate that because of the increased surface area, they may enable pathogens (i.e., bacteria) to seed in the tanks more easily than smooth bottomed tanks. While lumpfish adhesion to tank bottoms was highest in smooth bottomed tanks, altering bottom color provided a useful strategy to deter lumpfish from occupying the tank bottom. Smooth light bottom tanks had significantly lower lumpfish adhesion than the smooth dark tanks. Therefore, if using false bottom tanks is not possible, altering bottom color may be a feasible, economical solution for lumpfish facilities with limited budgets or

resources, achieved by painting the bottom of existing tanks with a light colored, nontoxic marine paint.

Tank Color: While tank color may affect where small juvenile lumpfish adhere, tank color does not seem to influence the growth, survival, or aggression of larger juveniles. In another study at the CML, 6 g juvenile lumpfish were grown out to 39 g in either blue, black, red, green, gray, or white tanks (Spada 2021). There were no significant differences between fish survival, growth rates, or aggression (fin nipping) in any of the different colored tanks.

At the CML, juvenile lumpfish are reared in flat-bottomed tanks with black sides and pale blue bottoms (Figure 5.1). Dark gray PVC panels are hung in the tanks to provide more surface area for the lumpfish to adhere to. We observe that there are more fish adhered to the panels and sides of the tanks than the bottom of the tanks, but fish still do utilize the tank bottom area.

Tank Cleanliness and Maintenance

Figure 5.2: Cleaning a tank
Using the vacuum siphon to clean juvenile lumpfish tank. Notice the T shaped head to the siphon. The head has weather stripping glued to the bottom edge to encourage fish to move. The siphoned water goes into a 1 mm mesh sieve to collect any live fish that get siphoned up.

Once dry diets become part of the daily feeding regime, excess feed will accumulate on the tank bottoms necessitating regular tank cleanings or water quality will deteriorate. Cleanings can be done by using a siphon to vacuum up the uneaten feed and fish feces. However, because some lumpfish will occupy the tank bottoms regardless of tank color, care must be taken to avoid these fish. At the CML, we use a custom-made vacuum made from pvc and tubing. At the bottom of the vacuum head and on the far side of the intake area, a strip of weather stripping is glued to increase the vacuum suction and to dislodge any waste materials from the tank bottom. To use it, the vacuum is positioned on the tank bottom, the siphon is started, and the vacuum is pulled very slowly from one area of the tank towards the person cleaning the tank (Figure 5.2). The siphon tube empties into a 1 mm mesh sieve positioned in a collecting

Figure 5.3: Sieve bucket
System used to collect siphon effluent when cleaning tanks. The 1-mm suspended sieve retains any larval fish siphoned up, while feed and feces mostly pass through.

bucket filled with the tank water (Figure 5.3) so that any live fish sucked up by the siphon are collected and can be returned to the rearing tank. As the fish grow in size and larger pellets are used, the vacuum head is not needed and a larger siphon can be used to clean the tanks.

Stocking Density, Size Grading, and Fish Aggression

Lumpfish grow relatively quickly in their first year of life, reaching approximately 35 to 70 mm TL (Martin-Robichaud 1991), are aggressive and establish social hierarchies within the tanks, and as a result, fish size within a cohort can be quite variable necessitating frequent size grading of fish to limit fin nipping and adjusting tank densities to regulate growth rates (Jonassen et al. 2018).

Moving Fish: Transferring fish from one tank to another can be done in several ways. A siphon can be a safe, useful tool to move small fish between tanks. For larger juveniles, various sized, small mesh, dip nets are helpful for transferring dozens to hundreds of fish at a time. Further, if panels are suspended in tanks, these can be relocated to clean tanks with any fish adhered onto them. However, keeping track of how many fish are being moved in batches can be difficult. If you calculate the mean weight of the fish by individually weighing a subsample, then the tared dipnet full of fish (and blotted to remove excess water), can provide an estimate of total biomass transferred. Alternatively, for small fish, taking a photo of the tank or receptacle the fish are gathered into can provide a count.

Stocking Density: There is no known standard stocking density that commercial lumpfish hatcheries use for growing juvenile fish at various sizes. Reported stocking densities range from 10 to 43 g/L until the lumpfish are deployed as cleanerfish in salmonid farms (Treasurer 2018). At the CML, we evaluated the effects of four stocking densities (40, 60, 70, 90 g/L) on the growth, survival, and aggression of small (mean initial size: 2 g; mean final size range: 6-9 g) and large (mean initial size: 13 g; mean final size range: 50-72 g) juvenile lumpfish over eight weeks (Spada 2021). Survival and fish aggression were unaffected by the different density treatments. Survival was 100% for all treatments (fish sizes, densities). Occurrence of fin nipping ranged from 1 to 21% but density did not significantly affect fish aggression within a single fish size class (small, large juveniles). However, aggression was significantly higher amongst large juvenile lumpfish (21%) in tanks at 70 g/L density than in small juvenile lumpfish (1%) at the same density. Fin nipping that did occur was minor and ranked <1 on a qualitative scale of 0 (no fin damage) to 5 (lack of caudal peduncle). For both lumpfish size classes, growth was affected by stocking density with fish growth increasing as stocking density decreased.

Size Grading: As the juvenile fish grow and visible fish size differences exist in the rearing tanks, size grading becomes important to disrupt any established social and feeding hierarchies. Also, if fin nipping is observed, size grading the tanks and reducing fish density may be beneficial. Adjustable, floating bar graders with smooth, rounded grading slots are easy to use with lumpfish (Figure 5.4). Using dip nets, you can transfer small batches of juvenile lumpfish into the grader situated in a new seawater filled tank. The small fish will swim down and through the grader slots while the larger fish will be confined to the grader and can be transferred to another tank designated for large fish. Some rocking of the grader is required as the lumpfish will stick to all surfaces and must be "encouraged" to swim around. Transferring the larger fish can be as simple as upending the grader into the large fish tank.

Figure 5.4: Floating adjustable bar grader
An adjustable bar grader used for size grading juvenile lumpfish. The grader should be gently rocked to keep fish swimming and not sticking to the grader.

Figure 5.5: Lumpfish furniture

Minimizing Aggression: Under intensive culture, lumpfish are prone to suction cup deformities, fin damage, and body damage, all of which can result in emaciated fish (Rabadan et al. 2021). Anecdotally, hatcheries report that lumpfish seem to be most aggressive around 5-7 g in size. While we did not observe that in the stocking density study at the CML in which we compared small and large juvenile lumpfish, larger juveniles under certain conditions may be more aggressive than their smaller counterparts (Spada, 2021). Stocking density, temperature, fish size, and lighting regime may all be factors that contribute to aggression in juvenile lumpfish. The lack of surface area in rearing tanks is, in part, attributed to an increase in aggression (Jonassen et al. 2018) so providing "furniture" or suspending plastic panels in the tanks is a common technique used by facilities (Figure 5.5). It is important to minimize aggression in cultured species to maximize production, profit, and ensure a high standard of animal welfare.

Record Keeping

As with rearing any organism, accurate record keeping is important for monitoring and tracking water quality and fish health, and for all personnel to understand what has happened and what is expected to happen. There should be standardized data sheets for each tank, filled out daily, and uploaded to a database. Key parameters to consider, depending on rearing systems, include:

Date
Fish age: degree days (eggs), days post hatch (juveniles)
Water quality: temperature, salinity, dissolved oxygen, ammonia, nitrate, nitrite, pH
Feed: type, size, quantity, frequency
Mortalities: quantity, size, possible cause
Cleaning schedule
Growth: routine subsampling
Any daily activities or observations
Who conducted the task

A good rule of thumb when it comes to record keeping is: “if you don’t write it down, you didn’t do it.”

In addition, detailed, written or video protocols and checklists are helpful for hatchery personnel.

Chapter 6 Nutrition

The culture of lumpfish use for sea lice control is relatively new, thus lumpfish nutritional requirements are also new and evolving. The original diet used for lumpfish growth was a standard marine diet containing 55% protein and 15% lipid with most of the protein and fat sourced from fishmeal and fish oil. However as feeding frequency, temperature, feed type, and alternative ingredients are investigated, but albeit still very limited, lumpfish diet formulations and feeding strategies are evolving. Current commercial lumpfish diets contain 57-59% protein and 15% lipid. Further study on lumpfish nutrition is needed to increase the scope of knowledge available to researchers and lumpfish producers.

Basic Diet Requirements

In a study at the University of New Hampshire's Coastal Marine Laboratory (CML) with small, juvenile lumpfish (mean initial size: 8 g; mean final size ranges: 65 to 76 g), we evaluated a series of experimental diets composed of varying inclusion levels of protein (50, 55 %) and lipid (10, 15, 20 %; Spada 2021). Overall, lumpfish grew well regardless of the protein/lipid diet formulation they were fed with overall mean percent growth ranging from 685 to 781% over the 10-week testing period. No differences were observed in feed conversion ratios (FCR), specific growth rates, mean weight gain, or overall percent growth. Lumpfish need a higher protein diet with lower lipid levels compared to salmonids. Despite this, when fed a salmonid feed (BioTrout) with only 47% protein and 24% lipid, juvenile lumpfish still grew, albeit at a significantly slower rate and with a higher FCR, compared to the lumpfish fed the experimental diets (Spada 2021). Final mean percent growth and mean weight for lumpfish fed the salmonid feed was only 394% and 41 g, respectively. An important distinction between the experimental diets and the salmonid diet was protein source; the primary protein source in BioTrout is soy.

However, as opportunistic omnivores, juvenile lumpfish can grow and perform better when fed diets that are a 50:50 mix of fish meal and plant-based meal than diets that are strictly plant-based protein or fish meal (Willora et al. 2020). In a study conducted by Willora et al. (2020), neither growth, muscle fiber cellularity, nor chemical composition was impacted in juvenile lumpfish fed a 52 % protein /14 % lipid diet where 50 % of the protein was fish meal and the other 50 % was a 1:1 mix of soy protein and pea protein concentrate. Fish fed this treatment were significantly longer, wider (fish height), and heavier compared to fish in the other diet treatments. Since lumpfish exhibit diet plasticity, growers may be able to cut costs and create feeds that are more sustainable, with less reliance of fishmeal, without adversely impacting fish growth. However, beyond these two studies (Willora et al. 2020, Spada 2021), juvenile nutrition studies in the hatchery are lacking. Lipid composition, lipid requirements, fatty acid requirements, astaxanthin, novel ingredients (single cell protein, insect meal, algal meal), amino acid requirements, and additional plant-based ingredients all need to be investigated in future studies.

Basic Feeding Strategies

Optimization of lumpfish feed efficiency is a recognized research need given that basic knowledge of juvenile lumpfish feeding frequency and rations is largely unknown, especially relative to temperature. Juvenile lumpfish < 120 g grow best at temperatures between 13 ℃ and 16 ℃, with smaller fish preferring the higher temperatures (Nytrø et al. 2014). Fish > 120 g prefer lower temperatures (~9 ℃) compared to the smaller fish (Nytrø et al. 2014).

Lumpfish prefer slow sinking pellets. If the fish are not fed often enough during the day, the food will sink to the bottom and if they are fed too often, only the most active fish will get fed. However, some lumpfish will eat pellets off the tank bottom.

Lumpfish facilities feed juveniles anywhere from 2% to 10% body weight/day, mostly in flow-through ambient temperature systems, with rations decreasing as fish size increases. A feeding rate of 3-4% is reported by Jonassen et al. (2018) but the derivation of these values is not reported.

There is some evidence that lumpfish fed with a satiation feeding strategy (diet provided over 3 days each week) have lower rates of cataracts and reduced gut inflammation compared to fish fed using a regular feeding strategy (diet provided over 7 days each week), however, growth was reduced until fish reached about 100 g (Imsland et al. 2019).

Diets can be administered by hand or with autofeeders. The smaller the diet size, however, the more affected it will be by moisture or humidity and the greater tendency it will have to clump up in autofeeders. At the CML, we use vibrating autofeeders (Figure 6.1) with diets as small as 0.2 mm but the feeders must be checked regularly, all clumped feed residue removed from feeder openings, and feeders readjusted. At the National Cold Water Marine Aquaculture Center (NCWMAC), they have similar issues with clumping feed but have had some success using the Fish Mate F14 feeder for diets as small as 150 µm.

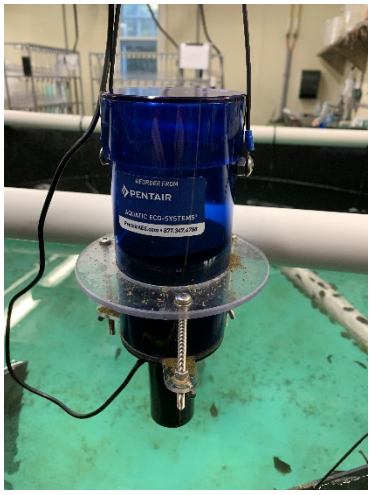

Figure 6.1: Vibratory feeder Juvenile lumpfish tank at the UNH Coastal Marine Laboratory with feed supplied via a vibratory autofeeder.

Feed Sizes for Various Sized Fish

Other factors to consider in feeding lumpfish are pellet size. If fish are fed a pellet that is too large, the pellet will not be consumed and removed from the system and if the pellet is too small, the fish will not be able to consume enough feed to support its growth. Most commercial manufacturers have a table that recommends the correct pellet size for the life stage of the fish which some lumpfish facilities have adapted for their own needs (see Table 6.1). Keep in mind that lumpfish grow relatively quickly in their first year of life so regular sampling of fish to track growth is recommended.

Table 6.1: Suggested feed sizes for lumpfish of varying sizes
Provided by G. McBriarty (Cooke Aquaculture).

Average Fish Weight (g)	Feed size (mm)
0.1	**0.3/0.5**
0.3	**0.3/0.5**
0.8	**0.5/0.8**
1	**0.5/0.8**
2	**0.8/1.2**
4	**1.2/1.5**
8	**1.5/1.8**
16	**1.8/2.0**
32	**2.0/3.0**
65	**3.0/4.0**
80	**3.0/4.0**
75	**4.0**
105	**4.0**

Chapter 7 Biosecurity and Potential Disease

At any fish rearing facility, the risk of experiencing a disease outbreak is always a concern. Fortunately, there are many steps that fish culturists can take to help reduce the risk of disease occurring at their facility. The basic principles used to reduce the risk of a disease outbreak are generally referred to as biosecurity. There are a number of references available for an overview of potential biosecurity options. A good place to start is the fact sheets available from the Regional Aquaculture Centers "Biosecurity on the Farm – Guidelines & Resources for Developing a Biosecurity Plan" (NRAC, Pietrak et al. 2010), "Biosecurity for Aquaculture Facilities in the North Central Region" (NCRAC, Dvorak 2009), "Biosecurity in Aquaculture, Part 1: An Overview" (SRAC, Yanong and Erlacher-Reid 2012) and "Biosecurity in Aquaculture, Part 2: Recirculating Aquaculture Systems" (SRAC, Yanong 2012).

Biosecurity

Every facility should develop its own unique biosecurity plan. A basic biosecurity plan will consider the layout, species being reared, and operations of a facility. It will identify where it might be best and easiest to reduce any potential risk that is identified and how that might be best accomplished. Finally, and most importantly the plan will be written down in some form. A facility's plan should not be static but should be evaluated on a regular basis and adapted as either resources, risks, or operations change. The Hazard Analysis and Critical Control Point (HACCP) methodology can be very useful in creating a biosecurity plan. The factsheet "Biosecurity on the Farm – Guidelines & Resources for Developing a Biosecurity Plan" (Pietrak et al. 2010) from the Northeastern Regional Aquaculture Center (NRAC) can be a good starting point for developing a plan for your facility. It is important to remember that a biosecurity plan does not eliminate potential risks, but it will reduce them.

Common Biosecurity Practices

There are some simple steps that can be taken as part of a biosecurity plan. These steps are only helpful if they are done as part of thought-out, coordinated approach.

Establish a relationship with a fish health professional: One of the most important steps you can take is to establish a relationship with a fish health professional. This may be a veterinarian, extension professional, fish health lab, or other similar professional. They will be able to help you develop your biosecurity plan and advise you on practical steps to take to reduce risk. They will also be able to help you, in the event of a disease outbreak, to identify the pathogen and develop an appropriate treatment. Just like our own health, it is often far less costly to take preventative steps than treat an outbreak. A good fish health professional will be able to work with you to determine and implement the best strategies for your facility and situation.

Cleanliness: Maintaining a clean dry facility can be effective in helping to prevent the spread of pathogens within a system. While it can be challenging to keep floors and surfaces dry, most aquatic pathogens can spread through water from tank splashes to the floor to feet or hands or equipment and then to other tanks. Cleaning empty tanks or systems as soon as possible, disinfecting them, and keeping them dry when not in use can help to prevent pathogens from spreading between groups of animals. Similarly, cleaning and disinfecting equipment such as nets, totes, rain gear, and other supplies immediately after use will help to prevent pathogen spread within a facility.

Equipment: Equipment can often act as a fomite, or a means of spreading pathogens between systems. Keeping equipment clean and dry as recommended above is one good step. However, where possible it is good to have separate equipment for each room or group of fish. For example, have separate nets that are only used in a given room or system or provide staff with boots that stay at the facility and are only worn there.

It is also important to consider what materials equipment is made from. Where possible, you should avoid equipment made from wood or other porous materials. Instead, non-porous materials such as plastic, metal, or fiberglass can be more easily cleaned and disinfected.

Footbaths: Footbaths are a common biosecurity measure. We unintentionally carry pathogens around on our feet as we move from one location to another over the course of our normal daily activity at a facility. While wearing footgear specific to the facility is excellent at preventing the unintentional introduction of pathogens from outside the facility, it does not help to reduce the potential spread of pathogens within the facility. It also only helps if everyone entering the facility wears facility specific footwear upon entering or puts on shoe covers. Footbaths placed at doors and entry points to rooms and the facility can help reduce the spread of pathogens coming into the facility and spreading within the facility. The other advantage with footbaths is that they are a constant visual reminder of biosecurity. Because biosecurity is only as effective as you make it (i.e., if people choose to step around the footbath, it will not be effective), the same is true if staff chooses to not follow established procedures and practices.

Figure 7.1: Footbath at an aquaculture facility

Disease-free Fish: One of the biggest opportunities to bring disease into a facility is in any new groups of fish that are brought into a facility. There are several potential ways to minimize this risk. Whenever possible, only bring in fish from facilities that have an established fish health

history free from pathogens. These facilities are often tested on a semi-annual or annual basis following established protocols set by federal, state, or local regulations. Alternatively, many of these authorities recognize the standards set forth by the American Fisheries Society (AFS) Fish Health Section Blue Book (USFWS and AFS-FHS 2014) as the standard for sampling. Maine is currently the only state with fish health guidelines for lumpfish (see Resources).

If you are unable to source gametes or fish from facilities with an established disease-free history, then the next best option is to quarantine the animals and send a portion of them off for disease testing. The number needed to be sent off depends on a range of factors and you should consult with your fish health professional in advance to determine the appropriate number to send for testing.

Finally, in some cases you simply will not be able to send a sufficient number of fish in for testing and still have enough left for the intended purpose they were brought into the facility. Before bringing these fish into your facility, you should consult with your fish health professional about the potential risk the fish pose based on their age, source, and your risk tolerance. These fish should be quarantined at your facility when they do arrive. You should always make sure you receive any required permits to import fish into the country, across state lines, or move them within the state. In many situations, the health status of the fish will be one aspect that regulators will consider before approving any fish movement.

How to Spot Sick Fish and Sending Samples for Testing

Good observation skills are the best way to spot sick fish. However, spotting sick lumpfish can be a little more challenging than with other species due to their sedentary nature at times. The most important aspect to spotting potentially sick fish is to really observe your fish when they are healthy. This will provide you with a good understanding of what their normal behavior is like. Do they swim to the surface when you open a tank lid? How do they respond to feed being placed in the tank? How much to they eat each day? How much uneaten feed is in the tank? How do they utilize potential shelter or surface area enhancements you might have in the tank? What color are they normally? These are some potential behaviors you should look at daily to understand your fish. Often some change to their appearance or behavior of the fish is the first clue to potential problems in the tank.

If you spot fish that are behaving in an unusual manner or have an altered physical appearance, often becoming darker in color, you should begin investigating if all the system parameters are operating within the normal and expected ranges. Is the water quality optimal? Is there more or less uneaten feed than normal? Are all the various systems functioning normally? If everything appears to be normal, then there is the potential for a pathogen to be the cause. Even if you discover an issue with the system, you should continue to monitor the fish closely after correcting the issue. Many pathogens, especially some common bacterial pathogens, are very common in systems and typically do not normally cause problems until the fish become stressed from some other issue. Finding and fixing any issues early may prevent opportunistic infections

from becoming established or enable the fish the ability to fight off the infection on their own. If you continue to see abnormal behavior or any mortalities, you should consult with your fish health professional. Likewise, if you notice a steady but low level of mortalities, or a sudden increase in mortalities, any new or worsening wounds, brown, black, or red patches/spots on your fish, you should consult with your fish health professional. It is likely that they will want you to send off one or more fish for diagnostics. Generally, it is not advised to send off dead fish unless they are freshly dead. The preferred options are to send off moribund (near death) fish or fish that are alive, but appear to be sick, along with a healthy or normal appearing fish. Selected fish should be humanly euthanized according to your facility's procedures. They should then be placed in individual labeled bags, packed on ice, and shipped overnight to the diagnostic lab or where directed by your fish health professional. The goal is to keep the fish cool, but you do not want them to freeze. NRAC has a good fact sheet on how to prepare your fish for shipping to a diagnostic lab titled "General Fish Health Management" (Bowser 2012).

Lumpfish Pathogens

There are a number of potential pathogens for lumpfish and the list is constantly expanding the longer the fish are reared in captivity. Below is an incomplete list of pathogens of either regulatory concern or known to have been found in the Northeastern US.

Bacterial Pathogens

Aliivibrio logei
Aliivibrio salmonicida
Aliivibrio wodanis
Photobacterium sp.
Pseudomonas anguilliseptica
Vibrio splendidus
Vibrio lentus
Aeromonas salonicida masoucida
Vibrio ordalii
Listonella pelagia
Vibrio anguilliseptica
Aeromonas salonicida
Moritella viscosa
Vibrio anguillarum 02β

Viral Pathogens

Viral Hemorrhagic Septicemia (VHS)
Lumpfish ranavirus
Lumpfish flavivirus
Infectious Salmon Anemia (ISA)
Infectious Pancreatic Necrosis Virus (IPNV)
Nodavirus

Parasites

Gyrodactylus cyclopteri (Pietrak and Rosser 2020)
Nucleospora cyclopteri (Mullins et al. 1994)
Trichodina sp. (Powell et al. 2018a)

Fungus

Exophiala sp.

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Resources

This section contains various resources that could be helpful to various operations in a hatchery. Things are organized by their primary use and links to potential vendors are provided where possible.

Mention of trade names, commercial products, or retailers in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture or the University of New Hampshire.

Modified Turbot Extender (From Pountney et al. 2020)

NaCl	4.0908 g/L
KCl	0.1118 g/L
$CaCl_2$	0.2996 g/L
$MgCl_2$	0.5807 g/L
NaHCO3	2.1002 g/L
BSA	10 mg/L
Glucose	36.032 g/L
pH	8.1
Osmolarity	400 mOsm/kg

Herring Ringer's (From Pountney et al. 2020)

NaCl	12.0386 g/L
KCl	0.5367 g/L
$CaCl_2$	0.2331 g/L
$MgCl_2$	0.3141 g/L
NaHCO3	0.0840 g/L
BSA	10 mg/L
pH	7.8
Osmolarity	405 mOsm/kg

Spawning Products

Commercial gonad macerator
Whirl Pak filter bags: SKU B01385
Hemocytometer
Disposable urinaylsis slides: https://www.fishersci.com/shop/products/quick-read-precision-grid-urinalysis-slide/22171725#?keyword=urinalysis%20slide or Kova® Glasstic® slide 10 with grid (Product # 87144)
Hematocrit tubes
Hematocrit centrifuge/rotor
Vented cell culture flasks
Spermcoat: https://www.cryogenetics.com/

Feeding Products

Brine shrimp eggs https://www.brineshrimpdirect.com/brine-shrimp-eggs/
Ori-Green (Skretting) https://www.skretting.com/en-us
Fish Mate F14 aquarium feeder: readily available online and at many pet stores
Vibratory Feeder https://pentairaes.com/products/aquatic-feed-feeders/aquatic-feeders/vibratory-feeders

Commercially produced Lumpfish Feeds

There are a few feed companies that manufacture diets specifically marketed for various lumpfish life stages, however, some of these diets may not be available in the U.S. yet.

Skretting: https://www.skretting.com/en/feed-for-aquaculture/?filter=Species.eq.Lumpfish&page=1&page_size=10
Biomar: https://www.biomar.com/en/uk/products--species/cleaner-fish/lumpfish/
Ewos: https://www.ewos.com/uk/products-and-services/lumpfish

Husbandry Products

Fish graders
PentairAES: https://pentairaes.com/floating-fish-graders-adjustable-31324.html
Aquaculture ID: https://www.aquacultureid.com/products/fish-grading-equipment/

State Lumpfish Health Testing Requirements (current on Dec 2021)

Maine is currently the only state with fish health guidelines for lumpfish.

Lumpfish Pathogens of Regulatory Concern to the State of Maine

Viruses	Testing Type	Pathogen Classification	Testing Method
VHSV	Active	Exotic	Cell culture
Lumpfish ranavirus	Active	Exotic	Cell culture
Lumpfish flavivirus	Passive/elective	Exotic	PCR
ISAV deleted	Passive/elective	Exotic	Cell culture or PCR
ISAV HPR0	Elective	Endemic/limited distribution	PCR
IPNV	Active	Endemic/limited distribution	Cell culture
Nodavirus	Active	Endemic/limited distribution	Cell culture
Any CPE causing agent		Reportable	Cell culture
Bacteria[1]	**Testing Type**	**Pathogen Classification**	**Testing Method**
Aeromonas salmonicida	Active[2]	Endemic/limited distribution	TSA
Listonella anguillarum	Active[2]	La02ß Serotype is Exotic	TSA marine
Piscirickettsia salmonis	Passive/elective	Endemic/limited distribution	Cell culture, histology, PCR

[1] Bacterial testing is only required for broodstock sampled by lethal means and to cease quarantine

[2]Testing requirement proposed to be as passive when lumpfish are vaccinated for these pathogens