CULTURE MANUAL FOR THE FLORIDA POMPANO *Trachinotus carolinus* (Linnaeus, 1766)

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Introduction

Purpose of This Manual

The Florida pompano is a particularly interesting species for foodfish aquaculture. It has long been regarded as one of the best fish as table fare and traditionally has commanded a high price at market. Commercial catch of pompano in the United States is seasonal, explaining their high price and demand when available. Although it is not well known geographically in areas outside of its native range, it is well regarded by folks who know it either from high end restaurants or from catching them by angling in the surf zone.

The intent of this manual is to provide beginning to advanced fish culturists with information necessary to produce Florida pompano *Trachinotus carolinus* from breeding through a harvest size product. The information contained within recounts decades of work by many aquaculturists with an emphasis on techniques used and developed at Florida Atlantic University's Harbor Branch Oceanographic Institute (FAU-HBOI). Beginning with the biological information for the species and a brief historical perspective of pompano aquaculture, the manual delves into detailed larval biology providing a complete pictorial catalog of development from prior to fertilization through metamorphosis and weaning to a prepared diet. This is followed by detailed descriptions of culture techniques for all life stages and includes key Standard Operating Procedures (SOPs) for the critical life stages through final harvest. Discussions of other critical topics include dietary requirements specific to pompano, the state of genetic selection programs for improvement of pompano for aquaculture production, and finally elements of business planning and marketing. Companion information to the manual exists on the HBOI Aquaculture Education and Outreach website at https://www.fau.edu/hboi/research/aquaculture-innovation/center-for-marine-and-warm-water-aquaculture/education-outreach/ and includes

downloads of presentations a video tour of pompano aquaculture and tools such as an economic model spreadsheet and 3D printer files of some specialty devices referenced in the manual. This manual is intended to be a living document and will be updated periodically with new editions that will include updated SOPs and new information in each of the section as it becomes available.

Florida Pompano Life History and Distribution

The Florida pompano is in the family Carangidae (the Jacks). This group of fish has a characteristic style of swimming involving swift "sub-sinusoidal" oscillatory movement restricted to the rear of the body and tail with little or no movement of the majority of the body - called carangiform swimming or locomotion (Breder 1926). Because of this they are characterized as fast-endurance swimmers and, as eloquently stated by Breder (1926), "their relatively small 'furnaces' and large propelling 'engines imply that they must consume their 'fuel' rapidly, if only to be able to provide room for more, that is, they run their 'boilers' at a high rate and on readily 'combustible' materials of high grade." This helps to explain the relatively high food conversion ratios seen consistently in this fish species relative to other species that are more sedentary. pompano are a schooling fish that lives in warm coastal waters of the eastern Atlantic from Massachusetts to Brazil. In the northern portions of its range pompano migrate in response to the seasonal changes in water temperature coming south in winter and moving north in the spring much like the "snow-bird" residents of Florida (Gilbert and Parsons, 1986). Florida pompano on the southeastern coast of the US are thought to spawn in the wild between February and October peaking in April to May and September to October on the southeastern coast of the U.S. but may spawn year-round in the Gulf of Mexico (Weirich et al. 2021). Their diet in the wild is primarily crustaceans and small mollusks hence the use of

mole crabs and clam strips as bait by anglers. Florida pompano is highly regarded as a food fish due to its mild flavored and flaky but somewhat oily flesh. It is considered to be one of the most desirable table fish and fetches a higher-than-average price at market based on a limited commercial fishery (Weirich et al. 2021).

Pompano Aquaculture History

The first attempts to culture pompano were initiated in Florida in the early 1950s and focused on pond production of wild-caught juveniles (Berry and Iverson 1967; Cuevas 1978; Fielding 1966; Moe et al. 1968). Additional efforts to culture pompano were conducted in Florida in the 1960s using ponds, coastal impoundments, and tanks (Finucane 1970a, 1970b, 1971; Iverson and Berry 1969; Moe et al. 1968). In the early 1970s, producing pompano using floating cages was evaluated in coastal Alabama (Swingle 1972; Tatum 1972, 1973), Texas (Marcello and Strawn 1972), and Florida (Smith 1973). In addition, commercial tank-based production was evaluated in the Dominican Republic, as well as in Florida (McMaster 1988) and in the late 1970s, pond-based polyculture of pompano and shrimp was assessed in Alabama (Tatum and Trimble 1978; Trimble 1980). Also, in the late 1970s and early 1980s work in Venezuela evaluated polyculture of pompano and shrimp using seawater tanks (Gomez and Scelzo 1982) and production of pompano in floating cages (Gomez and Cervigon 1987).

A common thread shared by most of these initial studies was poor growth and feed conversion of fish greater than 200 g (0.4 lb), usually coupled with poor survival, resulting in the inability to consistently produce fish ≥450 g (1 lb). This was attributed to a number of factors including inadequate diets, disease outbreaks, and sub-optimal water temperatures.

Regarding reproduction and larval culture of Florida pompano, initial efforts were made in Florida during the 1970s. Hoff et al. (1972, 1978a, 1978b) demonstrated that broodstock can be

conditioned to spawn by strip and volitional spawning methods under varying photothermal conditions via administration of human chorionic gonadotropin, and Kloth (1980) described the spawning behavior of female pompano that were induced to spawn. Although large quantities of eggs were produced in these groundbreaking studies, Hoff et al. (1978a) reported that fertilization rates were highly variable and many eggs exhibited abnormal development, which was attributed to poor egg quality. Hormone-induced spawning of photothermal conditioned broodstock pompano with mass production of eggs was also reported in the 1970s by a commercial operation in the Dominican Republic (McMaster 1988).

As per larval culture, Hoff et al. (1978a) attempted to rear Florida pompano larvae produced by hormone-induced spawning to metamorphosis (24 days post-hatch [DPH]) using a diet consisting of natural plankton, protozoans, rotifers, and copepod nauplii, followed by Artemia nauplii. Considerable larval mortality was observed at 8–10 DPH and only a small number of juveniles were produced. Similarly, initial methods for larviculture of pompano were developed at a commercial hatchery facility in the 1970s in the Dominican Republic using a live feeds regimen of rotifers followed by Artemia with metamorphosis occurring at 22 DPH (McMaster 1988).

In the late 1990s and early 2000s a renewed interest in development of aquaculture methods of Florida pompano occurred, which was largely due to advancements made toward culture of other marine finfish species (Tucker 1998). Weirich and Riley (2007) collaborated on a joint research effort of the United States Department of Agriculture (USDA)'s Agricultural Research Service and Harbor Branch Oceanographic Institute (HBOI) and demonstrated that pompano broodstock maintained under controlled photothermal conditions using RAS technologies could be induced to spawn volitionally year-round via hormonal (gonadotropin-releasing hormone

analog [GnRHa]) induction. Similar efforts conducted during this time period at Mote Marine Laboratory (Main et al. 2007), as well as commercial facilities, provided the basis for reliable and consistent reproduction of broodstock and production sufficient quantities of fertilized eggs to support larval culture.

Regarding larval culture, since 2000 research has documented and described development and growth of hatchery-reared pompano larvae (Riley et al. 2009); compared performance of larvae fed rotifers, Brachionus plicatilis, enriched with different commercial diets (Cavalin and Weirich 2009); assessed copepod, Pseudodiaptomus pelagicus, nauplii as initial prey for first-feeding larvae (Cassiano et al. 2011); evaluated different microparticulate diets on weaning success, growth, fatty acid (FA) incorporation, and enzyme activity (Hauville et al. 2014); demonstrated the importance of probiotics in larval nutrition (Hauville 2014); determined FA acid utilization of early-stage larvae (Hauville et al. 2016); and investigated the effect of dietary taurine to improve larval performance (Derbes 2017).

With respect to growout of juveniles and market size pompano, most research efforts in the last two decades have focused on fish reared using RAS technologies. Weirich et al. (2006) investigated culture densities and feeding frequency of pompano reared in RAS using juveniles captured from the wild. Although culture densities utilized were lower than that required for commercial culture, pompano were grown from 17 g (0.04 oz) to >450 g (1 lb) in 4–5 months and >700 g (1.5 lb) in 8–9 months. Later work conducted by Weirich et al. (2009) revealed that hatchery produced pompano (260 g or 0.6 lb at stocking) could be reared using RAS technologies at low salinity (5 g/L) to 1 lb in 2 months and from 570-630 g (1.3-1.4 lb) in 3.5 months using commercial-scale tanks. Based on these observations, additional experiments were conducted to further assess and refine methods for the culture of pompano at low salinities.

Results indicated that under higher densities low-level chronic mortalities at salinities below 5 g/L were mitigated by increasing the culture salinity to 12 g/L (Riche et al. 2012). Similar observations were noted at Mote Marine Laboratory, where researchers determined that salinities of 12–15 g/L were better than lower salinities for optimal survival and growth (K. Main, personal communication). Next-generation transcriptomics of fish reared under lower salinity conditions (3 g/L) and after salinity increase (12 g/L) indicated that genes related to osmoregulation, solute carriers, and oxidative stress were affected. In addition to this work, Wills (2013) demonstrated successful pompano production in a commercial-scale RAS at salinities beginning at 8 g/L and ending at >20 g/L. Early chronic losses that were apparently due to stress subsided once the salinity was increased in the system (Riche et al. 2012). Fish were grown from egg to harvest with all inputs accounted for to conduct an economic production model (Wills, 2013). The production cycle required 507 days from egg to harvest. Survival was 89.1% and 5,884 fish were harvested at a mean weight of 425 g or 0.9 lb (range 100–898 g or 0.2-2.0 lb). Studies at Mote and HBOI have focused on next-generation marine RAS that incorporates the principles of integrated multi-trophic aquaculture (IMTA; Boxman et al. 2015; Hanisak and Wills 2013; Laramore et al. 2018; Wills et al., 2012). The Mote IMTA has produced pompano and marine wetland plants (Boxman et al. 2015) while the HBOI experimental system incorporates tank-based culture of pompano or other marine finfish species (e.g., cobia and red drum) in conjunction with marine shrimp, Litopenaeus vannamei, and macroalgae (e.g., Ulva lactuca). Two interesting observations of this experimental IMTA system have included: (a) after 9 years of operation the system consistently only requires approximately 0.5% per day of makeup water (much of which comes from rainfall), and (b) there has never been any off-flavor issues regarding fish compared with fish produced in other studies using RAS.

Regarding nutrition, prior to the early 2000s, little was known about the nutrient requirements of Florida pompano. More recently, attention has focused on investigating optimum dietary protein, lipid, and energy utilization; digestibility of various feed ingredients; amino acid (AA) availability; and reduction of dietary fish meal (FM) and fish oil (FO) utilizing alternative plant and industry coproducts. More detail with respect to nutritional requirements of Florida and research that has been conducted is provided in the "Florida Pompano Nutrition" section below.

As far as commercial aquaculture of Florida pompano, integrated hatchery and pond-based production of Florida pompano has been reported by a commercial venture in Florida established in the 1980s (McMaster et al. 2004) and the first reported aquaculture-based production and international trade of this species were in 2004 from net pen culture in the Bahamas with juveniles produced in Florida (FAO 2020). However, commercial activity in the Bahamas ceased by 2007 after a series of hurricanes impacted the farm and hatchery. In 2006, production of farmed pompano began in Panama, followed by production from a commercial net pen operation in the Dominican Republic from 2009 to 2016. Presently, in addition to the aforementioned Florida-based pond operation, on a commercial scale, there exists one breeding/juvenile production facility based in Florida, a recently established RAS-based production facility also located in Florida, and a fully integrated net-pen operation located in Panama. With respect to marketable fish, the Florida RAS operation is initially targeting production of 60 m.t. of whole pompano/year while the net pen operation in Panama is currently producing approximately 250–300 m.t. of whole fish/year. Although, on a much smaller scale, there is also some production of Florida pompano for the ornamental trade as well (E. Cassiano, Personal Communication).

Gamete Biology

Hoff et al. (1978a) determined the optimal mean oocyte diameter for application of hormonal injections with HCG was between 580 and 718 µm (or mid to late stage 4; see Table 1 from Hoff et al. 1972) with the optimum diameter of 640 µm. Eggs at spawning (predominately by strip spawning) were 1200 µm but shrank by up to 200 µm within the first hour after spawning with the average diameter of fertilized eggs being 970 µm with a 254 µm oil droplet. The authors indicated that multiple oil droplets represented premature spawning (i.e., forced ovulation of immature oocytes). Their hormone regime was 0.83 IU/g of HCG (IU = International Units) split in two doses 24 hours apart for oocytes that measured 580-810 µm. This hormone regime was

Table 1. Florida pompano egg staging from Hoff et al. (1972).

Table 1. - Size and descriptions of live pompano eggs. Stages 1 through 5 drafted in part from Moe (1969)

Oocyte stage	Approximate size (μ)	Description
Primary	5 to 15	Oocytes irregularly shaped, embedded in ovigerous tissue, usually along periphery of ovarian lamellae; large nucleus.
Stage 1	15 to 30	Oocytes generally clear, irregularly shaped, embedded in ovigerous tissue along periphery of ovarian lamellae; reduced nucleus; nuclear margin not sharply defined; nucleolus moving to periphery of nucleus.
Stage 2	30 to 120	Found in most samples but predominantly during resting periods. Oocytes generally clear, shape round, attenuated or sharply angular; nucleus clearly defined; several nucleoli.
Stage 3	120 to 330	Early 3- Oocytes generally round with tinge of yellow; more yolk globules in cytoplasm and vesicles in nuclear area. Mid 3- Nuclear membrane becoming less defined; more globules in cytoplasm. Late 3- Nuclear membrane obscure; many small globules present throughout oocyte.
Stage 4	330 to 550	Early 4- Oocytes slightly yellow; nucleus not well defined; yolk vesicles and globules larger and numerous. Mid 4- Nuclear area filled with large vesicles coalesced near center; numerous small globules present in cytoplasm, forming light granular band 30 to 50 μ thick around oocyte. Late 4- Entire egg filled with large globules and vesicles; light granular margin reduced; no defined nucleus.
Stage 5	550 to 750	Early 5- Oocyte becoming opaque; globules granular, contrasted against clear vesicles; no defined nucleus. Mid 5- Entire egg opaque except clearing central area. Late 5- Clear oil globules beginning to form, often irregular in shape, contrasted against dark granular yolk.
Stage 6	700 to 1200	Early 6- Granular area becoming clear; three or more small oil globules present in irregular pattern. Mid 6- General clearing, few granular areas remaining; non-granulated yolk area reticulated, resembling cell divisions; single oil globule present, approximately 240 μ in diameter (blood frequently present in sample). Late 6- No granular areas; yolk losing reticulations and becoming clear.
Stage 7	900 to 1400	Egg clear; nucleus barely discernible; ovulated and easily stripped; scarcely visible in water; floats in 32 to 35 ‰ sea water.

viable for both strip spawning and to induce volitional spawning in some cases as well. The authors advised that spawning should be induced before the oocytes reach 700-900 μm or mid-

stage 5 to early stage 6 or the eggs will begin to regress. Their trials suggest that the entire gonadal cycle appeared to require 34-66 days from pre-spawning condition though resting to pre-spawning dependent upon the environmental conditions allowing for up to 4 or more spawns per female per year. Their fertilization rates were generally low but were as high as 76% and averaged 25% for all spawns reported.

Weirich and Riley (2007) demonstrated volitional spawning of pompano with oocyte diameters >500 µm using implants of 75 µg GnRHa pellets (Ovaplant ™, Syndel International, Inc., Vancouver, BC) in the dorsal lateral musculature. Their water temperature during spawning was 24-26°C (75-79°F), salinity was 34-35 ppt and photoperiod was 14L:10D with 200-300 lux light intensity at the water surface of their spawning tanks. They implanted their broodfish between 1500 and 1800 hours and spawning occurred 30-36 hours after implantation. Separation of fertilized eggs was done by floating collected eggs in a cone shaped container allowing removal of nonviable eggs. Total fertilization rates were between 4.5 and 63.7% with the floating fraction being between 71.3 and 99.3% fertilized among the nine spawns reported which ranged from 454,000 to 2,316,000 eggs collected per spawn. Hatch rates varied from 73.1 to 95.4% of the fertilized eggs. Mean number of eggs was 109.7 to 150.7 eggs/g of female during each year of their reported spawning trials. The eggs were approximately 1 mm in diameter and were counted at 1,200 to 1,300 eggs per ml.

Embryology and Larval Development

Whereas the larval development of the pompano has been thoroughly characterized by Riley et al. (2009), the embryo developmental stages have never yet been fully described for this species. A recent experiment, conducted through Harbor Branch Oceanographic Institute at Florida Atlantic University (HBOI-FAU) in Fort Pierce, Florida, produced the first images of

embryo development. Prior to hormone injection, females were checked for eggs using cannulation, while males were massaged to see if they released sperm. The female broodstock were induced to spawn using the Ovaplant-Syndel time-release implant and they spawned volitionally around 24 to 48 hours post injection. The eggs were then placed in 725 L tanks at 29-30°C in 35 ppt salinity water. The embryonic development of pompano eggs was followed from unfertilized eggs to hatch where pictures were taken every hour until hatch, which occurred around 24-25 hours post fertilization. The embryonic development was divided into 10 sections: fertilization, cleavage, morula, blastula, gastrula, cephalization, neurulation, cranial regionalization, tail lift (pre-hatch) and hatching.

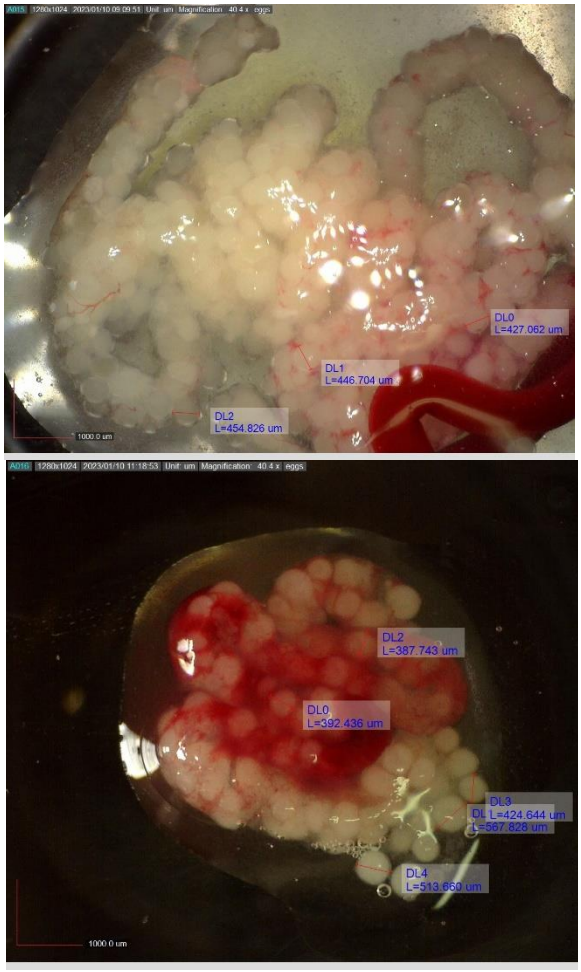

Figure 1. Collected ovocytes (unfertilized eggs) *via* cannulation on female *Trachinotus carolinus*. Scale represents 1,000 μm.

Unfertilized egg (Figure 1)

The average pompano unfertilized egg diameter was 451.86 ± 57.34 μm, and of cloudy off-white color. Slight variations across egg diameter are possibly due to differences in incubation conditions such as temperature, salinity and oxygen level and/or between individuals.

Fertilized egg

After fertilization, the floating egg absorbed water and became translucent, making it possible to observe embryological stages until hatch, under the microscope. Egg sizes increased

up to 966.5 ± 19.8 μm , when measured 13 hours post fertilization (hpf), with an oil droplet size approximation of 280.2 ± 11.3 μm. The fertilization rate was 78.6 ± 3.3 % while the hatch rate was 79.4 ± 15.7 %. These high rates resulted in dense aggregations of 1360.0 ± 155.7 eggs per ml.

Cleavage (0-2 hpf) (Figure 2)

The embryonic development starts once the egg develops a perivitelline space, which is filled with fluid, and meroblastic cleavage begins (Note: no picture was available since this occurs in the tank prior to egg collection). At this stage, starting right after fertilization, the unicellular fertilized egg starts to divide by mitotic divisions to form daughter cells called "blastomeres" or "cleavage cells" (Balinsky 1975, Mejri 2011). For the pompano, the timing of first cleavage wasn't reported, but occurs in the first 30 minutes post-fertilization when compared to other marine tropical fish species of similar embryonic development (Luan et al. 2016). The constant divisions of blastomeres will increase the cell number until it forms a mass of cells called the morula.

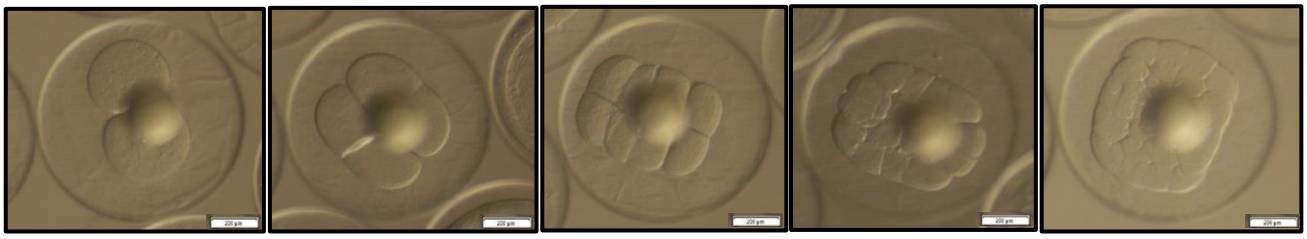

Figure 2. Florida Pompano embryo, at 2, 4, 8, 16 and 32 cell-stage during cleavage stage from 0-2 hours post fertilization (hpf) at 29°C (84°F). Scale represents 200 μm (Photos by Flavie Perron).

Morula stage (2-3 hpf) (Figure 3)

At 2-3 hpf, the blastomeres regroup as a mass of cells to form the blastoderm in the form of a blastodisc as we can observe by the blurry white mass. This mass, the morula, will then develop into fluid-filled mass of cells called the "blastula".

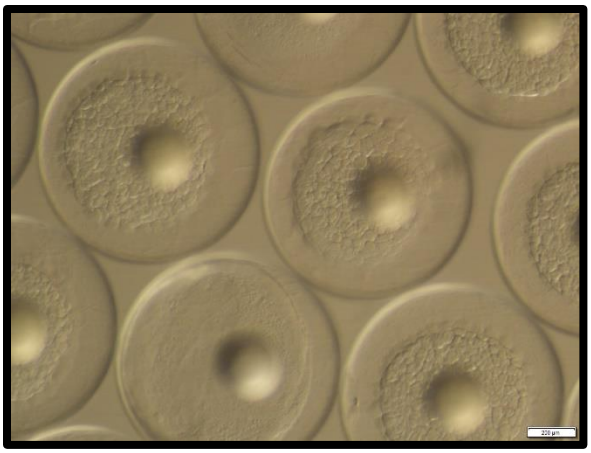

Figure 3. Florida Pompano embryo, morula stage at 2 hpf at 29°C (84°F). Scale represents 200 µm (Photo by Flavie Perron).

Blastula (3-4 hpf) (Figure 4)

Once we can no longer count the number of cells, after about 128 cells, the blastula stage begins. The peripheral periblast developed during this stage and surrounded the blastodisc while the blastoderm flattened uniformly (Figure 4A) (Mejri 2011). This stage is rapid, between 3 and 4 hpf, where the mass of cells (blastomeres) becomes tighter and concentrates on one side of the egg thus forming a disc at the animal pole (Figure 4B). This mass of cells will then start to invaginate to start forming the embryo, therefore beginning gastrulation.

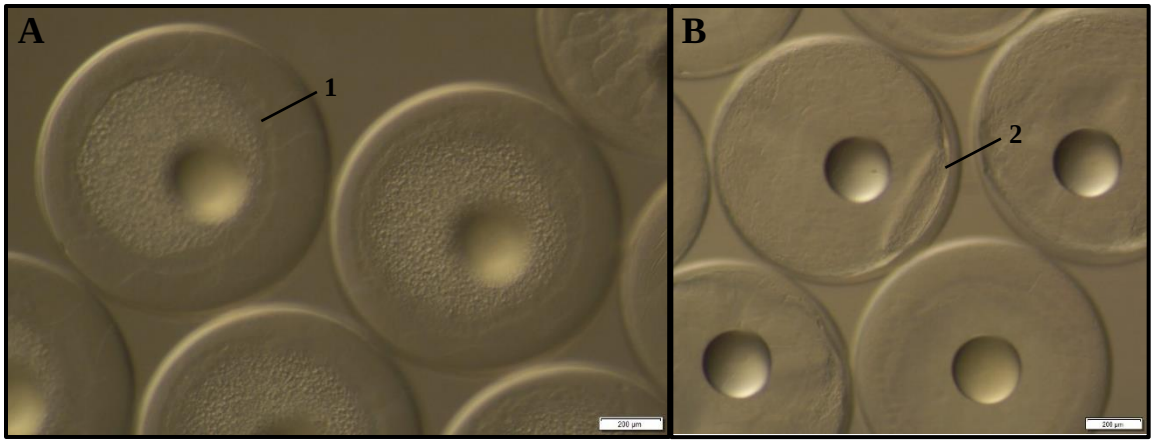

Figure 4. Florida Pompano embryo, early blastula stage at 3 hpf at 29°C (84°F) (**A**) with visible periblast (**1**) and late blastula stage around 4 hpf (**B**) with visible disc-shaped blastoderm (**2**). Scale represents 200 µm (Photos by Flavie Perron).

Gastrulation (5-9 hpf) (Figure 5)

During this stage, the germ layers (endoderm and mesoderm), necessary to form the embryo, will develop. The gastrula stage starts with cellular movements around 5 hpf where the blastoderm/blastodisc expands over the yolk with epiboly movements (Figure 5A) (Mejri 2011). It was possible to see the germ ring at 6 hpf (Figure 5B) and the embryonic shield (seen from

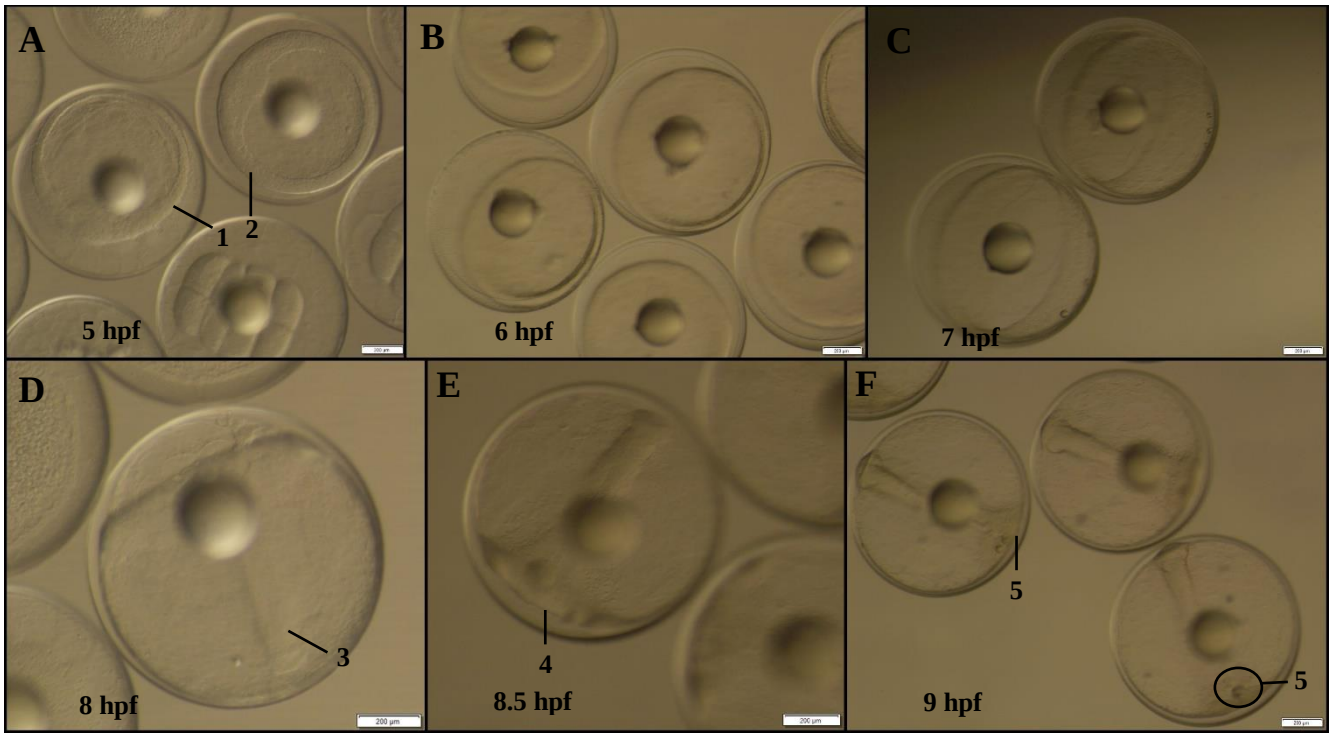

Figure 5. Florida Pompano embryo during gastrulation stage from 5 to 9 hpf at 29°C (84°F) where epiboly of the yolk by the blastoderm is observable: (**A**) germ ring and early embryonic shield are visible (**1, 2**); (**B**) extension of blastoderm, 50 % epiboly; (**C**) embryo formation commenced; (**D**) appearance of neural plate (**3**) and 75 % epiboly; (**E**) invagination of germ layers around blastodisc which closed the blastopore (**4**); (**F**) at 9 hpf the tail bud is visible (**5**). Scale represents 200 µm (Photos by Flavie Perron).

above; Figure 5C) around 7 hpf. At 7-8 hpf, the invagination of the germ layers around the blastodisc and the appearance of the neural plate (or notochord; Figure 5D) refers to the beginning of formation of embryo (Balinsky 1975). The blastopore, found at the edge of the blastodisc, will serve as a pathway for mesodermal and endodermal cells to get into the embryo (Balinsky 1975). Starting 9 hpf, the body axis of the embryo starts forming and it is possible to

observe a closing blastopore and the tail bud appearing. The blastopore is completely closed at 10 hpf and the presence of the tail bud results in the completion of the gastrulation stage (Mejri 2011, Srithongthum et al. 2021).

Neurulation and cephalization (9 hpf) (Figure 6)

Cephalization occurred at the same time as neurulation and will be described altogether. At 9 hpf, neurulation and cephalization started once the blastopore closed, tail bud appeared, and the embryo length was longer than the egg diameter. The neural groove/keel in the middle of the egg became larger at its anterior end to form the embryo's cephalic region thus defining the embryo's body axis (Conte et al. 1988). The eye rudiments were visible around 10 hpf on the head region followed by muscle segmentation, or somites, which appeared on the trunk of the embryo, particularly visible at 14 hpf.

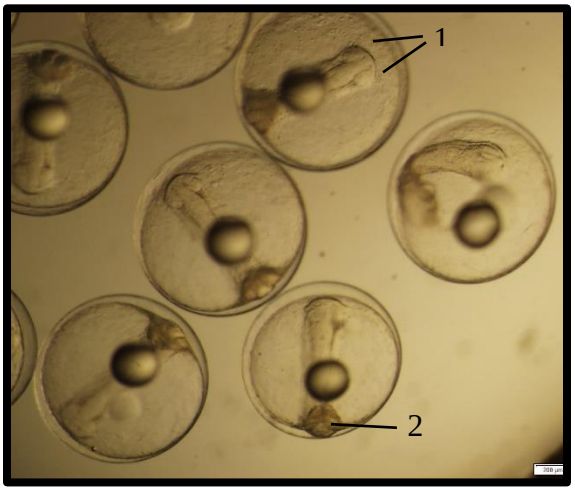

Figure 6. Florida Pompano embryo, neurulation and cephalization stages at 10 hpf at 29°C (84°F), showing the eye rudiments (**1**) on a developing cephalic region, muscle segmentation and tail bud (**2**). Scale represents 200 µm (Photo by Flavie Perron).

Cranial regionalization (12 hpf) (Figure 7)

Brain vesicles were differentiated through cranial regionalization, and optic lenses formed on the eye vesicles around 12-13 hpf. As development carried on, the head portion elongated and thickened, and the embryo's body grew in length, pigmentation started appearing over the yolk sac and the embryo at 16-17 hpf.

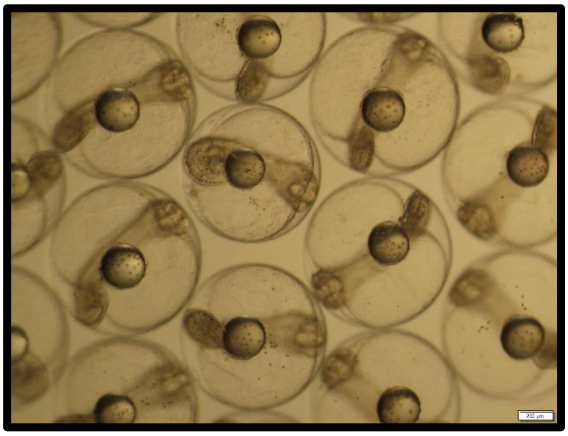

Figure 7. Florida Pompano embryo, cranial regionalization and development at 17 hpf at 29°C (84°F) where the optic lenses are formed, and pigmentation starts. Scale represents 200 μm (Photo by Flavie Perron).

Tail lift or prehatch (17-23 hpf) (Figure 8)

The prehatching stage starts when the tail lifts, meaning when it separates from the yolk which is around 15-16 hpf. Once the heart developed from a tube to a "S" shape form, the first heartbeat was between 16-19 hpf, resulting in the larvae's first movements. During this stage, the embryo keeps elongating its body and the formation of organs and muscle segmentation continues until hatch.

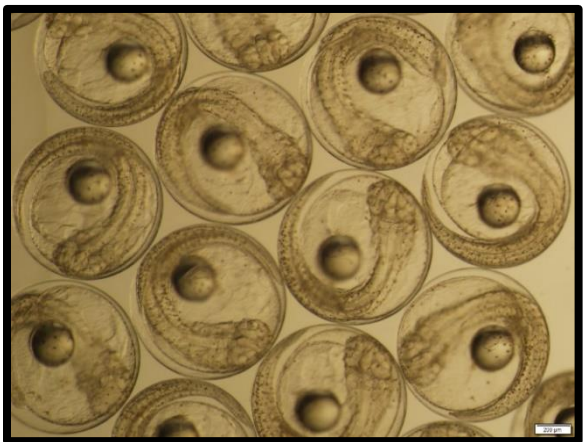

Figure 8. Florida Pompano embryo at 22 hpf at 29°C (84°F); tail separates from the yolk, first hearbeat and embryo's first movements. Scale represents 200 μm (Photo by Flavie Perron).

Hatch (24-25 hpf) (Figure 9)

At around 24 hpf, hatching begins as the larvae break free from their egg. At 25 hpf, hatching was completed, and the larvae's body straightens with the tip of their tail slightly raised and the newly hatched larvae has a yolk sac and an oil droplet which they consume for endogenous feeding until they develop the mouth and organs necessary for exogenous feeding.

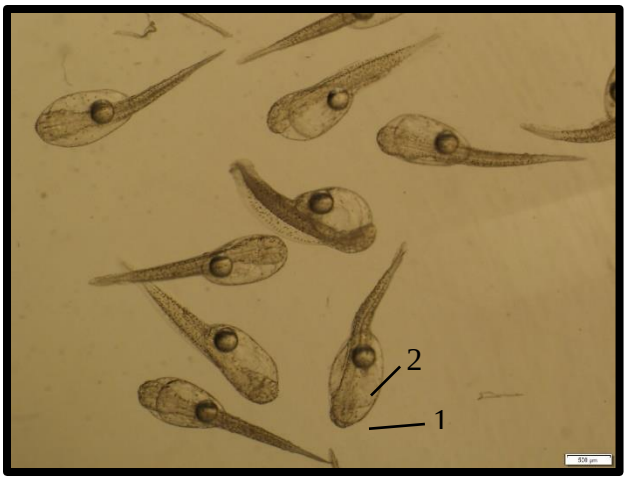

Figure 9. Florida Pompano newly hatched larvae at 25 hpf at 29°C (84°F) with a visible yolk sac (**1**) and oil droplet (**2**). Scale represents 500 µm (Photo by Flavie Perron).

Larval development

Prior to this manual, Riley et al. (2009) provided the most comprehensive larval development characterization for pompano to date and reported fertilized eggs as being 0.99 ±0.04 mm in diameter with a single oil droplet and noted that hatching of pompano eggs occurs at 30 to 36 hours after fertilization at 24- 26°C (75-79°F) in 34-35 ppt salinity water. Earlier observations by Hoff et al. (1978a) reported hatching at 25-38 hours

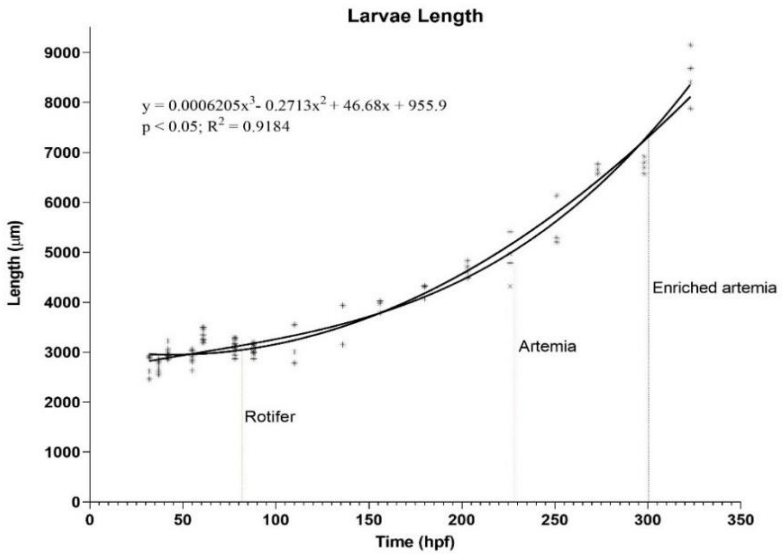

Figure 10. Trend analysis using stepwise regression showing nonlinear regression model for larvae length (cubic regression model) of Florida Pompano in relation to time in hours post fertilization (hpf) at 29°C (84°F). Data are presented as mean ± SD, and $n \geq 215$

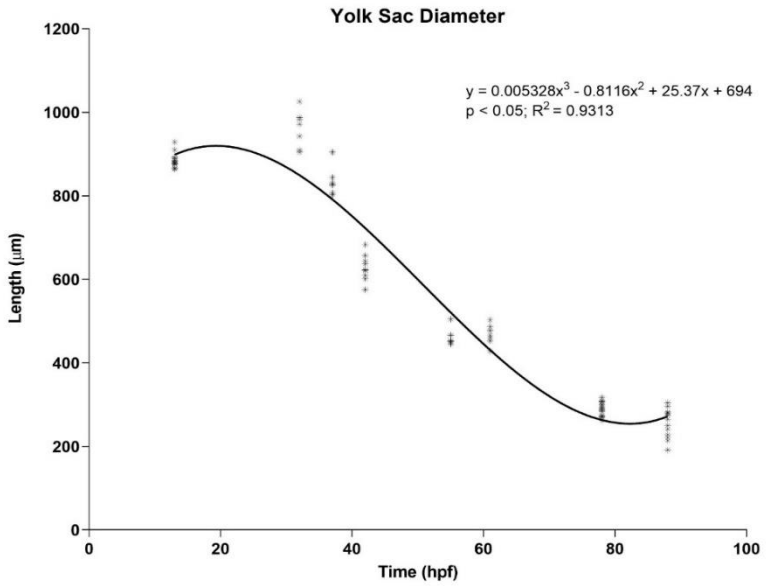

Figure 11. Trend analysis using stepwise regression showing nonlinear regression model for yolk sac diameter (quadratic regression model) of Florida Pompano in relation to time in hours post fertilization (hpf) at 29°C (84°F). Data are presented as mean ± SD, and n ≥ 150.

after spawning in 23.3-28.0°C (73.9-82.4°F) water with salinity at 34 ppt to maintain buoyancy. Riley et al. (2009) noted that the newly hatched larvae were 2.6 ±0.4 mm and were not well developed. They maintained strict water chemistry conditions and provided 16 h/d of white light initially at 300 lux at the water surface. During larval growout when water was greened using *Nannochloropsis* sp. light intensity was increased to 2,000 or 3,000 lux at the surface. At 2 days after hatch (DAH) pigment was forming and at first feeding 3 DAH larvae had fully formed their primitive gut that was connected to the anus and rotifers were present in the gut. The oil droplet and yolk were completely used by 5 DAH and the larvae were fully dependent on nutrients supplied by the rotifers (Figures 11 and 12). The authors noted

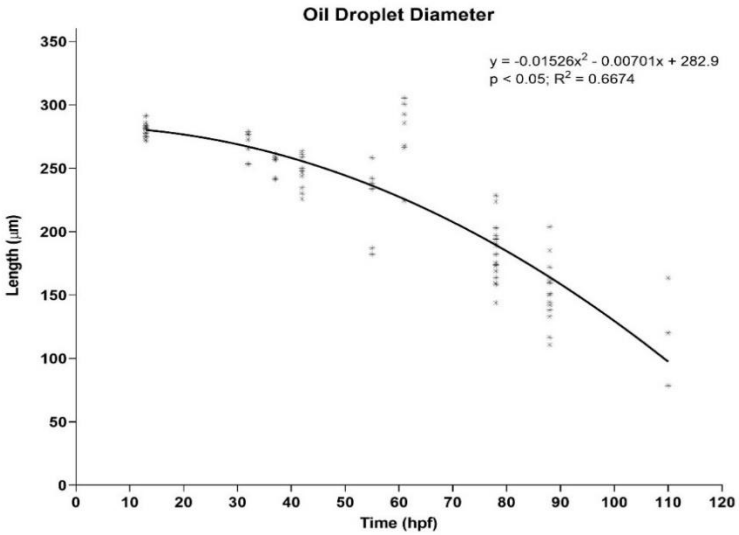

Figure 12. Trend analysis using stepwise regression showing nonlinear regression model for oil droplet diameter (cubic regression model) of Florida Pompano in relation to time in hours post fertilization (hpf) at 29°C (84°F). Data are presented as mean ± SD, and n ≥ 150.

fully developed gut at 12 DAH and they began feeding *Artemia* which were observed in the gut as orange material and especially by the presence of orange fecal matter. More recent work at HBOI (Fort Pierce, Florida) noted faster development of reared larvae at 29°C (84°F) than at the temperature used by Riley et al. (2009) (24-26°C; 75-79°F). Larvae at 29°C (84°F) began feeding at 2 DAH and their yolk sac and oil droplet were used completely after 4 DAH. Additionally, larvae reared at the warmer temperature began feeding on *Artemia* at 7 DAH, 5 days earlier than reported by Riley et al. (2009) at 12 DAH (Figure 15).

Notocord flexion was noted between 8 and 11 DAH (Figure 13A, B). This represents the passage of the larvae through metamorphosis and the beginning of formation of "adult" finnage

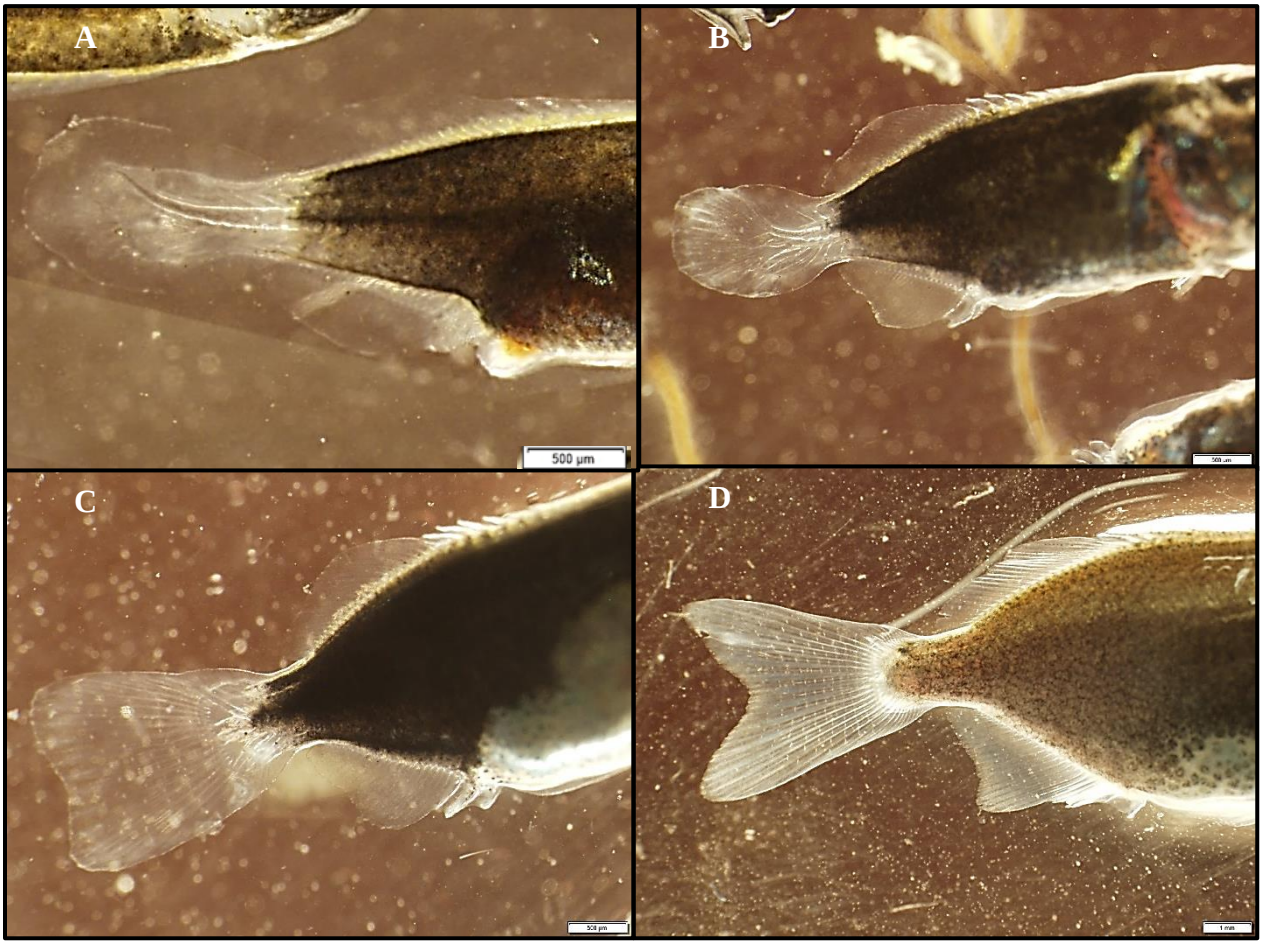

Figure 13. Development of the tail, with flexion (A) and post flexion (B) and development and distinction of "adult" rays and fins (C, D) at respectively 7, 9, 10 and 17 days after hatch at 29°C (84°F). Scale bars represent 500 µm for A, B and C, and 1 mm for D (Photos by Flavie Perron).

with complete ray counts and structures. Metamorphosis was noted to be completed between 18 and 20 days between the three trials that were observed (Riley et al. 2009) (Figure 13C, D). In the more recent work with rearing at HBOI occurring at 29°C (84°F), notochord flexion started at 7 DAH and was completed after 11 DAH and at 12 DAH, when larvae were weaned on prepared feed (Otohime™ in these instances), the larvae had "adult" finnage and rays. Metamorphosis was completed between 12 and 15 DAH, up to 5 days earlier than reported by Riley et al. (2009).

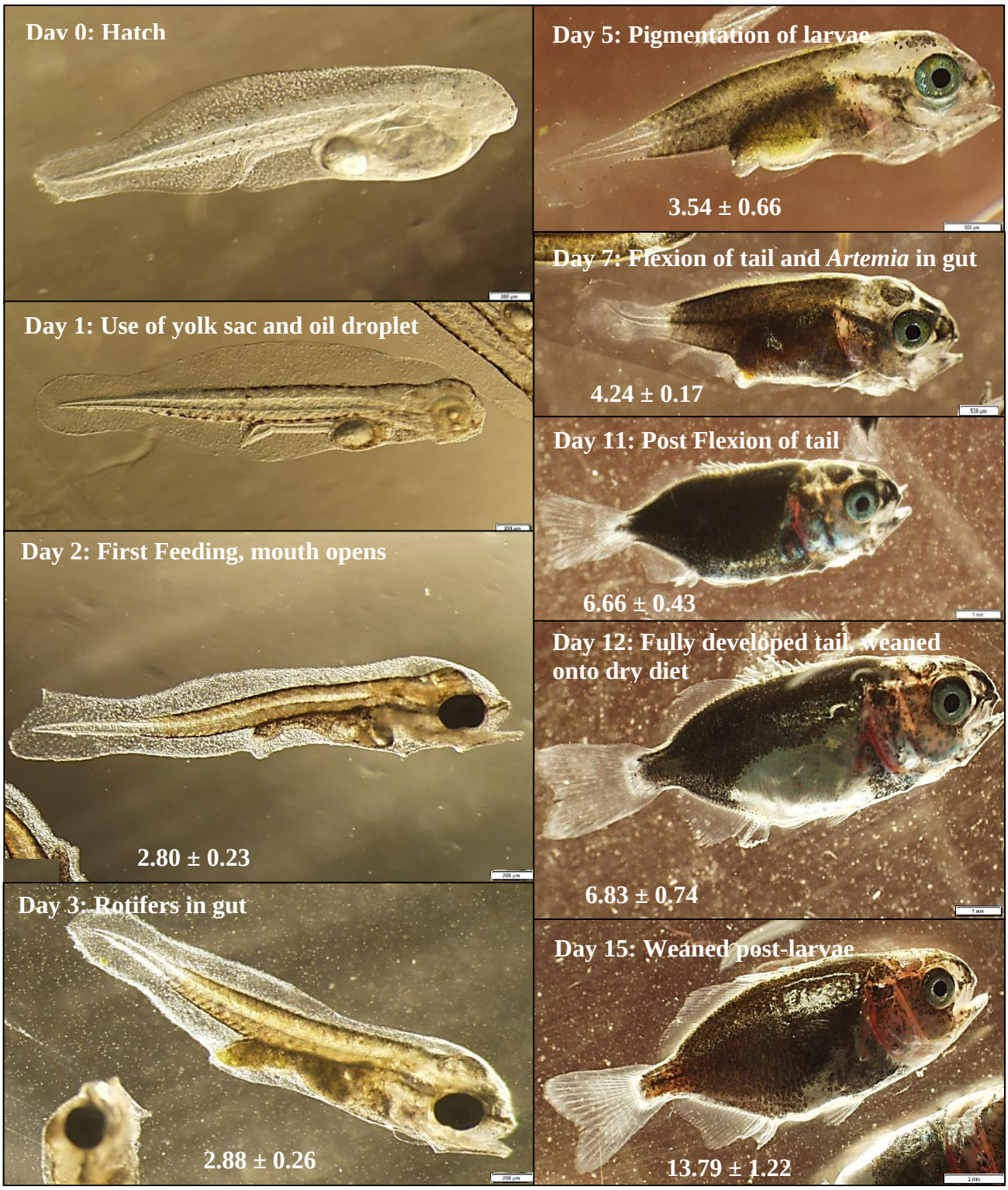

Figure 14. Development of newly hatched Florida Pompano larvae through weaning, 15 days after hatch at 29°C (84°F). (Photos by Flavie Perron).

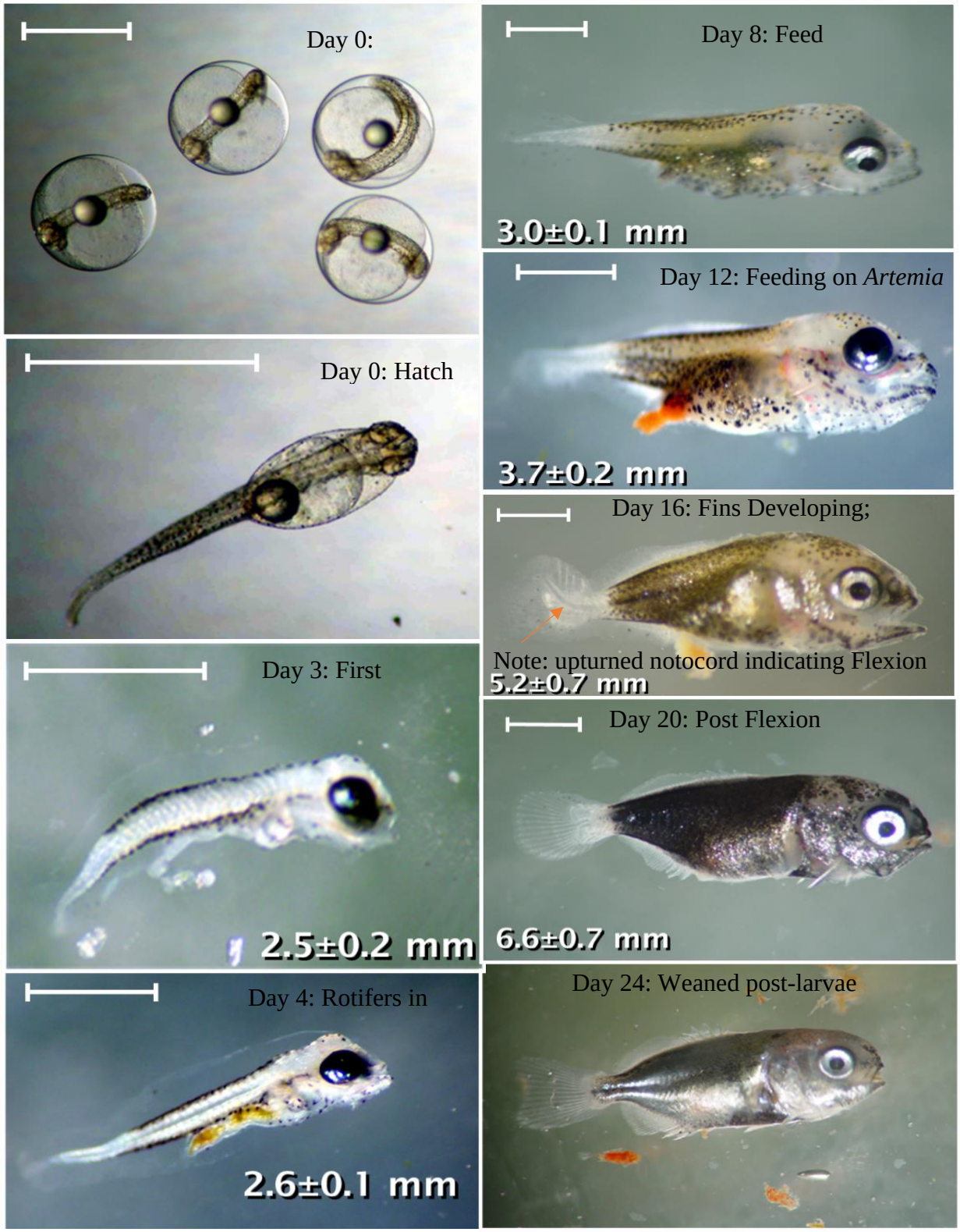

Figure 15. Developmental progression of Florida Pompano from just prior to hatch through weaning, 20 days after hatch at 24- 26°C (75-79°F). Scale Bars = 1 mm. (Photos by Charles Weirich).

Broodstock

Broodstock are the adult females and males used to produce the next generation of offspring for culture. Selection and care of broodstock is critical to the future operations of the farm as poor-quality broodfish yield poor quality eggs. In addition, suboptimal husbandry and conditioning also yield either no fertile eggs or poor-quality eggs. The systems, feeds and operational practices used in the broodstock area should be of the highest quality and most stringently applied. Personnel hired to manage and operate the broodstock and associated larval culture areas should be highly trained, detail oriented, trustworthy and competent.

Broodstock Holding System Design

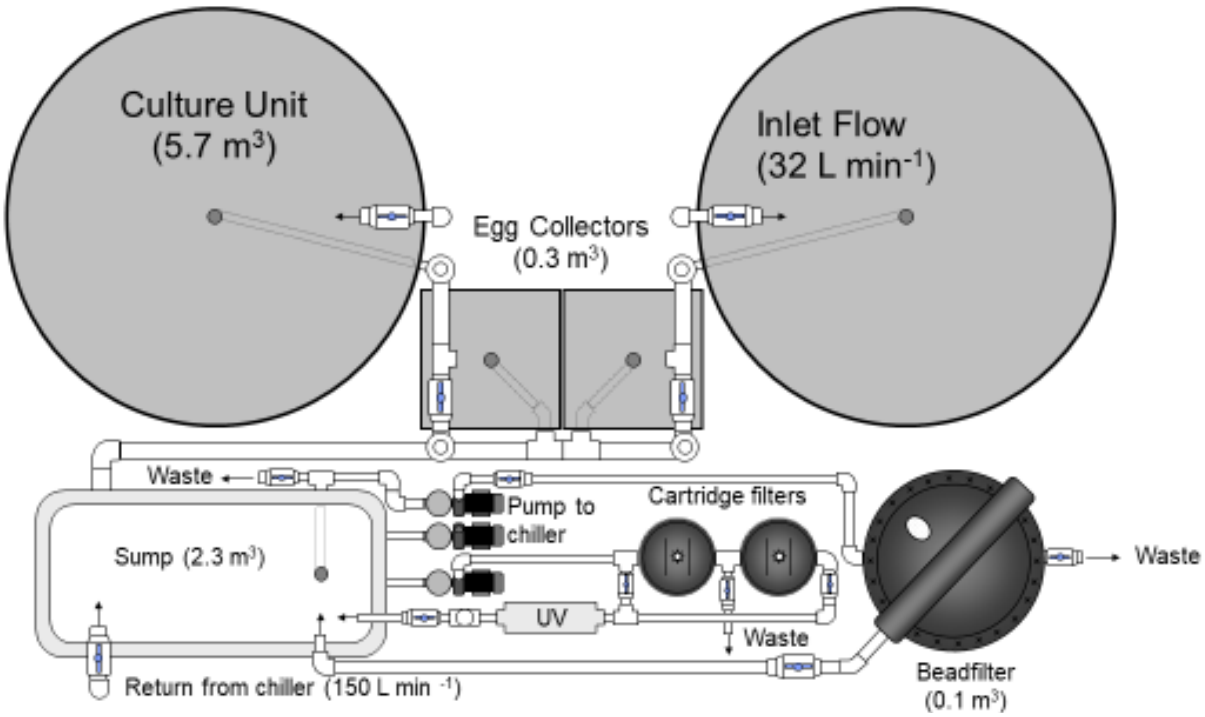

Figure 16. Broodstock holding and conditioning system design at HBOI (Figure by Chuck Weirich).

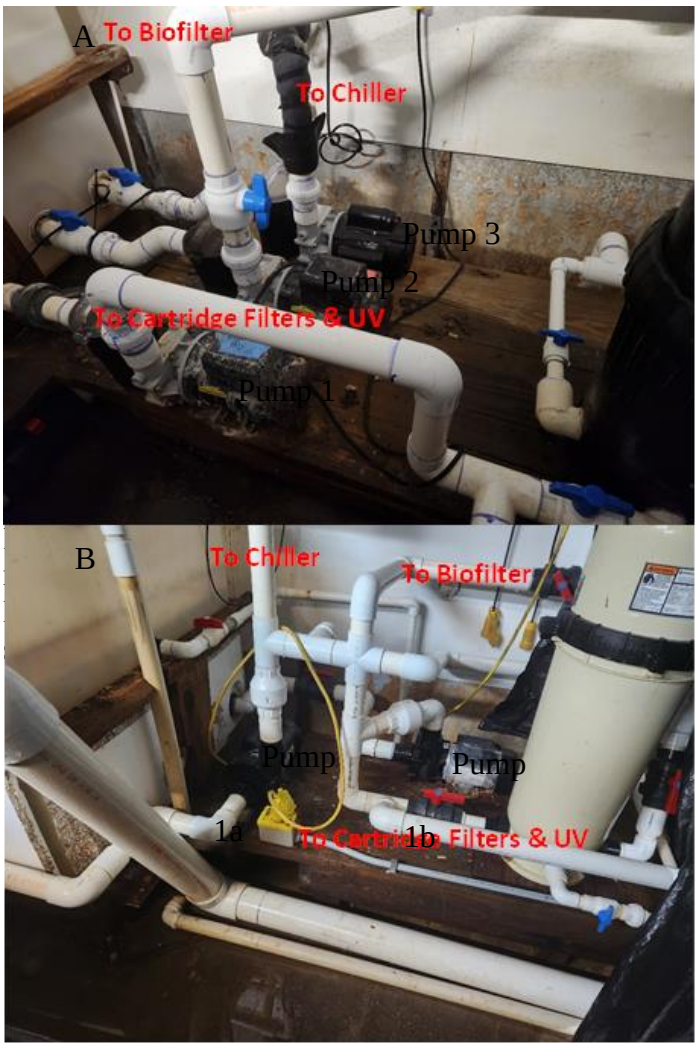

Figure 17. Photos showing two system plumbing designs currently being used at HBOI for Broodstock conditioning systems; A) design as depicted in Figure 16 using three pumps each operating its own loop, B) modified design as described in text using two pumps operating all three filtration loops.

processes and the pumps can be easily changed without having to cut and glue new pipe (Figure 17). The particulate filters in these systems are Pentair Senda 1000 pleated cartridge filters with 200 ft^2 of filter surface area, they are used for particulate removal and

At HBOI all of the broodstock culture systems are RAS-based designs. The systems have a general design that was developed by USDA-ARS that included three process loops each powered by one of three pumps (Figure 16). The design has been modified to include only two larger pumps that are manifolded together with each process loop manifolded from the pump outlet with valves to control flow to each loop (Figure 17). The pumps are plumbed with union connections and check valves so that a malfunctioning pump does not eliminate all flow through the system

Figure 18. Photograph of modification of cartridge filter plumbing showing parallel flow rather than serial flow as depicted in Figure 16.

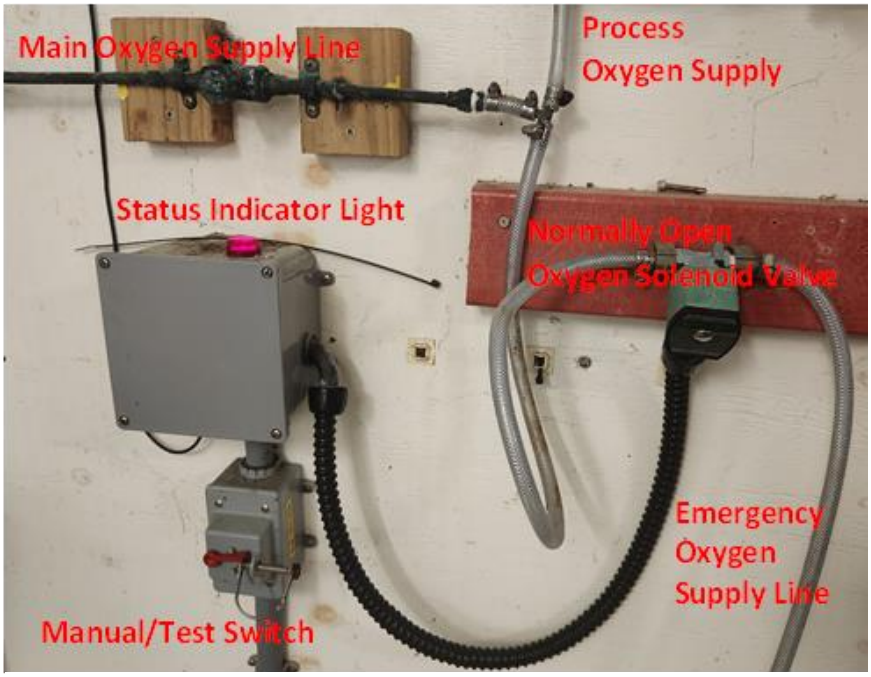

Figure 19. Details of simple failsafe emergency oxygen system using a normally open solenoid valve to supply oxygen to the tank's emergency oxygen diffusers.

to keep organic load in the tank down to a minimum. The second modification to the systems is in one of the three loops where rather than being in series (as depicted in Figure 16) the pleated paper cartridge filters are plumbed in parallel so that a clogged filter does not eliminate flow in the particulate filtration loop. The UV sterilizer is positioned after the cartridge filters in the same loop so that the water going through it is as clear as possible. The systems each have a Pentair Aquatic Eco-Systems Smart UV HO Sterilizer (Pentair Aquatic Ecosystems, Inc. Apopka, FL) with 120-watt bulb.

Figure 20. Dual sump/moving bed biofilter with MB3™ biomedia in one tank.

Biofiltration is accomplished by a Polygeyser™ bead filter (AST Aquaculture Systems Technologies, LLC, Baton Rouge, LA) in the second of

Figure 21. Example of one brand of Smart LED light that uses a standard Edison screw type socket.

the three loops. The third modification is conversion of one of the two sump tanks to a dual sump/moving bed biofilter with MB3™ biomedia (Innovasea Inc., Baton Rouge, LA, Figure 20). Temperature is controlled via the last loop using a 5-ton heat pump with a titanium heat exchanger. The photoperiod is controlled with light timers controlling LED strip lights in the hood of the tank and a Smart LED Light (such as the "Magic Light" see Figure 21) that is programed by an app on an IOS or Android device via Bluetooth. These inexpensive lights, available from Amazon.com, provide "sunrise/sunset" ramping of the light at the beginning and end of the daily light period and multiple lights can be synchronized to the same program over a tank (Note: not all brands of smart LED light provide the sunrise/sunset ramping feature at both lights on and lights off so verify this prior to use). The sunrise/sunset mode of lighting is essential to reduce stress on the fish since pompano will startle if the lights come on at full strength

Figure 22. Example flow sensor installation of paddle wheel type flow sensor (top) and HBOI 3D printed float switch flow sensor (bottom).

immediately. This could lead to the fish jumping out of the tank or hitting the sides of the tank leading to death by asphyxiation or blunt force trauma respectively. The 3,000-gallon maturation tanks are equipped with egg collectors, air and oxygen diffusers and the emergency oxygen system is operated by a normally open solenoid such that a power failure will automatically open flow oxygen to the tanks (Figure 19). Black fireproof poly-sheeting separates each RAS broodstock system. This allows each system to be staggered at different seasons in the conditioning and spawning cycle. Each broodstock system is equipped with an Apex® monitoring system (Neptune Systems, Morgan Hill, CA) that communicates critical system status continuously via the "Cloud" through an iOS or Android app, Apex®Fusion (Figure 23). The Apex® device monitors the power status of pumps and heat exchangers, high and low water levels in the sumps, water flow in the system, and system temperature. Alarms are sent out to key personnel if any levels fall out of optimum set points. The system employs two types of flow sensor: the provided paddle-wheel sensor, and a 3D printed float switch flow sensor designed by HBOI personnel (Figure 22; STL model for printing available on HBOI website, requires about 57 g of PLD filament costing approx. $1.25 per flow sensor to print) (Float Switch sensor

component is the “Anndason 6 Pieces Plastic PP Float Switch Fish Tank Liquid Water Level Sensor, Model No. : DP5200”; Max Contact Rating : 10W; Max Switching Current : 0.5A; Max Breakdown Voltage : 220V DC; Max Carry Current : 1.0A; Max Contact Resistance : 100m ohm; Temperature Rating : -10 / +85 Celsuis; Float Ball Material : P.P;Float Body Material : P.P; Cable Length : 39cm / 15.3" ; Switch Body Size : 65 x 22mm / 2.6" x0.87" Thread Diameter (Approx) : 16mm / 0.63" available from Amazon.com [search for B072QCHQ2P] price as of 6/05/2023 $13.99 for a package of 6; an equivalent brand and model may be suitable as well). Both flow sensors are connected to an accessory pipe that diverts a small amount of system flow going to the tanks as a “telltale” rather than monitoring the full flow of any one of the process loops.

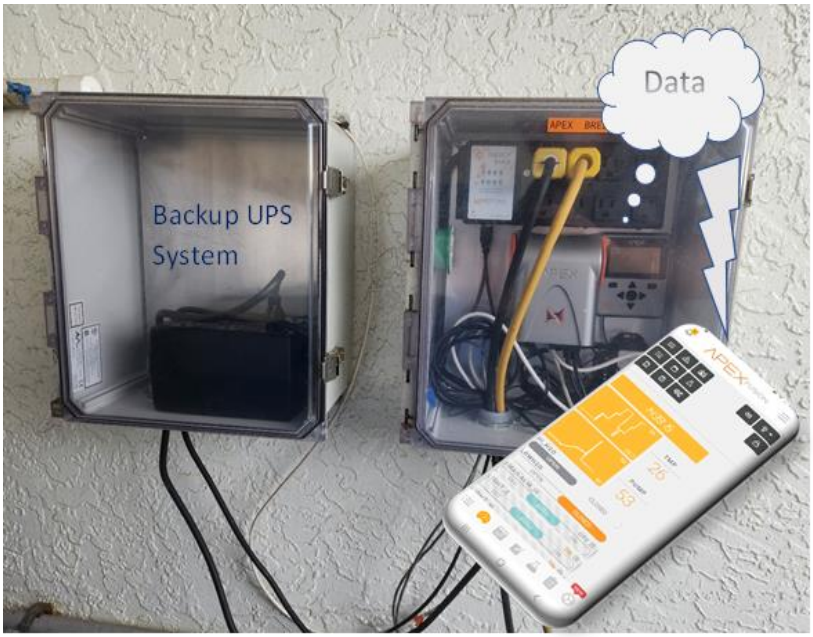

Figure 23. APEX® monitor and Alarm system installation with UPS battery backup added. Mobile app depicted by inset picture.

System Maintenance:

System maintenance checklist should be used that indicate tasks based on the frequency that they need to be accomplished (e.g., daily, weekly, annually). In general, sheets must require initialing by the personnel who completed each task once it is done so that all husbandry staff know that the task is completed and by whom. Knowing who completed the task is critical to determining why issues occurred so that standard operating procedure (SOP) changes can be made if needed. A culture of communication rather than repudiation is critical for efficient operation of complex RAS systems. No personnel should be reprimanded if a SOP is followed, and a breakdown occurs coincident to their completing a task. This leads to personnel hiding errors and the inability to adjust SOPs to reduce the chance of issues recurring. Example tasks (based on HBOI operations) and their suggest frequency of completion are:

- Pleated paper filter cartridges (Figure 24) are changed once a week or as needed based on reduction in flow to the tanks. Cartridges can last 1-3 years, depending on use with careful cleaning (Figure 25).
- Polygeyser™ bead filters are purged of solids collected in the bottom 1-2 times a day.
- Tanks are purged 1-2 times per day.
- Floors and concrete drains are cleaned with water once a day.
- The smart LED bulbs used for photoperiod control should be checked for operation and changed as needed.

Figure 24. Example pleated paper cartridge filter insert.

- Apex® probes need to be checked for proper operation daily
- Apex® monitoring probes need to be cleaned at least once a week.
- Diffusers including bio-weaves diffusers need to be checked daily and swapped with clean diffusers as needed. Dirty/clogged diffusers can be cleaned for later swap out.
- Photoperiod/Temperature cycles should be checked daily - a set photoperiod and temperature schedule is maintained for each broodstock tank according to their stage in the conditioning cycle, which will be discussed later.
- Water exchanges in these closed RAS systems only occur when fish are being sampled, moved (due to disease event) or if water quality parameters fall out of optimum levels. Otherwise, these are closed zero-exchange systems where water replacement is done only to compensate for evaporation.
- UV bulbs should be replaced once a year.
- UV quartz sleeves should be wiped down once a month.

- The sight glass on the side of the UV filtration unit should be checked daily to verify proper operation as the operator will be able to directly see when a bulb is out or if the intensity of the bulb is diminished. The material of the sight glass is generally designed to protect the user from exposure to UV-C irradiation, verify this with the manufacturer of the UV filters you use. CAUTION: Never look directly at a lit UV germicidal lamp without proper UV filtering glasses as the UV-C irradiation will quickly damage your eyes. Also, exposure of unprotected skin to UV-C irradiation will cause burns.

Figure 25. PVC stand constructed to facilitate pressure washing of pleated paper filter cartridges for reuse.

- Filter pressures should be checked at minimum daily, or better twice daily, they should not exceed manufacturer recommendations (operation 40 psi, max pressure is 50 psi in the case of the HBOI systems). Note: Gauges that last in salt environments are difficult to find so staff should also pay attention to the flow back to the culture tanks. If flow is decreasing and pressure gauges are not indicating high pressure the pressure gauge should be checked and likely replaced.
- Biological filter maintenance: Oxygen levels in the biofilter sump should not fall below 5 mg/L. The nitrifying bacteria species are aerobic. Anoxic conditions in the biofilter will kill the nitrifying bacteria colony. Keep the biofilter media in the dark if possible. Nitrifying bacteria are photosensitive. Make sure water going into the biofilter is prefiltered to reduce organic particulate waste that will lead to excessive

heterotrophic bacteria colonization of the biofilter and will cause anoxic conditions in the biofilter. It may be necessary to re-inoculate or change out old media for new seeded media periodically. The plastic biomedia will breakdown over the years into small pieces, especially if exposed to sunlight, UV or ozone. It is always good to have pump baskets on pumps to catch these small pieces as they break down so that they don't clog pumps. It is essential to keep the biofilter healthy.

- Tank cleaning: Daily cleaning of the brood tanks with fish in them involves rinsing off filter screens/socks, draining and rinsing out egg collectors and egg collector screens and wiping down viewing windows with a soft cloth and keeping them clean of algae and biofilm growth. The scum line in the tank can easily be removed with a scotchbrite™ or similar abrasive pad and sponge as long as it has been kept wet.
- Between spawning runs it is good practice to disinfect tank surfaces with chlorine bleach. This can be done by draining and rinsing tank with freshwater to remove any large particulate matter, algae, food residue, and other contaminants. Then a coat of jug strength chlorine bleach is applied to the tank surface with a sprayer or spray bottle and left to soak for a few minutes. All the surfaces of the tank should be scrubbed with scotchbrite™ pads or a good stiff brush (note: we advise using only composite plastic brushes as wooden brushes will disintegrate over time and are a potential disease reservoir). Rinse and repeat if needed. It is important to let the tank dry out afterward if possible. After refilling the tank, check to make sure there is no residual chlorine in the tank water. A chlorine test strips or HACH™ chlorine test kits are good for this. A sodium thiosulfate rinse can be used to dissipate any residual chlorine levels, be sure to test afterward.

Water Quality in Broodstock Systems:

Water quality parameters (salinity, dissolved oxygen, pH, and temperature) are recorded twice a day, AM/PM. Electronic meters are the most efficient method for this task (e.g., YSI D.O. Meter). Single parameter meters are preferred in our facility as we don't lose the ability to measure every parameter if one meter goes bad as can be the case with multi-parameter meters. Also, the probes on single parameter meters are less bulky. Total ammonia nitrogen, nitrite and alkalinity tests are conducted twice a week. We use HACH™ pillow pack reagents and one of the HACH™ spectrometers (e.g., HACH™ DREL). All water parameters are recorded on daily datasheets.

- *Dissolved oxygen (D.O.):* D.O. levels in the tanks should not fall below 5 mg/L (ppm). Air blowers should be maintained on a regular basis. The intake filters on blowers should be cleaned as needed. Air stones, Bio-weave™ diffusers (Bubblemac Industries, Inc., McAlpin, FL) and other diffusers should be swapped out for clean ones periodically. Fouled sintered glass air stones can be cleaned by soaking in 50% muriatic acid/50% freshwater for 5-10 minutes, then rinsed in fresh water, followed by a 50% chlorine/50% freshwater bath for 30 minutes to an hour. Cleaned air diffusers should be rinsed thoroughly with fresh water and allowed to fully dry before reuse. If pure oxygen is used (from Liquid Oxygen [LOX] or other sources) the oxygen diffusers can be cleaned as per manufacturer recommendations. Emergency oxygen diffusers should be regularly inspected and tested by manually activating the solenoid to verify proper flow. If flow is reduced the diffusers should be swapped with clean diffusers and cleaned for reuse.

- *pH*: If pH levels fall out of optimum range between 7.6-8, adjustments can be made with soda ash and sodium bicarbonate.
- *Salinity*: When a RAS is used, salinity levels typically rise due to evaporation. Adjustments can be made using freshwater to return salinity level to the optimum 30-35 ppt. The systems should be plumbed with both salt and freshwater supply lines to accommodate these adjustments. If incoming salinity is below 35 ppt then salt such as one of the synthetic salt blends can be used or solar salt (Sodium Chloride) with no additives can be used to raise salinity. Solar salt should be used for small changes as it doesn't contain trace minerals or other salts in ocean water. Be careful that any salt you use does not have yellow prussiate of soda (a.k.a., sodium ferrocyanide, a common anti-caking agent) or other additives as they can build to toxic levels in RAS.
- *Temperature:* Temperature can be controlled by various means including an immersion heater, a flow through heater, a building wide boiler/chiller with individual heat exchangers for each system, and HVAC style chillers or heat pumps with heat exchangers. Heat exchangers should be resistant to corrosion since they will be exposed to saltwater and extreme temperatures within their tubes. Generally, titanium heat exchangers are recommended even over high grades of stainless steel. Regardless of the system applied, it is important to make sure the system is running properly and water temperature is at the target level. Make sure probes are not corroded. If using HVAC style units ensure that freon levels in the unit are within the proper range and conduct regular preventative maintenance. Watch for rust on the unit parts and corrosion on cooling fins on condensers and evaporators. In an

insulated building air temperature can be regulated but this is not as practical in brood systems since the temperature changes required will affect all systems in a room and it can take more time to ramp up or down temperatures due to the passive nature of the method.

- *Nitrogenous Wastes:*
    - Ammonia: Levels are not to exceed 1.0 mg/L
    - Nitrite: Levels are not to exceed 2.0 mg/L
    - Nitrate: Levels do not become toxic until > 400 mg/L.

The control of nitrogenous waste levels is accomplished by having a healthy biofilter. The nitrifying bacterial community in the biofilter converts ammonia to nitrite (*Nitrosomonas* group of bacteria) and nitrite to nitrate (*Nitrobacter* group of bacteria). If the biofilter is not keeping up then water exchanges will be necessary, but this is a short-term solution and any issues with the biofilter must be addressed in the long term. A biofiltration system should be properly conditioned prior to housing fish. This is a process that requires 4 or more weeks (often 5-6 weeks depending on temperature) of monitoring water quality as a source of ammonia (e.g., ammonium chloride) is added along with sodium bicarbonate to stimulate the colonization of the biomedia by the nitrifying bacteria.

- *Other Important Water Quality Parameters:*
    - Alkalinity: Optimum levels fall between 50-300 mg/L
    - Hardness: Optimum level > 100 mg/L.

Adjustment of alkalinity is achieved with the addition of sodium bicarbonate (baking soda) and sodium carbonate (soda ash) to the water. Be careful when adding these

supplements because they will also adjust the pH of the water as well. Sodium carbonate will increase pH more than sodium bicarbonate since sodium bicarbonate has an equilibrium pH of about 8.3 and sodium carbonate has an equilibrium pH of about 11.6. Ca/Mg Hardness is typically sufficient when using seawater or synthetic sea salts but should be spot checked occasionally.

NOTE: Alkalinity in RAS systems changes dynamically relative to activity of the biofilter (bacteria in the biofilter consume bicarbonate) and the amount of CO_2 produced by the fish and bacteria in the system (CO_2 converts to carbonic acid which reduces pH and effectively Alkalinity). **Alkalinity, therefore, must be measured at least daily and maintained as above. If alkalinity falls below 50 mg/L the biofilter will shut down and will not effectively reduce ammonia and nitrite levels resulting in toxic buildup and eventually death of the fish**.

Broodstock Acquisition and Transport

Multiple sources of broodstock exist for fish farmers including purchasing brood size fish from other farms, capturing brood size fish from the wild (either with farm personnel or employing a commercial fisherman or biological specimen collector), or growing fish to brood size from stock on the farm. Regardless of the method used all state and federal certificates, permits, licenses, laws and rules must be adhered to so as not to put the farm in legal and financial jeopardy. Fines and/or jail time for “poaching” wild fish can be very steep and this type of activity also affects the already tenuous reputation of fish farming with the general public. The exact regulations for every state are beyond the scope of this manual but every farmer should investigate those that bind their operation.

Once a source of broodstock has been identified the animals need to be moved to the farm, or within the farm. Movement within a farm is straight forward but should be done very quickly to reduce stress on the animals. If personnel need to run to get animals between systems or areas of the farm in a net or it takes greater than 15 seconds to make the transfer the animals should be moved in water in a large bucket, tub, or tanks (for larger numbers) so that running is unnecessary. Running will lead to injury of workers and animals and should be avoided. The water in the container should be well oxygenated and if transport requires more than a couple minutes such as a compressed oxygen cylinder or other source of oxygen should be employed with a fine bubble diffuser. If transporting multiple groups in the same container (e.g., tank or bucket) the water should be changed regularly in the container to ensure the highest quality to reduce stress.

For transport between farms or from the wild to the farm a suitable transport vehicle that has pure oxygen available either using compressed oxygen or a LOX Dewar should be used. In some areas experienced fish haulers can be employed to haul fish if they are willing to haul in saltwater (many of their systems are designed only for freshwater). If the farm does not own this equipment but owns insulted large shipping boxes (a.k.a, dyno-boxes) they can be converted for use by drilling a small hole in the lid or upper edge of one of the sides just below the lip to pass an oxygen line through. The necessary equipment then is a tank such as the dyno-box, an oxygen source, an oxygen pressure regulator, an oxygen flow meter, oxygen distribution lines, a ceramic micropore oxygen diffuser, and a dissolved oxygen (D.O.) meter. Micropore oxygen diffusers are a bit more expensive than air stones but allow higher oxygen transfer efficiency and thus reduce the amount of oxygen needed and allowing a margin of safety in hauling time. This type of transport gear can be placed in the bed of a pickup truck and properly secured with heavy duty

ratchet straps. Note: the cam lock-style latches that come standard on dyno-boxes are not reliable and should be supplemented with ratchet straps as hold downs. One can also rent a large box truck that may not require a Commercial Driver's License (CDL) that will accommodate several shipping containers and oxygen bottles for hauling larger numbers of animals long distances. In summer or winter, it is helpful to have an enclosed truck to reduce temperature extremes.

When hauling it is advisable to stop within the first hour of beginning to haul to check that all straps and oxygen distribution systems are tight and in working order, and check that the oxygen concentration in the tank(s) is sufficient with the D.O. meter. Thereafter, periodic stops at a minimum every other hour should be done to check the hauling equipment and D.O. in the tanks.

Quarantine

Fish that have been moved to the farm from outside sources, from nonsecure areas of the farm, or in groups that have shown any disease symptoms should undergo a period of quarantine for a minimum of 30 days. During this period, the fish should be held in a quarantine area isolated from all other groups of fish until they are known to be clear of any parasites, bacterial infections or viral infections. Two example scenarios of quarantine are outlined here:

> Example #1 Disease-acclimation Procedures: Wild pompano will always come in with capture wounds. It is important that the quarantine systems are capable of a high turnover rate of clean water. This is necessary to reduce the likelihood of a secondary bacterial infection. If wounds continue to worsen after a few days with high water turnover, antibiotics may be needed to treat the fish. NOTE: The use of antibiotics is considered a last resort since practices should be taken that reduce antibiotic use in foodfish culture. Treatment with antibiotics

can only be done under the direction of a veterinarian based on the Veterinary Feed Directive (VFD). Prophylactic copper treatment is also recommended for possible parasite infections such as *Amyloodinium* sp. (see below) but must likewise be done under the direction of a veterinarian since it is not an approved treatment for food fish.

Example #2 Weening-acclimation Procedures: Wild pompano, as with other species, will typically not eat frozen food (a.k.a., cut bait) for the first week in captivity. To get them started, sometimes one must first feed them live shrimp for a couple of days. Once feeding has started, they can then be weaned onto frozen krill, etc.

Some fish will likely be lost from time to time during the quarantine and acclimation process. However, if there is sufficient clean water turnover and no overcrowding, the chances of losing fish are greatly reduced. Turbid water leads to bad water quality which results in stressed fish. Stressed fish have a compromised immune system and over time without relief this will result in sick and dying animals. Do not bring new fish onto the farm if insufficient space is available for quarantine and acclimation so that the quarantine systems are not overloaded.

Diet

Pompano broodstock have a high energy requirement, they are fed a minimum of 10% body weight per day (BWD) of a "cut-bait" or gel diet (see below). At HBOI we prefer the gel diet due to ease of handling and the ability to change the nutritional profile to meet the needs of different species of fish being conditioned. The amount of feed offered is adjusted based on the stage of the conditioning cycle (i.e., summer vs. winter) the fish are in. Fish typically eat more during the summer season of their spawning cycle (regardless of whether this is a natural season or an

artificially set season) and less during the winter season. Regardless of the feed amount, a daily ration is divided into 4 even feedings per day. The gel feed is made up of whole frozen krill followed by an enriched diet of INVE Fish Breed-M powder (INVE AQUACULTURE, INC., 3528 West 500 South, Salt Lake City UT 84104) mixed with whole frozen krill and un-flavored pork skin gelatin.

The recipe for the gel diet used at HBOI is:

500g whole frozen krill,

500g INVE Fish Breed-M,

80-120g pork skin gelatin.

NOTE: Fish Breed-M can vary in gelling capability and pork skin gelatin can be adjusted up or down depending on gelling which is affected by temperature and cooling rate; this comes with experience).

Using a high torque blender, mix these ingredients and add 1 cup of hot water while mixing. This mixture is then poured into aluminum trays to a thickness of ½ to ¾ inch thick. The mixture should be left to set up in the refridgeator for a few hours. The gelatin is then be cut to the desired size cubes using a knife of pizza cutter.

The cubes can be stored by freezing pre-weighed daily rations in airtight containers or freezer bags. These daily rations can then be defrosted in a refrigerator the day before use for the next day's feedings. This enriched gel diet is typically fed for the two months prior to bringing the brooders into their spawning season (i.e., during the conditioning period). They are then fed the enriched diet through the entire spawning season. Note that other manufacturers produce gel diets and other producers have reported good results with these other diets as well. In addition, Frank Hoff's 1996 book "Conditioning,

Spawning and Rearing of Fish with Emphasis on Marine Clownfish" (ISBN 978-0966296013) has a recipe for gel diet that has been used for a variety of marine fishes.

Conditioning

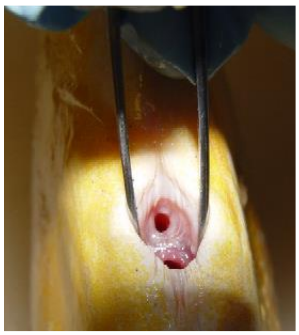

Figure 26. Female external anatomy showing urogenital opening (above) and anus (below) (Photo: Chuck Weirich).

Successful spawning conditioning includes not only a proper diet as above but also control of the broodstock maturation cycle through photo-thermal manipulation. A typical pompano spawning cycle is a 26-week process. Using this 26 week cycle the spawning group will remain in spawn season conditions for about 6 to 8 months. Temperature and light cycles are changed every week during the 26-week photothermal cycling period and then held steady during the 6 to 8 month spawning season. Once a conditioning cycle has begun it should not be interrupted unless there is a problem with the fish. For example, if fish become sick during a cycle it is important to hold the fish at their current photothermal stage, and do not

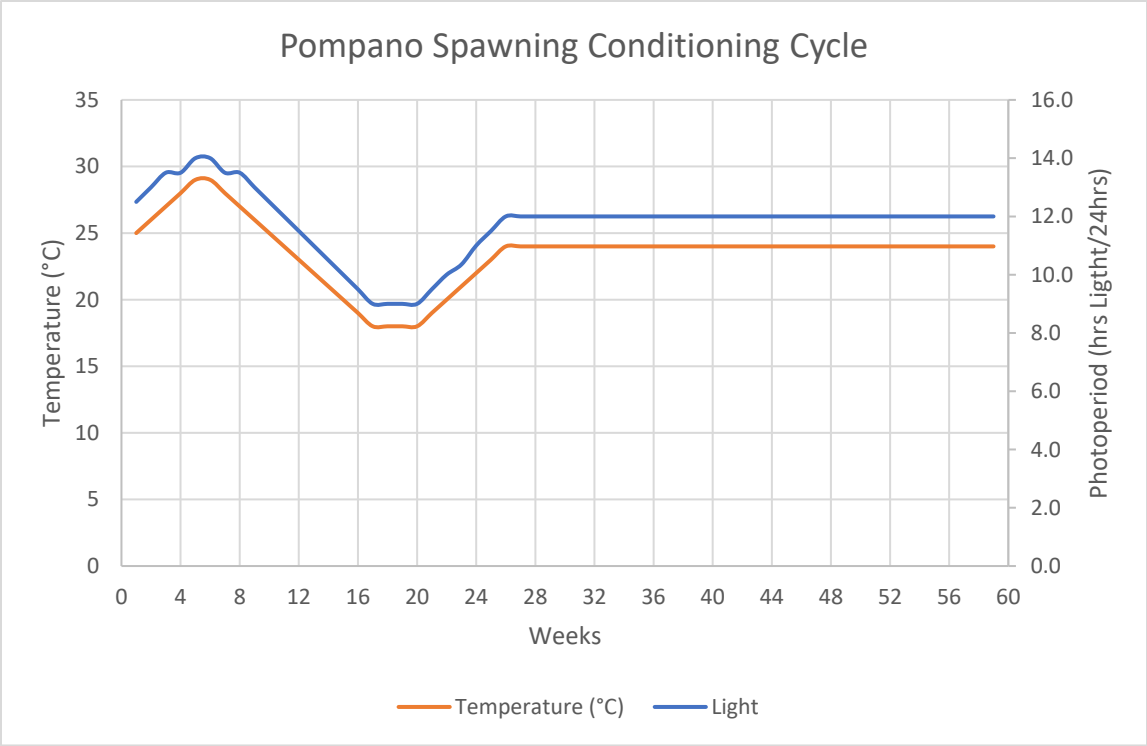

Figure 27. HBOI suggested Florida Pompano broodstock photothermal conditioning cycle. Orange line (bottom line) represents the changes in temperature in degrees Celsius over time and the blue line (top line) represents the changes in photoperiod specifically the hours of light per 24-hour period.

advance the group any further. Once they are healthy again and eating well, the cycle can resume. If the fish must be relocated to a clean tank the temperature and light cycle of the new system must match the old system prior to moving. pompano responds well to artificial photothermal conditioning cycles. They are spring spawners and will spawn when the temperature is 24℃ (75°F) and the photoperiod is 12 hours light and 12 hours dark (Figure 27).

Figure 18. Sampling eggs with Teflon tubing.

Gamete Sampling

Sampling of oocytes can be achieved using a 10 cm section of 20-gauge Teflon tubing attached to a 3 ml syringe to provide suction or some prefer a longer piece of 20-gauge Teflon tubing with suction provide by mouth (Figure 28). Males can be checked for running sperm by applying gentle but firm pressure laterally with the thumb and forefinger and moving from the anterior dorsal aspect of the abdomen to posterior ventral surface aiming for the urogenital opening. A stream of viscous sperm will flow in actively spermiating males (a.k.a., “running ripe”) (Figure 29). Do not use excess pressure

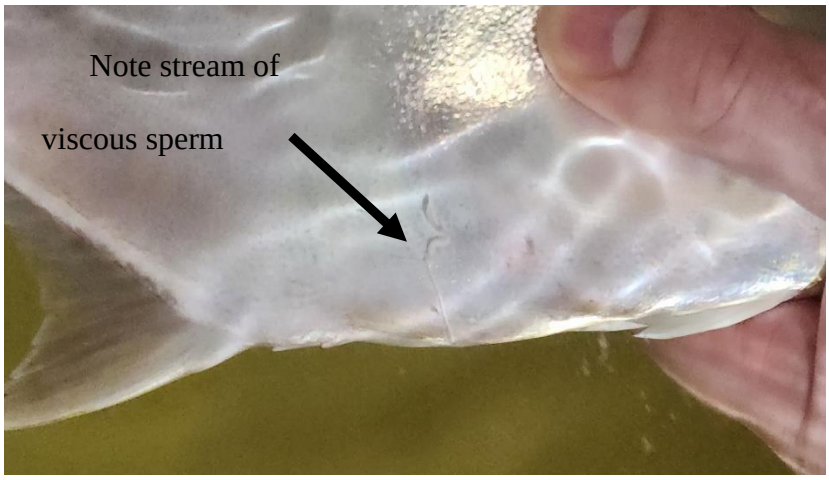

Figure 29. Gentle pressure applied to expel sperm while checking a male Florida Pompano. The male depicted would be considered in “running ripe” condition.

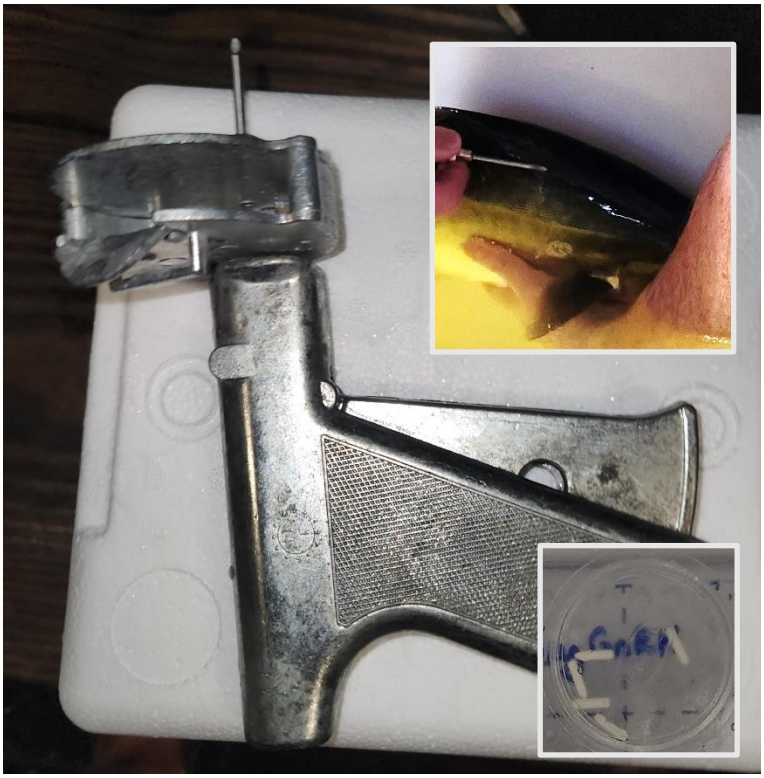

Figure 30. sGnRHa implants (insets; implanting gun, bottom, and injection site (inset, top).

when sampling sperm as pompano have a very small gut cavity and internal organs can easily be damaged if excessive pressure is used.

Spawning

Not all fish spawn naturally on their own in captivity (a.k.a., volitional spawning) and the Florida pompano is one of those species. This may be due in part to fish not experiencing specific environmental conditions/cues in captivity compared to their natural spawning grounds or perhaps other factors such as density and tank size. However, pompano will ovulate with hormonal manipulation once the ovaries are mature, large enough, and have been properly conditioned so that the eggs are sufficiently advanced in development to ovulate. From an aquaculture standpoint, the key to maturation is not to have the fish spawn spontaneously, but to have control over the timing of spawning events to meet production cycles. The photothermal cycling is used primarily to bring the oocytes to the point of maturation. At this point the application of a hormonal manipulation induces the cascade that leads to ovulation and thus spawning occurs. In pompano, to date, only the use of hormones is known to allow the controlled stimulation of final maturation and spawning. This is as opposed to red drum, for instance, where temperature pulses will do the same without hormonal

intervention. The result is control over the timing of egg release (controlled spawning). Once the pompano broodstock have reached a state of gonadal maturation (vitellogenesis), the hormones can be administered to achieve a spawning event. To determine this the key metric for determining readiness in pompano is when more than half of the oocytes (pre-spawned eggs) in a sample reach a minimum of 400-500 µm in diameter (Figure 31). The cost of USB digital electronic microscopes that allow observation and measurement of eggs is dramatically lower nowadays and is the system used at HBOI (Figure 32). We also use a well-type microscope slide for examining samples as it prevents the sample from moving around on the slide excessively during examination.

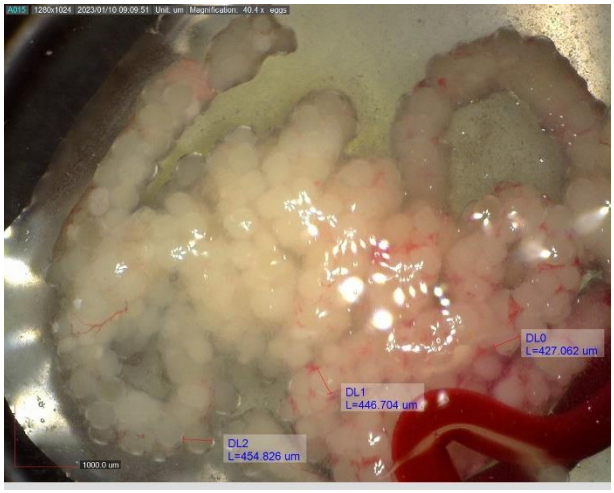

Figure 31. Example of a pompano egg sample showing measured eggs in the 400 to 500 µm range.

For hormonal induction at HBOI we historically have used the Syndel Ovaplant sGnRHa (salmon Gonadotropin Releasing Hormone analog) implants. This implant is designed to administer 75 µg sGnRHa/kg of body weight and will give the fish an initial burst of gonadotropin followed by several days of lower levels (Note: Syndel

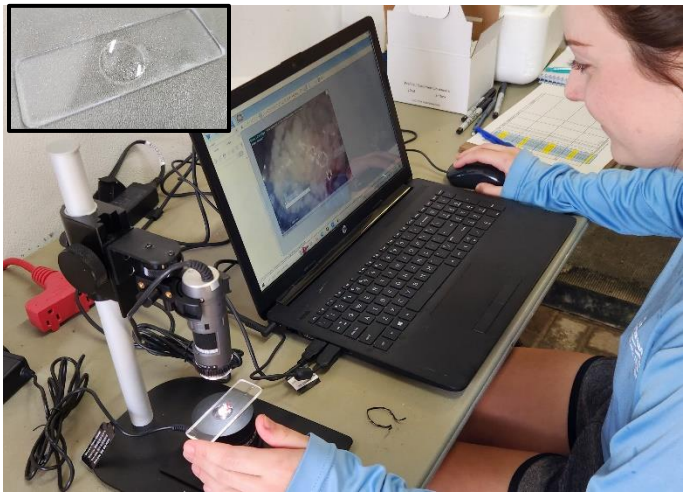

Figure 32. Technician measuring pompano egg sample using an electronic microscope and PC computer, a well-type microscope slide is depicted in the inset picture (Photo by James Masterson, HBOI)

is no longer producing this implant but rather is producing a 150 µg implant, they do not recommend splitting implants but others have had success doing so). Florida pompano (a high energy fish) will generally only yield one viable spawn at 1.5 to 2 days (~36 hrs) after the hormone injection. In contrast for other species, this may result in several consecutive days of spawning from a single implant. To administer the implant the operator injects the hormone implant in the shoulder meat of the fish as an intramuscular (IM) injection, just forward of the dorsal fin toward the head region using a specialized implanting gun (Figure 29).

Figure 33. Example HCG vial (inset) and injection site for intermuscular injection. Note: placement of thumb allowing massaging of injection site while administering injection to help HGC solution perfuse into musculature.

HCG (Human chorionic gonadotropin) is a hormone that comes as a premeasured dry powder packaged in a vial (Figure 33). It is reconstituted with bacteriostatic water (generally packaged in a separate vial with the hormone vial) and can be administered to males and females. Females receive the dose as an intraperitoneal (IP) injection (i.e., in the abdominal cavity) (not depicted). The IP injection sites are in the soft tissue areas behind the pectoral and pelvic fins. Care must be given to direct the injection at an oblique angle to the body so as not to damage any of the internal organs. Males will receive the injection as an IM shot in the dorsal musculature similar to the implants for females. It is more common to use HCG hormones for the males and GnRH implants for the females. Dose rates of HCG typically are 1,000-1,500 IU/kg (IU = international units).

Figure 34. View of egg collector showing nylon screen filter elements and bubble screen in place ready for a spawn once flow is diverted.

After fish have been injected with hormones in anticipation of a spawn the brood tanks should be configured such that the spawn can be captured and does not end up in the filtration system (in the case of an RAS) or down the drain (in the case of a flow-through system). In the systems used at HBOI a series of valves are opened and/or closed to divert the flow from the tanks through the egg collectors before the water continues to the filtration. The egg collectors are configured by inserting all needed filtration elements that retain the eggs and the oxygen bubble screens that keep the eggs alive (Figures 34 and 35).

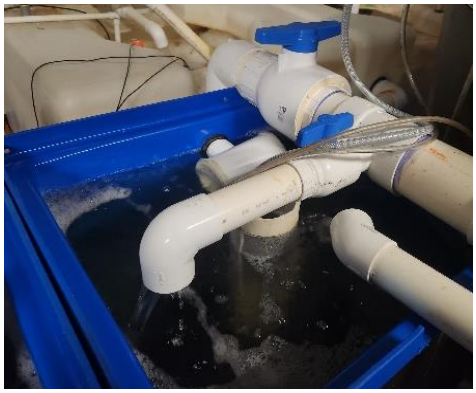

Figure 35. Egg collector box with flow diverted from tank in preparation for egg collection.

Hatching

Gamete Collection and Processing

The egg collectors are checked each morning after hormone treatment to determine if a spawn has occurred. Florida pompano generally will spawn in the early morning about 36 hours after hormone treatment. The floating eggs will have moved from the tank into the egg collectors with the diverted flow. Generally, it will take a couple hours after eggs have been observed to let all eggs in the system move into the egg collectors from the tank. This also gives time to prepare the hatchery to receive the eggs. Once all is prepared the eggs are removed from the egg collectors using fine mesh dip nets and moved quickly into the hatchery tanks or into egg separators if sinking eggs are still mixed in with the floating eggs or precise enumeration of the

dead eggs is desired (Figures 36 and 37). When used, the egg separators are filled with system water that was previously adjusted to the target of 34-35 ppt salinity so that live eggs will float. The eggs are allowed to sit briefly in the separator until clear layers have formed with the live viable eggs floating at the top and the dead non-viable eggs in the bottom of the cone. The valve is carefully opened, and the dead eggs collected in a container and enumerated to determine the proportion of dead eggs for hatchery records. The majority of the excess water can then be purged from the cone but care should be taken that the flow isn't opened to the point that a vortex forms causing live eggs to be eliminated from the cone at the same time. Finally, the live egg layer can be collected in a volumetric cylinder or beaker to determine the volume of live eggs. There are approximately 1,000 eggs per

Figure 36. Example of the style of net used to collect eggs from egg collector box.

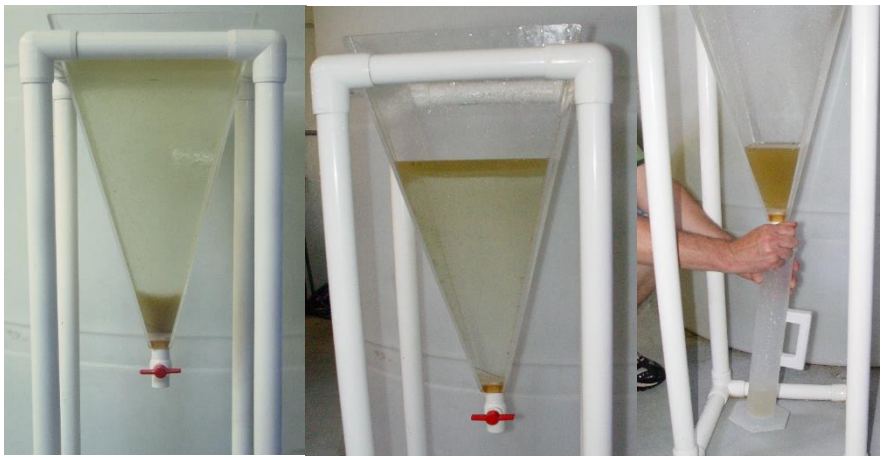

Figure 37. Series of pictures showing (left to right); separation of dead eggs (sinking) and live eggs (floating), post-removal of dead eggs, and moving live eggs for enumeration into a volumetric cylinder (Photos by Chuck Weirich).

Figure 38. Eggs floating after air flow and oxygen flow are turned off.

Figure 29. Skimming eggs directly from egg collectors.

ml for pompano so multiplication of the volume in ml by 1,000 gives an approximation of the number of live eggs (e.g., 40 ml of eggs x 1,000 eggs per ml = 40,000 eggs). For more precise enumeration 1 or more 1 ml subsamples of eggs can be taken and counted using a microscope to determine the exact egg count per ml for a given spawn.

Note: the separation cone step can be eliminated with care by turning off the air and oxygen flow to the egg collection tanks to allow in situ separation (Figure 38) and removal of the floating eggs directly. This is done carefully and providing sufficient time so that the flow within the collector settles and only live (i.e., floating) eggs are being collected and that the netting is done with care so as not to "stir" the water in the tank excessively disrupting the floating egg layer (Figure 39). The eggs can then be enumerated directly in a beaker and stocked into the larval tanks. This reduces many steps but also slightly reduces the accuracy of estimating percentage of hatch (Figures 40 and 41).

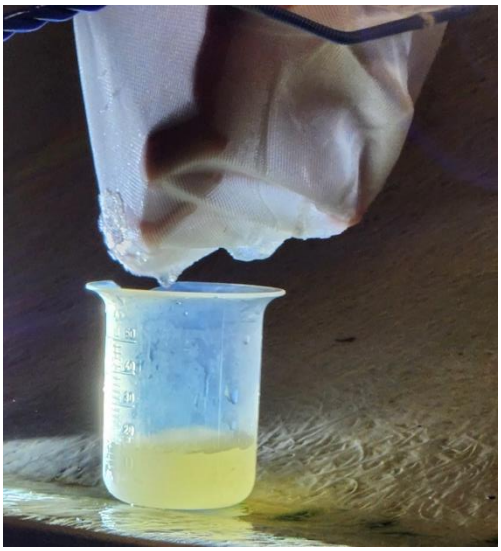

Figure 40. Carefully placing eggs from the collector in a beaker for volumetric enumeration.

Figure 41. Stocking enumerated eggs into the larviculture system tanks to hatch.

Hatching System

Eggs are enumerated volumetrically to be stocked directly into the larval culture tanks for hatching (Figure 40). This eliminates the need to move the fragile larvae from a hatching system to the larval culture systems after only a few hours. Eggs are stocked at a rate of 50-75 eggs/L of tank volume directly into the larval tanks depending on production goals.

Larval Culture (~0g to ~0.5g)

System Design

Figure 42. Commercial scale RAS based larviculture tanks for warm water marine fish with pelagic eggs and larvae.

Either a closed recirculating larval tank system or a flow through system can be used for larval culture of pompano depending upon the water source available. If flow through is used care should be taken to ensure the water quality is sufficient for the hatchery stage, or that supplemental filtration and treatment is used to ensure good water quality. At HBOI closed recirculating systems have been used successfully for multiple marine fish species including Florida pompano for several decades. Currently at HBOI, there are two larval RAS, each consists of 8 (725 L working volume; 192 gallon) tanks (16 tanks total, Figures 42 and 43). Each

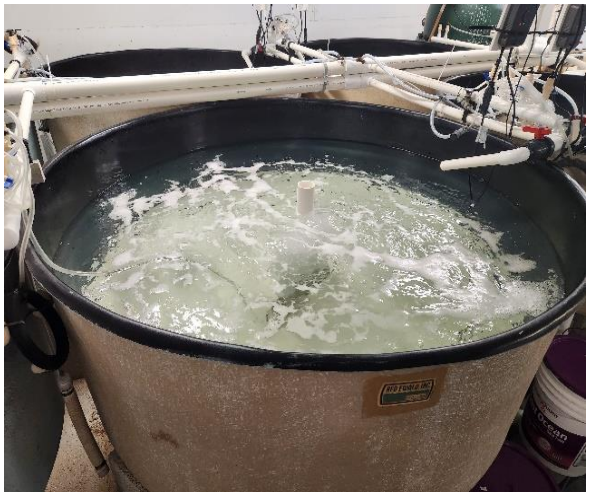

Figure 43. Closeup view of larval rearing tank. Note: light colored bottom and black sides.

Figure 44. Screens (left to right 250 µm and 500 µm), bubble curtain, and slotted pipes (from left to right 800 µm and 1,200 µm) used during the larviculture period for pompano from egg through weaning.

system is equipped with 5-10-micron socks for particulate removal, UV to provide a minimum of 30,000 $\mu w \cdot cm^{2 \cdot -1} \cdot sec^{-1}$ of 256 nm UV irradiation for sterilization and 3 ft^3 Polygeyser bead filter for solids and biofiltration and a fluidized bed filter/sump with MB3™ biomedia (Innovasea Systems, Inc, Baton Rouge, LA) for additional biofiltration. Temperature is controlled with a titanium inline heater. Overhead fluorescent lighting is maintained 24/7 through the whole larval process. The tanks are equipped with air and oxygen diffusers. Each larval system is equipped with an Apex® monitoring system (previously described). The device monitors power status of pumps and in line heater, high and low water levels in the sumps and system temperature. Alarms are sent out if any levels fall out of optimum set points. Center standpipes with filter screening are used in tanks with a 250 µm screen filter pipe used from Day 0 until about Day 9 (Figure 44). Standpipes are then switched to a 500 µm screen filter pipe. When post-metamorphic larvae look large enough, standpipes are again switched to slotted 2” pipes made from Schedule 40 PVC well screen that we source from a local irrigation supply house. The two slot sizes are used sequentially (i.e., 600 µm/ 0.020” slots followed by 1200 µm/0.040” slots).

Live Feeds

Production and use of live feeds is a very complex and critical process in the culture of larval pompano. Staff performing these tasks should be well trained and strictly follow standard operating protocols (SOPs). Often variations in the feeds and culturing the live feeds make identifying culture issues difficult. Having SOP checklists and keeping good records of all data is critical to troubleshooting. Not all staff members are "cut out" for live feeds culture due to the strict detailed nature of the processes. A staff member who is good elsewhere on the farm and then assigned to live feeds production may not fare well with the tasks at hand and should be reassigned to other tasks if they aren't showing the necessary aptitude. This should not be seen as a reflection on the work ethic of staff members, it is just the nature of live feeds culture. Without a well-performing live feeds crew, larviculture of pompano is not possible.

Copepods

Copepods *Pseudodiaptomus pelagicus* have been experimentally successfully used as a first feed for pompano resulting in slightly better growth and larval survival (Cassiano et al. 2011). Cassiano et al. (2012) also showed feeding both copepods and rotifers (i.e., rather than just rotifers) during the first three days of exogenous feeding conferred a degree of stress resistance to the pompano larvae. When feeding copepods, a fine mesh standpipe screen (50 µm) is required to retain them in the larval tank (Cassiano et al. 2011).

Rotifers

There are currently three "types" of rotifers commonly available in the market for marine fish culture in the USA currently. L-Type (*Brachionus plicatilis*) that reportedly range in size from 150-280 µm, S-Type and SS-Type (both are considered to be *Brachionus rotundiformis* but likely represent multiple species*)* that reportedly range in size from 85-180 µm and 70-160 µm respectively.

The current standard for pompano larviculture is to use enriched S-type rotifers, *Brachionus rotundiformes* as the first feed until transition to *Artemia*. Generally, this rotifer ranges in size from 85 to 150 µm, so a 50 µm screen is suggested to capture the widest size ranges. The S-type rotifers are grown at 25 ppt salinity, 29.2-29.5℃ (84.6-85.1°F) and at a pH of 6.5-8.0. Air and oxygen should be used to keep D.O. levels over 100% saturation. Rotifers are typically fed an algal paste feed such as Reed Mariculture Rotigrow Nanno™ (Reed Mariculture Inc., Campbell, CA), which is a *Nannochloropsis* microalgae paste. It can be kept frozen for two years or refrigerated for three months. It is sold in 1 L bags, jugs or 10 L cubitainers. Rotigrow should be refrigerated after thawing and opening and should be used within 1-2 weeks. Rotifers are fed Rotigrow daily along with the nutritional supplement (e.g., INVE S.parkle; INVE Aquaculture, Inc. Salt Lake City, UT). The supplement should be kept in freezer all the time and expires in a year. Timers and automatic peristaltic pump feeders are used to feed the algae paste preparation over a 24-hour period (preparation method described below). S.parkle supplements are manually fed three times a day. There is a three-day turnover on a Rotifer tank from stocking to harvest. S.parkle can be used as a sole diet if Rotigrow is not available and is mixed in larger batches and fed via the peristaltic pump feeder containers as would Rotigrow (see below).

Rotifer Daily Maintenance Standard Operating Procedures Used at HBOI:

NOTE: Much of the information in this section is specific to the systems at HBOI to provide a working example and can be adopted *as is* if systems are used that are similar in size. However, adaptations would need to be made for systems that are larger or smaller.

HBOI Rotifer Culture system:

Rotifer culture at HBOI is done as a batch culture process. 400 L (105 gallon) cone bottom rotifer culture tanks are used that are filled to 250 L (66 gallons). The feeding containers are 4 L

(1 gallon) polycarbonate food storage containers, “beakers,” that are available from most restaurant supply companies. These feed containers are filled to a volume of 3 L (3/4th gallons) The tanks each have four air stones with thumb-wheel type valves and fine bubble diffusers to keep D.O. levels high. They are plumbed with a joint manifold to allow draining the rotifer cultures from a selected tank into a centralized harvest tanks that has very fine screens to filter the rotifers (see Figures 46-50 and text below for details).

First steps in the morning to start the day:

- Add a ½ to full cap of defoamer (e.g., Proline® Defoamer, Pentair Aquatic Ecosystems, Apopka, FL, USA) to each tank if you notice considerable foam on the surface of the tank before doing anything else.
- Remove Rotigrow Nanno paste from the refrigerator and shaken well before using.
- Water parameters should be checked before using. The Parameters should be DO≥100% of saturation; Temp.~29.4±0.5°C (~85±1°F); Salinity~25t±5 ppt; pH>7.5 to 8.5.

Counting rotifers to determine colony density: Fill a 10 ml graduated cylinder with a 10 ml sample from the rotifer culture tank to be sampled. The sample should be taken from a consistent spot in the tank (e.g., always sample from a quadrant away from the aeration). Dilute that sample in a 100 ml graduated cylinder rinsing the 10 ml graduated cylinder several times into the 100 ml graduated cylinder with clean water (saltwater) using a wash bottle. This ensures all rotifers are transferred. Top off the 100 ml graduated cylinders to the 100 ml mark with clean saltwater. The diluted samples should then be poured into a clean 100 ml beaker and homogenized by pouring back and forth between a second clean 100 ml beaker at least 4 times. Immediately withdraw a 1 ml subsample from the homogenized beaker. Place the 1 ml subsample in a Ward counting

wheel's groove allowing separation between each sample. Repeat sampling with homogenization in between two more times resulting in three 1 ml samples from the rotifer culture tank around the counting wheel's groove (Figure 45). Add three drops of Lugol's iodine solution to each sample on the counting wheel. This will kill and stain the rotifers making the counting process easier since the rotifers darken and stop moving. Count the number of rotifers in each subsample under a dissecting microscope by slowly turning the wheel while tallying using a hand counter ("clicker"). Average the number of rotifers counted per 1 ml subsample and multiply by 10 (the dilution factor) to calculate the number of rotifers/ml in the rotifer culture tanks. Repeat this process for each rotifer culture tank. Log these densities on the farm's

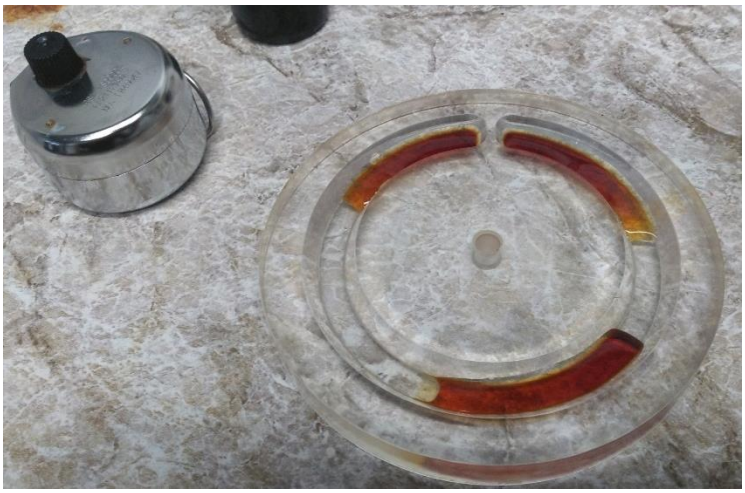

Figure 45. Ward Counting Wheel with three 1 ml samples of rotifers stained with lugol's solution.

Figure 46. Rotifer culture tanks. Note detail inset at bottom of Peristaltic pump for metering feed to the rotifers from the feed container.

Daily Rotifer Culture Datasheet (see appendix).

Example: counts =35, 37 and 39; average is 37; 37 x 10 = 370 rotifers per mL

After the rotifer counts are completed, rinse all graduated cylinders, beakers and counting wheels in the sink and store them on a drying rack for use the next day. Counting wheels can be stored upside down out of the way on the table in the microscope work area.

Microalgae paste feeding: Remove empty feeding containers from the peristaltic pumps and clean with freshwater in the sink. Use a scrub pad if needed to remove old algae paste. Turn the containers upside down to dry out. Also remove air stones and the peristaltic pump strainer assembly on the suction tubes. Be careful not to break the strainers or air stones as they can be very brittle. Rinse these off in the sink. Reassemble the strainer assembly on the suction hose. The fouled/used air stones should be placed in a chlorine bath for 2-3 hours minimum. An extra pump-strainer basket helps with keeping small items together in the chlorine bath. After the 2-3 hour soak time remove the strainer basket with the stones and hang on side of the chlorine bath to dry overnight. Clean air stones from the prior day can be used to reassemble the air lines. Fill each feeding container with saltwater to the proper volume for a day's feeding (a distinct black sharpie mark aids in visualizing the fill volume quickly from day-to-day). The 400 L rotifer tanks are filled to 250 L. The feed containers are filled to a volume of 3 L and the peristaltic pump is adjusted to deliver this volume over the 18 hour period (e.g., 10 a.m. to 4 a.m.). Place a new air stone and peristaltic pump suction tube with strainer attached into the feeding container. A chlorine test strip is used to make sure there is no residual chlorine before adding the feed. Add a small amount of sodium thiosulfate to the water if there is residual chlorine detected.

Add the microalgae paste at the amount needed depending on the density of rotifers in the tank. Although package instructions are not followed at HBOI, individual site results may vary. The HBOI rotifer culture method has been developed through years of trial and error and is dependent upon rotifer density. The formula used determines the volume in ml of Rotigrow Nanno to add to the Feed container = (((rotifer density in number/mL)/10) x 2). For example, if density in the tank is 400 rotifers/mL, drop the zero (i.e., divide by 10), so 40, then multiply by 2 (40 x 2 = 80). So, based on this calculation 80 ml of Rotigrow Nanno paste will be added to the feeding container. Use a graduated cylinder to measure microalgae paste volume. The Rotigrow Nanno is a cleaner product than some brands and foaming issues generally are not seen. If foaming has been an issue, add ½ to full cap of defoamer to the feeding container. Refrigerate unused Rotigrow Nanno paste for the next day.

Adding S.parkle feed supplement: Remove the container of S.parkle from freezer. Typically, at HBOI 9 g of S.parkle is fed each day to each tank. This is split into 3 separate 3 g feedings per tank per day; am, noon and pm. Fill a blender with 200 ml of lukewarm (20-40°C; 68-104°F) 1 µm filtered freshwater per tank and add the correct amount of S.parkle. Doses for multiple rotifer tanks can be blended at the same time. Blend for at least 5 minutes. Decant the blended S.parkle mix into a measuring cup for feeding while keeping any foam that formed out of the measuring cup. Feed 200 ml to each tank slowly. Repeat the procedure for the noon and afternoon (4:30-5pm) feedings. Rinse everything off and hang them on the drying rack between feedings.

Rotifer harvest and restocking:

Rotifer culture tank preparation: The day before harvesting, fill any new tanks to be stocked with saltwater and freshwater using the 1 µm filter and adjusting the salinity to 25 ppt. Place the

Figure 47. Disinfection Barrel with 10% sodium hypochlorite solution. Note the surplus pump basket for small part disinfection hanging on the outside of the basket for the parts to dry.

halfway with saltwater, mix and let dissolve. Meanwhile, add a cap full of defoamer to the tank that will be harvested. Clean up the dirty algae paste feeding container setup with a scrub pad. Turn it upside down and let dry out for use the next day. Remove the air stone, rinse and place in a basket hanging in chlorine

salinity meter in the tank as it is filled to adjust it to the correct salinity. Fill each 400 L tank that is to be stocked to the 250 L mark. Add 12-150 ml of sodium hypochlorite bleach at jug strength to the tank. Turn on the aeration so there is a light flow of air. Do not turn on the pure oxygen until the next morning when you are going to harvest. A microalgae paste feeding container with a new air stone and strainer assembly can also be set up for the new rotifer tank(s). Don't fill the feeder containers with saltwater until the following morning.

The morning of harvest, weigh out and add 16 g of sodium thiosulfate to a 500 ml beaker. Fill the beaker

Figure 48. Detail of Rotifer harvest tank (inset on lower left) and placement at end of culture tank drain manifold. Prefilter sieve is shown on the stand to the left of the harvest tank in the main picture. Note: Main drain manifold valve is the red handled valve to the left of the rotifer harvest tank It is shown resting outside of the harvest tank in these photos.

disinfection barrel. (NOTE: at HBOI a covered 80 L polytank is maintained with a 10% sodium hypochlorite solution for disinfection of nets and other equipment. A surplus pump basket screen with a hook fashioned from wire that hangs from the rim to hold small parts that need to be disinfected, see Figure 47). Remove the ceramic weight (if used) and strainer assembly. Rinse off in the sink. The ceramic weight can be added to the disinfection barrel's basket with the air stone. Clean the peristaltic pump filter strainer in the chlorine bath and soak for 20-30 minutes and remove and let dry in a 1 L cup to protect it from being damaged.

Add the dissolved sodium thiosulfate to the new, chlorinated rotifer tank (make sure the sodium thiosulfate is substantially dissolved). Let the sodium thiosulfate mixture mix with the chlorinated water in the tank for a minute or two. Also add a cup full of sodium bicarbonate to the tank to help buffer the alkalinity as sodium thiosulfate is acidic.

Prepare rotifer harvest tank: Begin to fill a polypropylene harvest tank (Figure 48) with salt water until saltwater flows out of it. Inspect a 50 µm harvest sock for damage, split seams or holes. Make sure that the seam is facing outward, so rotifers won't get caught in it. Place a 750 µm prefilter sieve (described below) inside the harvest sock (Figure 49) being careful not to scratch or rip the harvest sock. The prefilter sieve is constructed from a ring of 12 inch PVC pipe that will fit inside the filter sock with the 750 µm nylon filter cloth stretched tight and glued in place with epoxy or hot glue. A piece of ½ inch pipe is passed through two holes in the

Figure 49. Detail of prefilter (inset at top) and its placement in the filter sock of the harvest tank.

top to act as a brace to hold it in place in the harvest sock. Turn on the pure oxygen and set to 3 Lpm on the flow meter. Do not turn on the air yet. Have the water flow just trickling out/slow flow of the harvest tank. While the harvest tank is filling check that the residual chlorine is gone in the new rotifer tank(s) to be stocked with a chlorine test strip. Follow the directions for the brand of test strips used, at HBOI test strips are stirred in the tank for about 30 seconds. Also, check all the feeder containers with chlorine bleach test strips as well. If any color develops on the test strip (for the brand we use they turn pink/purple), add more sodium thiosulfate to completely neutralize the chlorine. Close the main harvest valve to the rotifer tank drain manifold (see red valve attached to a flex hose in Figure 48) and open the bottom valve on the tank that was just dechlorinated (these are blue on the HBOI system) (Figure 50). After a few seconds close the tank valve for the new tank. This step will fill the culture tank drain line manifold with some water that contains residual sodium thiosulfate that will dechlorinate any chlorine bleach that might have leaked out over night from the new rotifer tank into the rotifer tank drain line. Residual chlorine bleach anywhere in the system during the harvest and restocking process will kill the rotifers. Open the main harvest valve to the drain manifold (red valve on the HBOI system) and let the thiosulfate water drain out of the manifold into the floor drain.

Figure 50. 400 L Rotifer tanks on stand. Note: Harvest drain line manifold at bottom with individual valves for each tank (blue)

Finish preparing the new rotifer tank for re-stocking: Inoculate the new rotifer tank and algae paste feeder bucket with algae paste. Measure out 10 ml of algae paste into a graduated cylinder. Add the volume to the new rotifer tank so that the new rotifer culture has feed immediately after stocking. Measure out 20 ml of algae paste and add that to the feeder bucket (after day 1 feed the calculated amounts from above based on the counts) (NOTE: the 30 ml (10 ml + 20 mL) indicated is calculated based on the stocking density of 150 rotifers per ml (150 rotifers per mL/[10*2] = 30 ml of algae paste). Rinse the graduated cylinder into the tank. Defoamer may or may not be needed at this point. Double check that the heater is on for the new tank and unplug the heater from the rotifer tank that is going to be harvested. CAUTION: A plugged-in heater in an empty tank is a fire hazard.

Rotifer harvest: Make sure all the valves are closed underneath the rotifer tank drain manifold for all the other rotifer tanks (otherwise you will get back flow into the empty tanks when you open the rotifer tank to be harvested). Make sure the main harvest drain valve on the hose by the polypropylene harvest tank is closed. Open the valve at the bottom of the rotifer tank that is being harvested. Open it slightly and let air bubbles purge slowly out of line before opening it

completely. Then go to the main harvest drain hose. Purge just a small amount of the harvest water out of the line into the floor drain by slightly opening the harvest drain valve briefly. This is done to purge any sludge present in the bottom of the rotifer tank that came down when the tank valve was opened. Attach the harvest hose in the hose clamp on the side of the harvest tank and point the hose down into the prefilter sieve. It helps to wedge it under the PVC handle piece on the prefilter sieve. Open the main harvest hose valve about ¾ of the way to get a moderate steady flow. At HBOI, the valve is marked with a sharpie line to help with visualizing the position since this whole process is fairly quick and the following steps are done in quick succession. Open the airline on the harvest tank (HBOI's is also marked with a sharpie at the correct position). The aeration should look like a "light hot tub flow" of air bubbles. Control the clean water flow from the saltwater supply line (again our valve is marked with a sharpie). At this point the harvest tank is receiving flow from the saltwater supply line and the rotifer culture tank drain line manifold. The saltwater flow helps to keep the harvested rotifers in the sock from impinging on the fine mesh and helps to clean the rotifers throughout the harvest. Adjust the saltwater flow by opening or

Figure 51. Removing rotifer harvest sock from the polypropylene harvest tank for transfer to a 5-gallon graduated harvest bucket.

closing the valve as needed. The harvest sock will need to be constantly checked at this point. As the rotifers get dense in the bag, they will clog up the side of the bag. Frequently gently massage the bag (typically every 20-30 seconds or so) to keep the rotifers in the water column and off the sides of the bag. When the rotifer culture tank being harvested is empty the flow from the drain manifold will stop. Take the harvest hose out of the polypropylene harvest tank prefilter sieve and pour the last bit into the drain. The hose can be left in the drain. Carefully remove the PVC prefilter sieve and rinse any scum on it into the sink. Place the prefilter sieve out of the way and let it dry.

Prepare a graduated 5-gallon harvest bucket by filling it with about an inch or two of saltwater. Drop the standpipe on the polypropylene harvest tank by rotating it on the bulkhead fitting. As the water drains out, keep massaging that bag to keep the rotifers suspended. When the water level gets low, use a glass 1 L beaker to move three or so beakers full of rotifers to the graduated harvest bucket to allow space in the harvest sock. Remove the harvest sock with the remaining rotifers and move it over to the graduated bucket and gently invert the leftovers into the bucket and rinse the sock with the saltwater hose. Return the sock to the polypropylene harvest tank for cleaning later. Top off the graduated bucket to the 13 L mark. Turn off the saltwater hose. Move the bucket of rotifers onto the platform and place the oxygen diffuser into the bucket and decrease the oxygen flow to a light flow 0.5-1.0 Lpm adjusting by eyeball so as to provide good oxygen flow but not to “boil” the rotifers with excessive agitation. Turn off the air flow to the empty polypropylene harvest tank. Shut off the air and oxygen flow meter to the now empty rotifer culture tank.

Stocking the new rotifer culture: Conduct a rotifer count on the rotifers in a graduated harvest bucket using a similar method for counting the culture tanks except you will be using the L cups and 1 L graduated cylinder. Fill the 1 graduated cylinder to 900 ml mark and add a 10 ml sample from the bucket. Be sure to take the sample in a location away from the oxygen flow. Rinse the small-graduated cylinder with clean water and top off the L graduated cylinder to the 1 L mark. Pour between the two 1 L beakers and mix 4 times to homogenize the rotifers. Take the three 1 ml samples homogenizing in between for the counting wheel well as described above. Count the rotifers in each sample, average them and multiply by 100 this time (as there is a different dilution factor). This will be the estimated number of rotifers per ml in the harvest bucket.

Example: 84, 83, 88; average is 85 x 100 = 8,500 rotifers per mL.

Inoculate rotifers in the new tank: To calculate the volume of rotifers to add to the new tank from the harvest bucket the formula is: (150 rotifers per ml stocking density x 250,000 ml volume of the culture tank)/ rotifers per ml in the harvest bucket (as calculated above). The result will be the volume in ml you will measure from the harvest bucket to add to the new tank. Divide this result by 1,000 to convert the volume to L.

Example: (150 x 250,000)/8,500 rotifers per ml= 4,411 ml/1,000=4.411 L.

Use appropriately sized volumetric cylinders and/or beakers to add this volume of concentrated rotifers from the bucket to the new rotifer tank.

Begin the S.parkle feedings for the new tanks immediately. For the size tank used at HBOI, 3 g per tank three times a day like the other tanks is used.

Final steps:

If no feeding is planned with the rotifers discard the extra rotifers in the graduated bucket and rinse everything. Otherwise, save the rotifers for enrichment and feeding to the larval fish (see below). Make sure air and oxygen are turned off in the polypropylene harvest tank. Clean and rinse the harvest tank and carefully clean the rotifer harvest sock by rinsing it with clean freshwater.

Clean the rotifer culture tank that was just harvested. Attach a vinyl hose to the freshwater outlet (HBOI's is installed above rotifer tanks for convenience). Then complete an initial rinse down of everything (don't open the valve fully just open enough to get a good flow for rinsing). Hand clean the tubing and each air stone by rinsing them with freshwater. Lay each line up out of the tank as they are cleaned. Do the same for the heater and oxygen diffuser. Removing everything from the tank will make it easier to clean with nothing hanging in the way. Use the scrub brush to scrub all the scum off the walls of the tank. Rinse with freshwater. Finish up with a long handled stiff bristled scrub brush to scrub the bottom of the tank. Do a final rinse of everything. Re-hang the air stones and other parts back in the tank and let all sit to dry overnight.

Feeding larvae and rotifer enrichment: The balance of the rotifers harvested are ready for enrichment for feeding (Figure 52). Rotifers are fed to the larval pompano in the larval tanks at a rate of 5-6 rotifer per ml. This number can be calculated as in the following example: 725 L working volume larval tank = 725,000 ml volume. To feed 5 rotifer/ml multiply 725,000 ml x 5 rotifers/ml = 3.625 million rotifers per tank per feeding. This feeding is done four times per day. Therefore, each 725 L tank requires 3.625 million rotifers per feeding x 4 feedings per day = 14.5 million rotifers per day per tank. To ease the process of feeding stock each 5-gallon enrichment bucket with 15 million rotifers minimum to allow a bit extra. A density of up to 20-25 million is permissible. Keep in mind that at densities higher than this it is difficult to maintain

adequate water quality in the bucket overnight even though they will be cooled down and refrigerated after enrichment. For this example, at HBOI a density of 15 million rotifers per bucket is used for four larval tanks (i.e., four buckets). This will total 60 million rotifers that will be needed from the harvest to reach the desired amount for enrichment.

Fill each bucket up with 10 L of saltwater. Each bucket should have an oxygen diffuser and air stone for aeration during the enrichment.

Calculate the volume of rotifers to move to each bucket for the 15 million density.

Example: If your harvest bucket density is 10,000 rotifers per ml that would equate to 1.5 L of rotifers added to each bucket to reach the 15 million rotifer stocking density (i.e., 1,500 ml x 10,000 rotifers per ml = 15,000,000 rotifers).

Stock the enrichment buckets with the calculated volume. Add the required oxygen and air supplies to keep the rotifers alive.

Add the enrichment product desired. At HBOI we use Ori-Green™ (Skretting USA, Tooele, UT). Ori-Green should be stored in the freezer between uses.

Ori-Green addition depends on the density of rotifers in the bucket. For 15 million rotifers use 4 g per bucket (16 g total for 4 buckets). Mix the 16 g Ori-green in 1 L of salt water (250 ml per 4 g) for 1-2 minutes in a blender. Turn blender off and let the mixture hydrate for 5 minutes then turn the blender back on for an additional 1-2 minutes for a final mix. Divide the 1 L of mixed enrichment evenly between the 4 buckets (250 ml per bucket).

NOTE:

**For 20 million rotifers it would be 4.5 g per bucket.

**For 25 million rotifers it would be 5.0 g per bucket.

Figure 52. Rotifer enrichment area setup.

Figure 53. Re-filtering enriched rotifers to clean out excess enrichment product.

After enrichments have been added top-off the bucket volume to 12 L and let the buckets sit for 2.5 hours. Check the D.O. and make sure it is high (at least 160% of saturation).

After the 2.5-hrs enrichment period, flush the excess enrichment out of the buckets. One at a time, move the buckets back over to the polypropylene rotifer harvest tank (Figure 53) and pour back into the harvest sock. Make sure the saltwater and oxygen are turned back on before the rotifers are poured back into the sock in the harvest tank. Let the rotifers in the sock flush until the water clears up. Move rotifers back to the bucket and fill to 12 L again with salt water. Bring the bucket back to the rotifer enrichment area and add the oxygen and air diffusers back to the bucket (Figure 54). Repeat for the other buckets.

Chilling the rotifers must be done so they do not metabolize the enrichment product overnight. Start the chilling process by putting frozen water bottles into each bucket. Three bottles for each full bucket is generally sufficient. Switch them out for new frozen bottles once

they melt. After the second batch of bottles melt remove them and put the buckets in the refrigerator to cool down the rest of the way. The refrigerator must be modified to allow the oxygen and air lines to pass into the refrigerated compartment. Consult a qualified refrigeration technician and any manuals for the refrigerator being modified to ensure that no refrigerant lines are punctured while drilling through the cabinet walls. Set the refrigerator to 10°C (50°F), the temperature should not go below 9.0°C (48°F).

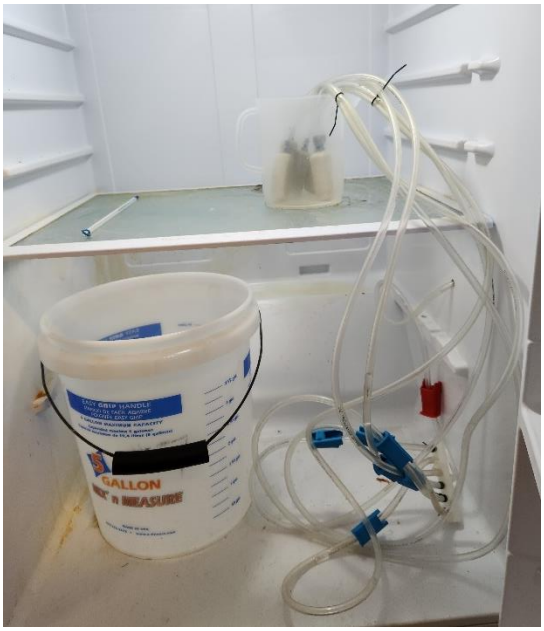

Figure 54. Refrigerator cabinet with oxygen run inside for cold storage of enriched rotifers or Artemia overnight.

NOTE: Before chilling down the buckets of rotifers you can do the morning feeding of the larval fish tanks.

Rewarming the rotifers before each feeding must be done so that the rotifers aren't shocked by a rapid temperature change and will swim normally. Remove the whole bucket to be fed from the fridge. Move the chilled rotifers to the enrichment setup area. Provide oxygen and air. Put heaters in the bucket and let them warm up to ~28°C (82°F), approximating the temperature of the larval culture tanks. Check the temperature with a thermometer periodically. When the target temperature has been reached the measured rations can be fed to the larvae.

Greening and feeding larval tanks: It is necessary to green the larval tank water before feeding. The green color provides a contrast to the rotifers so the fish larvae can see them. At HBOI Sanolife® GWS powder (INVE Aquaculture, Inc., Salt Lake City, UT), is used. Add 15-20 g of the Sanolife® GWS powder to 1 L of seawater and mix for a minimum of 5 minutes with a blender. Divide the mixture evenly among all the larval tanks. Repeat as many times as needed to

achieve a Secchi disc turbidity between 19-24 cm. The warmed rotifer rations may then be fed to each larval tank as described above.

Artemia

At HBOI the GSL (Great Salt Lake) 90% hatch brand of *Artemia* cysts are preferred due to their high hatch rate. They come in sealed cans that are stored in the freezer for extended periods (NOTE: storage can be as long as two years in the freezer in their original sealed can, upon opening a can they will take on moisture and over time this will reduce the hatch rate). Each can contains 425 g of cysts. The price of *Artemia* has increased over time so it can make sense to maintain a good supply on hand. This may particularly be important given that the primary domestic source of *Artemia* cysts is the Great Salt Lake which has been shrinking in size and some predictions are that it may dry completely within the next half-decade compromising future supplies.

Artemia Decapsulation, Hatching and Enrichment protocols:

Decapsulation recipe:

(NOTE: the process is time sensitive so be sure all components are on hand and premeasured)

(CAUTION: use necessary personal protective gear since caustic chemicals are used for this process)

For decapsulation you will need:

A ~17 L cone tank with valve at the bottom (Figure 55)

(Available from https://floridaaquafarms.com)

Freshwater to hydrate the *Artemia* cysts.

20 g of NaOH in 800 ml of freshwater

60 g of sodium thiosulfate in 1 L of freshwater

2 L of chilled chlorine bleach

2 L of chilled seawater

- Hydrate a whole can of *Artemia* cysts for 45 minutes to an hour by soaking the cysts in freshwater. (NOTE: for partial cans adjust volumes and weights of ingredients as necessary maintaining the same ratios)
- Add hydrated *Artemia* to decapsulation cone.
- Add 2 L of chilled seawater.
- Add NaOH and chlorine bleach.
- Turn on air to the cone and let the decapsulation process commence.
- After about 2.5- 5 minutes the color should change from grey, brown to bright orange.
- Add sodium thiosulfate to neutralize the chlorine once this color change occurs.
- Drain decapsulated *Artemia* cysts into a mesh screen bag and rinse thoroughly with clean freshwater for 5-10 min.
- Put rinsed decapsulated *Artemia* cysts back into cone.
- Add 1-2 L of saltwater
- Drain the *Artemia* and saltwater into a three-L jug and top off to 3 L.

Figure 55. *Artemia* decapsulation cone with stand setup in a sink dedicated to the process.

- Place jug in refrigerator for storage. Store no longer than a week in the refrigerator.

Hatching Artemia: The working volume of the HBOI *Artemia* hatching tanks is 300 L (80 gallons) using the same 400 L cone bottom tanks as for rotifer cultures. The only difference in the setup is that *Artemia* do not require the peristaltic pumps. Heaters, air and oxygen supplied by diffusers are used. NOTE: locate the *Artemia* tanks in an area separate from the rotifer tanks. If *Artemia* find their way into the rotifer tanks they will prey on the rotifers and cause problems with the rotifer cultures. The *Artemia* hatching tanks should be filled with chlorinated water similar to the procedure above for new rotifer tanks. Dechlorinate the water as above with sodium thiosulfate. Set air and pure oxygen diffusers in the tank. Turn on heaters (set to 30°C; 89.6°F). Add decapsulated *Artemia* to the tank. Maximum density would be one 3 L jug per tank which is one full can of *Artemia*. (425 g of cysts per can prior to decapsulation). Turn on overhead lights and put a clear acrylic cover over tanks to prevent splashing. Water flow from the aeration in the hatching tank should be rolling strongly to aid in hatching. Typically, it takes 16 hours to hatch but it may take as long as 24 hours.

Artemia Enrichment: First instar *Artemia* do not require enrichment, but later instars should be enriched. Enrichment of *Artemia* at HBOI is achieved using INVE A1 DHA Selco® (INVE Aquaculture Inc., Salt Lake City, UT). Enrichment requires 24 hours. Follow the manufacturer's instructions on the bottle. Mix by hand for 1 to 2 minutes in a blender with freahwater. Add enrichment to *Artemia* hatching tank directly. Keep temperature at ~29°C (~84°F). Enriched *Artemia* can be chilled and cold stored as with rotifers. The hatchery should have at least two modified refrigerators, one for each live food species. Each refrigerator at HBOI can hold four 20 L (five-gallon) buckets.

Larviculture and Weaning

Prior to stocking fertilized pompano eggs into the larval hatching and rearing system tanks, the larviculture water is chlorinated to 30 ppm with chlorine bleach. ***CAUTION: the water supply from the RAS loop is turned off to prevent chlorine from reaching the biofilter.*** The chlorine is allowed to gas off a few days before the spawn is anticipated. Any residual chlorine is removed with the addition of sodium thiosulfate after testing with chlorine test strips. Once a fertile spawn is retrieved from the brood system egg collectors it is transferred to the larval hatching and rearing system tanks. The egg collectors that are part of the HBOI broodstock systems (see above) allow the viable floating eggs to be skimmed from the surface directly from the collectors while the non-viable eggs settle to the bottom when aeration to the collector is turned off (see broodstock system description and operation above). Eggs are skimmed from the collector using a very fine mesh dip net and directly enumerated into graduated beakers (Figure 56). The production scale hatching/larviculture systems at HBOI are based on a unit with eight 725 L (192 gallon), working volume, larval tanks (Figures 42 and 43). A volume of 35-40 ml of fertilized eggs is stocked into each larval tank. Since one ml of fertilized eggs as collected from the egg

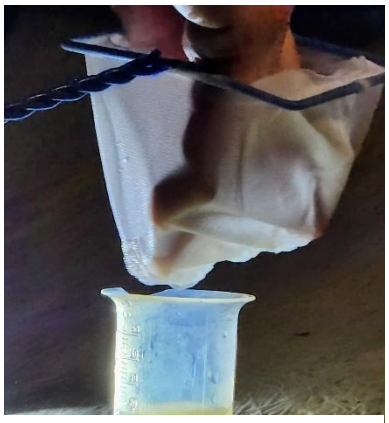

Figure 56. Measuring fertilized eggs for stocking into tanks.

Figure 57. Handheld Secchi disc device used to measure turbidity due to the greening compound (detail of device inset).

collectors is about 1,000 eggs (i.e., 35,000-40,000 eggs) this results in a stocking density of 50-55 larvae per L of water. Observation of the eggs after stocking will help to assess spawn quality. As a general rule-of-thumb, floating eggs in the hatchery tanks will usually indicate a quality spawn; mid-water suspended eggs can, but not always, be an indication of a poor-quality spawn. Never dump a spawn until egg quality is assessed and certain to be bad (see embryo development section in this manual to assess proper development). During the hatching period the larval tanks are static and not receiving flow from the biofilter loop and other system components. A small bubbling of pure oxygen from an air stone is used to keep the D.O. over 100% saturation. 250 µm filter screens are put into the drain in the center of the tanks. The next day when the eggs hatch (approximately 24 hrs), they are designated as Day 0 (0 DAH). The larvae will live off their oil droplet and yolk for the next two days as yolk-sac larvae. On the afternoon of Day 2 (2 DAH), the culture water is greened, and the first feeding of enriched rotifers is introduced to the larval tanks (Figures 57 and 58). The yolk's oil droplets have been used up or will be by the evening of Day 2. The larvae will start actively feeding on the enriched rotifers that evening. A slight recirculation trickle is started on the larval RAS system at approximately 1 l/min (or 1 turnover per 12-hour day) flow rate. A bubble screen ring is set around the center drain screen and started to keep the larvae from

Figure 58. Adding greening agent to the larval tanks.

getting impinged on the outflow screens. Enriched rotifer density is maintained at 5 to 6 rotifers per ml of tank volume by feeding 4 times a day. Water greening is also maintained to keep Secchi disc reading in that 19-24 cm range (Figure 57). The handheld Secchi disc device is used by lowering it into the water while observing from directly overhead until the black ring is no longer discernable and then raising it until the ring is just visible. The measurement is then noted on the devices ruled markings. The recirculating flow rate is gradually increased over time but should not exceed 6 L/min (or 6 turnovers per 12-hour day). The best practice is to watch how the larvae react to the flow rate if the larvae are being moved around the tank (i.e., not holding position) decrease the flow accordingly. The bubble screen flow rate should also be gradually increased as should the oxygen flow to maintain D.O. Bubble screen aeration should be high enough to keep larvae away from the screens but not too high that the larvae get pushed into the side of the tank where they will be damaged by the force of the water. Again, it is best to watch the larvae as they get pushed back by the ring of air bubbles and adjust the bubble screen flow accordingly. It is desired that the larvae are able to right themselves and swim away before hitting the side of the tank. NOTE: enriched live feeds (both rotifers and *Artemia*) lose their nutritional value over time if not consumed and it is important to flush out excess quantities.

At 6 DAH, set up the first *Artemia* hatch. Anticipate needing enough 1st instar *Artemia* to feed at 0.5 L *Artemia*/ml of tank volume. Begin to watch for the 250 µm screens to start to clog from the greening powder and switch screens for clean ones to maintain sufficient flow from the tanks. Siphoning bottom debris/wastes for the first time may be required. Watch how the larvae react to the siphon. Siphon into a catch bucket, not directly down the drain, and if a large number of larvae are being sucked into the bucket by the siphon stop and try again the next day. Larvae

are not quite strong enough to avoid the siphon yet so this process must be done with care and vigilant observation in the siphon bucket.

At 7 DAH the first 1st instar *Artemia* feeding will occur. Initially 1st instar *Artemia* and rotifers will be co-fed. However always feed the *Artemia* first when the larvae are at their hungriest at each feeding time. Follow up the *Artemia* with the enriched rotifer feeding. Keep that regime and watch how the larvae feed to ensure that they are consuming all of the *Artemia* fed. Do not over feed with *Artemia.* Set up a 10-micron sock on the tank inflows to catch the extra *Artemia* and filter them out of your system. Do not allow *Artemia* to grow as they will compete with the pompano larvae for food. They can and will outcompete the larvae if densities are left unchecked. Anticipate the *Artemia* feed rate to increase to 1-2 *Artemia*/ml density by 8 DAH. Therefore, double hatch for the 1st instar *Artemia* ration for

At 8/9 DAH the drain screens can be switched from 250 µm to the 500 µm screens. Maintain enriched rotifer and 1st instar *Artemia* feed rates. Continue to watch how the larvae are eating. If they are taking the 1st instar *Artemia* well, stop feeding enriched rotifers by 10 DAH and discontinue greening. Set up to begin producing enriched *Artemia* tank on Day 9 and start feeding newly hatched 1st instar *Artemia* and enriched *Artemia* on Day 10. Feed rates for the mix will be at 2 *Artemia*/ml. Increase *Artemia* feed rates to 3 *Artemia*/ml for the mix of 1st instar and enriched *Artemia* when you observe that the 2 *Artemia*/ml rate is no longer sufficient (i.e., the larvae are cleaning them up quickly). Siphon tank bottoms as needed.

At 12 DAH start to introduction of a dry microparticulate feed is initiated. At HBOI Otohime brand B1(Reed Mariculture, Campbell, CA) is used as the first size to start weaning to dry feed. Introduce dry feed before each feeding of *Artemia.* Stop feeding newly hatched 1st instar *Artemia* by 13 DAH and only feed enriched *Artemia.* Keep feeding dry feed (just 1, 2-gram test

feedings) and enriched *Artemia* (3-4/ml feed density) for the next few days. As larvae with full guts become evident from the initial dry feed additions, start to phase out the enriched *Artemia.* Pompano will usually be completely weaned onto dry feed by 20 DAH. Depending on the batch this may be between 18 to 22 DAH. At this stage, provide dry feed diet to apparent satiation at each feeding. Feeding should now be done every half hour to hour throughout the day. Siphon tank bottoms as needed, as feed and feces will build up quicker on the dry feed. You will be able to switch to the progressively larger sizes of slotted drainpipes in sequence as the postlarvae/juveniles get large enough (0.025 inch then 0.04 inch, and so on). Once the fish are fully on dry feed they are termed as "weaned juveniles" and will likely also have undergone complete metamorphosis (see larval developmental stages above).

Juvenile Culture; a.k.a, Nursery Production (~0.5g to 100g) (C. Robinson, C. Weirich, P. Wills)

Juvenile culture and production grow-out to harvest size are fundamentally similar in that the fish from this point on are being fed progressively larger sizes of feed to match their ability to ingest progressively larger pellets as they grow (see Table 3 below). Densities in the juvenile system tanks are optimized based on a given production plan but are generally lower in biomass than in production tanks where densities should be maximized without compromising growth (Weirich et al. 2009). HBOI's experimental units typically don't exceed 15 kg/m^3 of biomass in juvenile systems (described below) and this amounts to 225 to 400 fish per tank at 50 g or 115 to 200 fish per tank at 100 g depending upon which system is being used (0.8 m^3 or 1.4 m^3 tanks). Juvenile systems on a commercial farm would, by necessity, need to be much larger (e.g., 8 m^3) to accommodate the numbers needed to fit a particular farm's production plan. In this example at

the densities used at HBOI, an 8 m^3 tank would accommodate up to 2,400 fish up to ~50g or 1,200 fish up to 100 g before being moved to larger tanks for further grow out.

Individual age groups (a.k.a., cohorts) of fish during this period can be graded in order to keep size distributions as even as possible. They should not be graded too frequently (i.e., no more than once every other week) to allow sufficient time in between gradings to recover from the handling stress. In the future as selective breeding program comes to fruition the need for frequent grading will likely be reduced. It is likely that two to three gradings during the juvenile culture period (5 g to 100 g) will be required to maintain cohorts with similar size distributions. Designing a juvenile system with many smaller tanks rather than a few larger tanks facilitates the ability to grade and keep size groups split from a cohort separate for further grow-out. It should be recognize that a farm needs to optimize the number of juvenile rearing tanks based on cost and management of lots for their own production plan.

Juvenile Nursery System Design

At HBOI there are two RAS systems, one with 24 0.8 m^3 (210 gallon) tanks, and the other with 18 1.4 m^3 (370 gallon) tanks, each with its associated filtration and temperature control systems. The filtration systems of both systems are identical as the total system volumes are comparable. The filtration for each system consists of a microscreen drum filter with a 60-µm screen, two 7.5 ft^3 moving bed bio reactor type biofilters, two 6 ft^3 Bubble bead filters, and two UV filters to provide 30,000 $\mu w \cdot cm^{-2} \cdot sec^{-1}$ of 256 nm UV irradiation. The bubble bead filters are operated for solids filtration primarily and are backwashed once daily alternating between the two filters for morning and afternoon backwashes. Each system is also fitted with a heat pump with a titanium heat exchanger to provide temperature control within ±2°C year-round. As

mentioned above, this system is designed for research scale juvenile production and not for commercial production and may need to be upscaled to meet a particular farm's production plan.

Production (100g to 640g) (P. Wills, C. Robinson, R. Baptiste, C. Weirich)

System Design

HBOI designed and operated a large 99 m^3 (26,150 gal) commercial-scale demonstration production RAS system with a single 77 m^3 (20,230 gal) tank and associated filtration systems (Figure 59). Filtration includes a large 60 µm drum screen filter, a 12 m^3 MBBR, a 300 gpm protein fractionator, a UV providing 30,000 $\mu w \cdot cm^{2 \cdot -1} \cdot sec^{-1}$ of 256 nm UV irradiation. The drum screen filter's backwash water supply inlet has two FV1 bag filters to polish the backwash water (these require a change of the filter bags daily). Based on experience with the system design shown in Figure 59, the biofilter should be scaled up to a biofilter chamber (e.g., 15 ft diameter tank rather than 12 ft) that can hold a minimum of 380 ft^3 of media to provide a carrying capacity over 40 kg/m^3. A larger scale operation may want to design their system with multiple tanks that

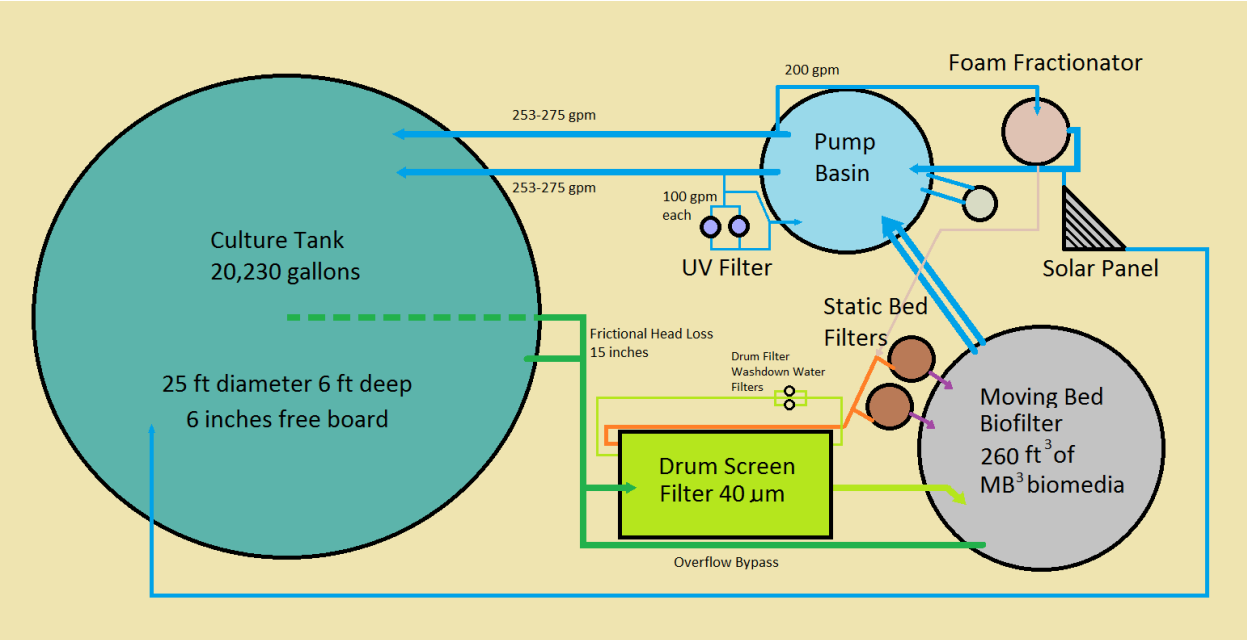

Figure 59. Demonstration production RAS system at the HBOI aquaculture park in Fort Pierce FL.

will hold production lots representing the amount needed for periodic harvest quantities which would dramatically alter the system design. The design presented is only one of many possible iterations of system design possible. It is recommended to hire a professional RAS engineer to assist with system design that meets the farm's production goals.

Table 2. Example costs for construction of the HBOI demonstration RAS system for Florida pompano production using a biofilter increased to 450 cubic feet of MB3 media. Costs are in US dollars in 2013 at the time of the demonstration system's construction. Costs do not include labor.

Commercial Scale Demonstration System, Scaled Up Biofilter				
Quantity	Description	Supplier	Cost per	Total Cost
1	Faver 40um microscreen drum filter	AES	22,085.45	22,085.45
1	25'x6' 20,200 gal Culture Tank	Dolphin Fiberglass	14,500.00	14,500.00
2	1.5 hp Axial Flow Pump	Carry Mfg	5,739.17	11,478.34
1	PS250 Protein Skimmer	Solar Components Inc	6,450.00	6,450.00
450	MB3 Biomedia	W-M-T	21.28	9,576.00
1	solar array	Solar Components Inc	4,792.00	4,792.00
1	760 Watt UV sterilizer	Aqualogic	3,995.00	3,995.00
1	15'x4' Tank - Biofilter	Dolphin Fiberglass	5,410.00	5,410.00
1	YSI 5200 DO monitor w/ 10 m cable multi probe and software	AES	3,450.00	3,450.00
1	Plumbing materials	multiple	3,000.00	3,000.00
2	S63 regenerative blowers	AES	1,228.00	2,456.00
20	Micropore diffusers	AES	113.00	2,260.00
4	36 inch Wave Vortex Chambers	AES	1,061.00	4,244.00
1	Electrical materials	multiple	2,000.00	2,000.00
1	8'x3' Tank - Pumping Basin	Dolphin Fiberglass	1,285.00	1,285.00
4	oxygen solenoid valves	AES	285.69	1,142.76
1	1/2 hp titanium pump	AES	967.00	967.00
1	Pacific Ozone Ozone Generator	AES	948.00	948.00
20	3' bioweave Air diffusers	AES	45.11	902.20
1	Lumber materials	Local Supplier	800.00	800.00
2	3" pressure relief valves	AES	358.89	717.78
10	oxygen flow meters 0-8 L/min	AES	61.50	615.00
2	FVI Bagfilter w/ gauge and bleedvalve	AES	302.85	605.70
2	JP1 3/4 hp pump	AES	258.36	516.72
2	3/4" Float valves	AES	251.03	502.06
400	Concrete Blocks	Local Supplier	1.20	480.00
1	Hanna Instruments ORP controller	AES	263.00	263.00
3	Totalizing water meters	DV controls	62.85	188.55
1	1/2" polyethylene screen full roll	AES	160.00	160.00
15	Moisture-B-Gone Dessicant	HDLtd.com	9.02	135.30
1	Pressure Gauges 0-100 psi	AES	68.00	68.00
10	200 um filter bags	AES	6.20	62.00
		Total projected costs per System		**106,055.86**

Feeding

Figure 60. Automatic feeders on experimental production tanks at HBOI-FAU, Fort Pierce FL.

Feeding can be done by hand or with automatic feeders. Pompano digest and move food through their gut very quickly and have a measured gut clearance rate of about 3 hours (Riche 2009). Therefore, it is possible to feed them up to every three hours throughout the daytime feeding period. It is generally not necessary to feed more than four times per day as there is no demonstrated growth benefit (Weirich et al. 2006). Weirich et al. (2006), however, did demonstrate higher growth in pompano fed to satiation four times per day over those fed twice per day to satiation. Automatic feeders that are programed to dispense feed at regular intervals can reduce labor requirements (Figure 60). However, without monitoring, automatic feeders can overfeed the fish on days when they satiate quickly. Excess uneaten feed puts an undue burden on filtration systems in a RAS and must be avoided. Currently, there is a push to develop feeding systems that use artificial intelligence (AI) machine learning algorithms with machine vision to improve precision of automatic feeding (Zhou et al. 2017, Hu et al. 2022). Similar systems are reportedly being deployed in net pen culture with commercialized systems being advertised as available. They are still generally regarded as experimental in RAS. The conditions for deployment of AI based feeding systems in RAS, surprisingly, can be more difficult than in net pens due to higher turbidity reducing the application of underwater optical methods. This technology is being rapidly developed, though, and should be available in the near future. Use of surface feeding detection rather than underwater detection may allow this

technology to be used in RAS tanks and ponds (Hu et al. 2022). Once deployed these methods will allow satiation feeding and reduced reliance on feeding to a percentage of the body weight per day improving efficiency of feeding.

In the meantime, the use of a standard feeding table is the preferred method for determination of feed rates at different sizes during grow-out (Table 3). Feeding tables rely on good information on the size of the fish being fed, so periodic sampling (e.g., biweekly) is necessary to establish and track growth in each production unit. In addition, daily removal and recording of mortalities is required for tracking populations size and to monitor for disease or water quality issues. Pompano do not cannibalize dead individuals, so removal of mortalities is more accurate than with species that do cannibalize their dead and moribund tank-mates (e.g., red drum *Sciaenops ocellatus*).

For pompano, the preferred pellet type for RAS culture is a floating pellet followed by an extruded slow sinking pellet. Floating pellets allow observation of feeding behavior and easier recognition of when satiation has occurred, helping to reduce waste feed in the system. When hungry, pompano will feed aggressively at the surface and will also swim around the tank at a faster pace than during non-feeding periods. Sluggish feeding, especially during the first feeding of the day can indicate other issues in the system such as poor water quality or the onset of a disease issue and should be noted, monitored and followed up on promptly. Even when automatic feeders are deployed producers should take time to monitor feeding and nonfeeding activity since, as a rule, any abnormal behavior will provide clues to issues with the fish that should not be ignored.

Table 3. Suggested feed table showing feed sizes and feed rates by age and weight throughout production cycle from egg to harvest for Florida pompano. For larval period there is necessary flexibility in changes of feeds dependent upon growth rates (see description above) for the growout diets (20 g fish and up) use floating pellets preferentially or alternatively slow sinking pellets.

Age or Weight (g)	Feed Type/Size	Feed Rate
Hatch – Day 2	N/A	N/A
Day 3-6	Rotifers (R)	R 5/ml
Day 7	Rotifers & 1st instar *Artemia* (A)	R 5/ml & A 1/mL
Day 8 &9	Rotifers and 1st instar *Artemia*	R 5/ml & A 2/mL
Day 9	1st instar *Artemia*	1st instar A 4/mL
Day 10	Enriched *Artemia* (EA)	EA 4/ml
Day 11-15 (or until weaned)	EA and Otohime B1	EA 4/ml & B1
Day 16-19	Otohime B1	Satiation
Day 20-22	Otohime B2	Satiation
Day 23-25	Otohime C1	Satiation
3-10 g	#1 crumble	40% body weight per day (BWD)
10-20 g	#2 crumble	10%
20-50 g	1.5 mm	4-8%
50-100 g	2 mm	3-3.5% BWD
100-250 g	3 mm	3 % BWD
250-454 g	6 mm	3 % BWD
454-680 g	8 mm	2.5% BWD

Grading

In a commercial operation that has access to multiple large tanks (as opposed to the single tank available in the HBOI demonstration system) it is possible that the fish could be graded during grow-out to correct for size disparity in culture tanks. Data on body width versus size by weight was collected and length-weight relationship was calculated as; weight (g) = 7×10^{-6}(Fork Length in mm)$^{3.206}$. Body width by weight was predicted by the equation Body Width (mm) = 7.874× Ln(weight in g)-10.375 (Figure 61). From this a regression equation was developed and used to predict suggested standard "minnow" bar grader sizes (in 64^{ths} of an inch) for different weights of pompano between 5 g and 600 g (Table 4). Grader sizes needed in practice may vary based on the condition of the fish under a particular farm's husbandry practices.

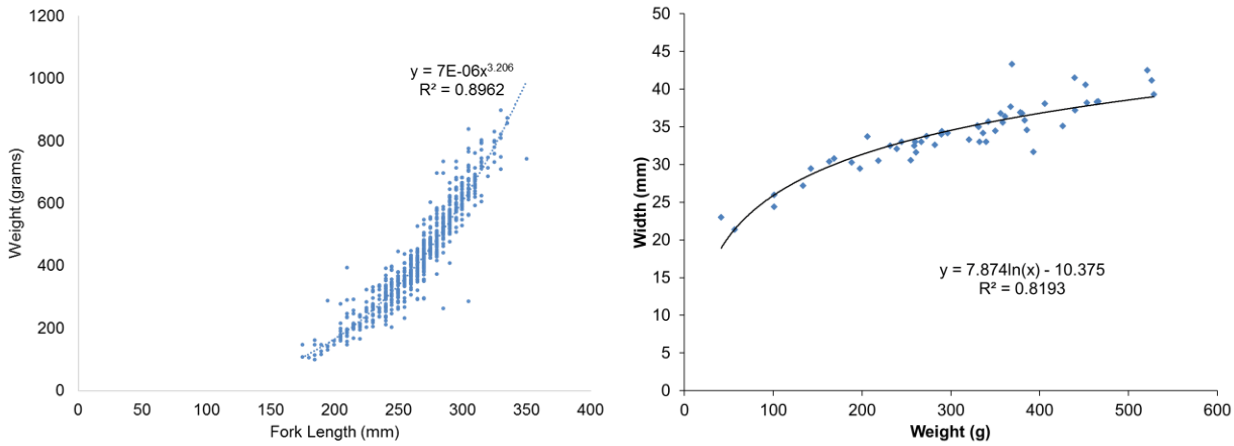

Figure 61. Length weight relationship (A) and Body width by weight (B) relationship for Florida Pompano grown in a commercial scale RAS on a prepared pelleted diet.

Table 4. Suggested bar grader gap sizes (64th of an inch) to separate Florida pompano by weight.

Fish Weight (g)	Slot Width (mm)	Grader size (x/64")
5	2.3	8/64
10	7.8	20/64
20	13.2	33/64
30	16.4	40/64
40	18.7	44/64 or 48/64
50	20.4	50/64 or 52/64
100	25.9	64/64 or 66/64
200	31.3	78/64 or 80/64
300	34.5	84/64 or 88/64
400	36.8	94/64
454	37.8	94/64 or 96/64
500	38.6	97/64*
600	40.0	101/64*

*/ Non-standard grader sizes that are likely not available from a manufacturer

Harvest

Off-Flavor Mitigation

One of the key goals of sustainable high density recirculating aquaculture of food fish is the production of high-quality consistent products that are as acceptable to the consumer as the "wild" product they are used to eating. Otherwise, benign organisms (e.g., bacteria, fungi) in high intensity RASs, thrive due to characteristically high nutrient loads and produce compounds such as geosmin (GSM; *trans*-1, 10-dimethyl-*trans*-9-decalol), and 2-methylisoborneol (MIB; 1-*R*-exo1,2,77-tetramethly bicycle-[2-2-1]-heptan-2-ol) as byproducts. Compounds such as these are absorbed and accumulate in the fat rich tissues of fish grown in these systems causing them to have offensive earthy and/or musty flavors (commonly called off-flavor). If some mitigating action is not taken prior to sale, these off-flavors reduces consumer acceptance and could render the final products unmarketable. Reduction of the circulating concentrations of these compounds in an RAS at the end of a production cycle will allow them to be purged from the fish's tissues rendering the fish "on-flavor" and saleable.

This can be achieved by thorough cleaning of all parts of the system to remove accumulated waste and heterotrophic bacteria growth, reducing or halting feeding, and flushing the system with clean water during a purging period. It can also be accomplished by moving fish to a separate purging system that is maintained with clean water to allow the fish to purge of the off-flavor compounds prior to sale. As part of a program to control off-flavor in salable product fish should be taste tested in order to determine the presence and degree of off-flavor present. For reference of off-flavor analysis see the Southern Regional Aquaculture Center (SRAC) Publication No 431 "Testing Flavor Quality of Preharvest Channel Catfish" which provides a detailed description of flavor testing in fish that is relevant in most respects to any other fish

including pompano. Note that typically the predominate off-flavor producing compound in RAS is MIB that produces a musty flavor in the product.

Florida Pompano Nutrition

There are numerous publications describing the species within the Carangidae family that are suitable for aquaculture as: golden pompano (*T. ovatus*), silver or blunt pompano (*T. blochii*), Indian (*T. mookalee*) and Florida pompano (Webber and Riordan, 1976; Weirich et al., 2006; Jayakumar et al., 2014, Liu, Liu and Hussein, 2019; Alfrey et al., 2022).

Global pompano production was approximately 173,000 MT in 2019 (FAO, 2021) with Asia accounting for 99% of this production. Unfortunately, a relatively high percentage of this annual tonnage is produced in an unsustainable manner by today's standards. While the species readily accepts compounded feeds from early life-stages through market size, much of the current global production is estimated to be fed a variety of fresh fishery products. This is due to the proximity of Asian aquaculture farms to artisanal fisheries as opposed to commercial feed mills, making the fresh fishery products a more economical feed source in many Asian coastal aquaculture locations.

Pompano is a carnivorous species with specific requirements for protein, fat and vitamins/minerals depending on its life stage. Protein requirement is typically as high as 40-50%, being the most expensive ingredient when formulating aquafeeds. Alfrey et al., (2022) recently demonstrated the ability to simultaneously reduce and remove fishmeal and fish oil from the diets of pompano reared in intensive recirculating systems. These diets utilized a suite of novel aquaculture ingredients designed to allow nutritionists the ability to formulate diets that meet or exceed the known nutritional requirements of pompano without depending on marine-based fish meals and fish oils. These ingredients included enzyme treated and hydrolyzed plant proteins, marine algae oil, and single cell proteins. While the costs of such formulas are currently reflective of low-scale production of the novel ingredients, the study clearly demonstrates the

validity of using these ingredients. Surely, increased demand for these novel and sustainable ingredients will drive commercialization followed by economy of scale pricing.

Early studies on pompano nutritional physiology evaluated protein and energy requirements for this candidate aquaculture species (Weirich et al., 2006; Riche, 2009). Then, numerous studies have determined the effects of supplying protein and energy from a large variety of common aquaculture ingredients including terrestrial animal and plant products (Lech and Reigh, 2012; Rossi and Davis, 2012; Rhodes et al., 2015). In addition, a key nutritional component like taurine was revealed (Salze et al., 2016). These nutrition studies continue today as new and novel ingredients geared towards increased sustainability to proliferate ingredient supply chains (Habte-Tsion, 2022).

Carnivorous species like pompano do not have a specific requirement for carbohydrates inclusion in diets (NRC., 2011). However, there are several studies showing beneficial effects of carbohydrate utilization in fish formulas (Boonanuntanasarn et al., 2018; García-Meilán et al., 2020; Sari et al., 2022; Takahashi et al., 2018). It is well known that carbohydrates are an effective source of body energy, high quality binding ingredient and economical nutrient for aquafeed diets. These biding properties of carbohydrates gain relevance during the extrusion process varying the consistency of the pellet and the digestibility of the carbohydrates. It is also known that when carbohydrates are not provided in the diet, other energy-yielding nutrients, such as proteins and lipids are catabolized to produce energy. However, an inappropriate dietary carbohydrate inclusion will lead to reduced growth performance, higher feed conversion ratio, fat liver deposit and higher mortality (Hemre et al., 2002; Zhou et al., 2014). Plus, fish ability for carbohydrate utilization greatly varies depending on the species, carbohydrate source, dietary inclusion level, feeding habits and habitats (García-Meilán et al., 2020; Nafees et al., 2022; NRC,

2011). For these reasons, determining a correct carbohydrate source to tailor a commercial diet for a specific species has become a hotspot in the fish nutrition industry. Research at HBOI gears towards optimizing a feed formula for sustainable and profitable culturing of pompano. This formulation will include an appropriate carbohydrate source, sparing the use of high-priced proteins or lipids for energy consumption. To this end, HBOI geared efforts towards evaluating the effects of different carbohydrate sources namely, whole wheat grain flour, wheat starch, whole corn grain flour, corn starch and dextrinized corn starch to determine pompano growth performance. The results of this study revealed that whole wheat grain flour is the best carbohydrate source for pompano presenting highest weight gain and feed efficiency. In fish fed with whole wheat grain flour, biochemical parameters on blood-liver and hepatic gene expression also suggested an appropriate use of carbohydrates towards energy consumption sparing the protein utilization towards growth. These results are of critical importance when formulating a well-balance diet for pompano because an appropriate carbohydrate inclusion will lead to cost reduction of formulated diets, lessen ammonia excretion, improve protein consumption and increase farm profitability (Coutinho et al., 2018; Peres and Oliva-Teles, 2002; Wilson, 1994).

Unique to several marine and freshwater species that specialize in preying on crustaceans, including the Carangidae family, is the presence of granular teeth and bony plates in the buccal cavity. These evolutionary traits cause pompano to often crush feed pellets as opposed to gulping them whole. This behavior has led to the anecdotal belief that pompano may require a feed with specialized physical characteristics better able to remain mostly intact through this crushing feeding behavior. Novel feed manufacturing technology developed by the USDA can impart a

texture more resembling animal flesh and can be adopted to commercial aquafeed manufacturing (Liu et al., 2021).

Infectious Diseases of Florida Pompano (Gonzalo Illán, C. Robinson and P.S. Wills)

Pathology, prevention, and treatment:

The aquaculture of pompano in high-density situations is relatively new, so the overall reporting of diseases that affect them is relatively low. However, as the commercial culture of this species increases, new diseases will likely be identified or become prevalent.

Infectious diseases represent the most critical disorders in aquaculture because they can increase the production costs due to increased mortality and/or morbidity, decreased growth, cost of treatments (Nowak 2007, Asche and Bjørndal 2011, Dixon, 2012) and prevention measures (Lafferty et al. 2015, Blanco et al. 2020). Diseased or infected fish may also present an off-putting flavor or appearance, reducing their market value (Moran et al. 1999, McClelland et al. 2002, Nowak 2007).

After an extensive review, the present manual features some aspects of the pathology, prevention and treatment for 17 diseases or health-related conditions that affect pompano and other related *Trachinotus* species. It also describes some features related to the etiology of their causative agents (viruses, bacteria, and parasites), as well as their geographical distribution (Table 5).

According to the literature, two types of viruses may currently pose a threat to pompano aquaculture: an iridovirus and a nodavirus. The Red Seabream Iridovirus Disease (RSIVD), detected in farmed pompano in Central America (López-Porras et al. 2018), is characterized by the formation of hypertrophic cells in the fish's internal organs (Roberts 2012). Affected fish show lethargy and severe anemia, which leads to significant mortality (Inouye et al. 1992, Nakajima and Sorimachi 1994). On the other hand, the Nervous Necrosis Virus (VNN), isolated

from Golden pompano (*T. ovatus*) and Snubnose pompano (*T. blochii*) in Asia, can cause an extensive vacuolation of the nervous tissue (Nakai et al. 2009, Ransangan et al. 2011), leading to whirling swimming, as well as lethargy, anorexia (Grotmol et al. 1999, Xu et al. 2012) and mass mortality among younger fish populations (Pakingking et al. 2011). The only way to minimize the risks associated with viral infections is through prevention, and some methods are already implemented with good results, such as water disinfection and physical treatment (e.g., by UV, chlorine), disinfection of fertilized eggs (e.g., by peroxide or formalin), and genetic selection of resistant strains. Commercial vaccines are also available for RSIVD (Aquavac 2021) and VNN (Pharmaq 2021).

Bacterial infections are also one of the primary causes of disease in many aquaculture systems. Different groups can cause septicemia. Aeromonas hydrophila is problematic in culture conditions characterized by poor water quality (Hanson and Grizzle 1985, Austin 1999), high temperatures (Nieto et al. 1985), and physiological stress (Bullock et al. 1971). The affected fish show ulcerative hemorrhages on the skin and congestion/necrosis of internal organs (Huizinga et al. 1979, Miyazaki and Kaige 1985). Warmwater marine *Pseudomonas* spp., abundant in eutrophic waters (Austin 1998) can also originate septicemia. Infected fish show similar clinical signs to *Aeromonas* septicemia (Roberts 2012). *Pseudomonas alcaligenes* has been isolated from the pompano in the US (Hawke 1976) and the Golden pompano in China (Xia et al. 2015). Some antibacterials (e.g., oxytetracycline, approved for aquaculture by the FDA) are allowed to treat infection by these bacteria on certain species and via off-label prescription by a veterinarian. Other issues to consider come from the development of resistance by bacteria and the difficulty of treating fish that are not actively feeding due to illness (i.e., the fish have gone off feed).

Also known as granulomatous ulcerative dermatitis or pseudotuberculosis, photobacteriosis caused by *Photobacterium damselae* subspecies *piscicida* is also characterized by severe septicemia (Romalde 2022), externally noticeable by the formation of deep ulcers and muscle lysis (Kubota 1970a, Love et al. 1981). Young and physiologically stressed farmed fish seem more susceptible to this disease (Kusuda and Yamaoka 1972, Austin and Austin 2007) with outbreaks becoming more frequent during the warm summer months (20-25°C) (Paperna and Zwerner 1976, Toranzo et al. 1991). In Asia, it affects the culture of the Golden pompano (Xia et al. 2015).

Vibriosis, caused by multiple *Vibrio* spp. (Schiewe 1981), is the most common and significant bacterial disease in marine farmed fish (Rasheed 1989, Roberts 2012). Two main types of vibriosis have been described in pompano (*Trachinotus* spp.) worldwide: 'dermatitis vibriosis' (also known as boil or ulcer disease, a hemorrhagic septicemia) by *Listonella anguillarum* (syn. *V. anguillarum*) and *V. vulnificus*; and 'gastroenteritis vibriosis' (*vasculitis*) by *V. harveyi* and *V. ponticus*. In both cases, the causative agents are usually part of a healthy environment and fish-associated microbiome (Kanno et al. 1990), becoming primary pathogens under stressful husbandry conditions (Anderson and Conroy 1970, Austin 1999). When this happens, fish show signs of disease, such as redness, skin necrosis, and ulcers in the first case, and gastroenteritis, ascites, meningitis, and vasculitis in the second. Eventually, they become debilitated, showing signs of anemia, lethargy, go off feed (Stoskopf 1993a, Colwell and Grimes 1984) and die in high numbers (Saeed 1995, Yii et al. 1997, Roberts 2012). As a preventive measure, when fish show their first signs of vibriosis, freshwater dip baths (5-10 min) (FAO 2009a) can help to reduce the load of bacteria. Immunostimulants (e.g., Levamisole) (Kajita et al. 1990) and probiotics (e.g., other *Vibrio* and *Aeromonas* spp.) (Austin et al. 1995b) have also

been successfully used. A commercial injectable/oral vaccine against *L. anguillarum* (serotypes O1 and O2α) has proved effective in some warmwater marine species, such as the yellowtails (*Seriola* spp.) (Merck 2021). The use of oxytetracycline, effective against some *Vibrio* spp., should be prescribed by a licensed veterinarian and administered in the feed under the requirements of the veterinary feed directive (VFD), following the isolation/identification of the causing microorganism. Since fish will go off feed, administration of the antibiotic in fed must be started early before they go off feed so diagnosis should not be delayed if any signs are evident.

Another bacterium that may be present in aquaculture settings worldwide is *Tenacibaculum maritimum* (syn. *Flexibacter maritimus*) which causes saltwater myxobacteriosis. This microorganism, a normal inhabitant of the fish mucosa and usually associated with mucoid surfaces and biofilms (Roberts 2012), becomes highly pathogenic under poor water quality conditions (Handlinger et al. 1997, Kusuda and Kawai 1998, Austin 1999). Characteristic hemorrhagic ulcerated patches (Handlinger et al. 1997, Wakabayashi et al. 1986) can be easily recognized on the skin and tail (‘tail rot’) of the infected fish, which also may show signs of suffocation (FAO 2009a). Severe mortality by this organism has been observed in farmed pompano in the US (Hawke 1976) and Snubnose pompano in Asia (FAO 2009a). Commercial vaccines have shown effective for coldwater marine fish species, such as salmonids and Turbot *Scophthalmus maximus* (Hipra 2021), and oxytetracycline can also be used under the necessary caution (Baxa et al. 1988b, Soltani et al. 1995).

Mycobacterium marinum, a Ziehl-Neelsen positive rod (Dulin 1979), is an environmental opportunistic pathogen (Arakawa and Fryer 1984) with a worldwide distribution (Nigrelli and Vogel 1963, George et al. 1980, Van Duijn 1981) and is also abundant in the biofilms of

recirculating aquaculture systems (Steed and Falkinham 2006). In the US, it has been isolated from the internal organs of the pompano (Aronson 1926, Yanong et al., 2010), where granulomas are easily recognized (Gauthier and Rhodes 2009, Weerakhun et al. 2010). Although high mortalities have also been registered (Snieszko 1978, Weerakhun et al. 2007), low-level chronic conditions (Yanong et al. 2010) and loss of market value (Knibb et al. 1993) are the main concern. Within the same phylum but belonging to a different family, *Nocardia seriolae* is a Gram-positive intracellular actinomycete with branching morphology (Kudo et al. 1988) that causes abscesses/ulcers in the skin and muscle as well as lesions in the kidney and spleen (Kariya et al. 1968, Kudo et al. 1988) of the Golden pompano (Xia et al. 2015). As preventive measures, a clean environment (Kusuda and Nakagawa 1978), removing the affected fish, and disinfecting the rearing facilities (Roberts 2012) are critical in preventing the disease. Also, experimental vaccines are under development against nocardiosis, but with limited success (Itano et al. 2006, Austin and Austin 2007). Some antibiotics, such as chloramine T (approved by the FDA for aquaculture) have traditionally been proven partially effective in treating fish mycobacteriosis (Dulin 1979, Kawakami amnd Kusuda 1989).

A number of fish protozoan parasites with a direct life cycle also find the perfect conditions to thrive in a temperate/warm water recirculating system. Although the symptoms and severity of the disease they induce may vary depending on the parasite itself, the husbandry conditions, and the water quality, they all share some common features. They are usually attached to the external surfaces (skin, gills, fins, eyes) of the fish and feed on the epithelium and subjacent layers, causing irritation and excessive mucus production (Nigrelli and Ruggieri 1966, Van As et al. 1984). Severe infections can lead to necrosis, ulceration, hemorrhages, and hyperplasia (Dickerson 2006, Roberts 2012), and damaged tissue can be easily infected by other

opportunistic pathogens (e.g., fungus). Behavioral changes (Main et al. 2007), going off feed (Noga 1996), and growth inhibition (Sanmartín Durán et al. 1991) are consequences of delayed or lack of treatment.

Typical ectocommensals on warmwater fish are found on the skin and gills of the pompano in the US (Williams 1974a). Two protozoan groups of ciliates (subclass Peritrichia) can pose a serious threat to the fish's health during the summer months (Carvenia and Speranza 2003) and under poor water quality (e.g., eutrophic conditions) (Brown 2000, Oldewage and Van As 2006), especially among younger or stressed and weakened individuals (Paperna 1991, Basson and Van As 2006). These are *Scyphidia* sp., a large (~100 µm long) sessile ciliate, and the trichodinids (Family Trichodinidae), disc-shaped motile ciliates with an adhesive disc and a denticulate skeletal ring to glide and attach to the fish skin.

Cryptocarion irritans, another ciliate protozoan also known as *marine white spot*, is even more relevant. Its feeding stage, a large trophont (up to 450 µm), can be easily recognized as whitish 'blisters' on the fish skin, eyes and gills (Paperna 1991). Considered an obligate parasite (Colorni and Diamant 1993), it is responsible for severe skin and gill damage (Cheung 1993b, Dickerson 2006), with earlier stages of fish more susceptible to the disease (Glazebrook and Campbell 1987, Leong 1994). Its life cycle, which includes a cyst stage on the bottom substrate of the tank which releases infective free-swimming theronts (Ogawa and Yokoyama 1998), is temperature-dependent (optimal at 25-30°C) (Colorni and Burgess 1997). With worldwide distribution, infections have also been reported in farmed pompano (*T. carolinus, T. blochii, T. goodei* and *T. ovatus*) In the Americas (Sinderman 1977a, Gómez-Gaspar 1987) and in Asia (FAO 2009a, Zhong et al. 2020). In some cases, successful management includes breaking the parasite's life cycle of infection (e.g., with good cleaning routines that will eliminate the resistant

and/or infective stages from the tanks) (Colorni 1987, Dickerson 2006). Formalin baths have also proved effective against cryptocaryonasis (Cheung 1993b), and quinine derivatives in hypersaline conditions have been shown to kill *C. irritans* theronts (Huff and Burns 1981). A disease of importance that is common in many warmwater marine fishes is *Amyloodinium* sp. (a.k.a., Amylo or marine velvet disease) and is also of particular concern in pompano (Williams 1974a, Gómez-Gaspar 1987). Amylo is a parasitic dinoflagellate with a three-stage life cycle. The trophont stage, up to 350 µm in size, lives on the fish's skin and gills where it feeds (Lom and Lawler 1973, Paperna 1980). When mature, they fall off and become a benthic cyst which undergoes reproduction forming large numbers of free-swimming "swarmers" called dinospores. The dinospores swim into the water column and locate a host to begin the cycle again as trophonts. Fish that have been infected with this parasite will show obvious signs of infection, such as behavioral changes (e.g., flashing or scratching on the tank surface) (Main et al. 2007) and will go off feed (Lawler 1977a, Noga 1996), signs of irritation and respiratory distress (Cheung 1993b). Outbreaks resulting in high mortalities are relatively frequent (Trimble 1979). Water temperature notably affects the parasite's life cycle, with an optimum between 23-30°C (73-86°F) (Lom and Dykova 1992, Noga and Levy 2006), with interruption of spore formation above 35°C (95°F) and below 8°C (46°F). Optimum salinity is 10 to 60 ppt but division occurs as low as 1 ppt and halts above 80 ppt. Upon close inspection of the fish, white bumps on the fish skin will be apparent. A skin scrape and gill biopsy (a.k.a., gill clip) is needed to verify the diagnosis by identifying the parasite on the fish. Once the parasite has been properly identified treatment can be initiated. There should not be any delay in conducting a diagnosis or starting the treatment if Amylo is suspected. As with any disease, quick action is necessary to lessen severity and losses.

Any disease treatment that is used should be determined in consultation with a veterinarian that is qualified to work with marine fish. Many treatments (especially antibiotics) can only be used when prescribed and incorporated into the feed under the laws governing the Veterinary Feed Directive (VFD), which is administered by the FDA. Check the FDA website and the Aquatic Animal Drug Approval Partnership Program (AADAP) website for information about approved drugs and the Investigational New Animal Drugs (INAD) program. A copper treatment at a dose rate of 0.20-0.35 mg/L of free copper by dosing with a chelated copper or copper sulfate is an effective treatment. This treatment can be administered to the whole system and should last for at least 21 days. Be sure to add the copper treatment to the culture water slowly. Treatment of Amylo with copper is not approved for food fish. Another treatment option that is approved for other diseases is a formalin bath at 250 ppm with a one-hour exposure time (Birdsong and Avault 1971). Hydrogen peroxide treatments (75 ppm) also seem to eliminate or reduce Amylo infection (Montgomery-Brock et al. 2001). Short freshwater baths may also help to reduce the infestation by the feeding stages of *A. ocellatum* (Lawler 1977b, Smith 1999). After the bath treatment, it is important to move the fish to a clean tank free from infection. Before moving the fish and after treating them with formalin, a freshwater dip of ~5 minutes is customary. The theory behind this treatment practice is that once the fish is exposed, mucous production increases causing the parasite to be sloughed off in the mucous. Care must be taken with formalin treatments because it is harmful to the gills if fish are overexposed. In addition, there is anecdotal evidence that the dinospores can be controlled in a RAS by ensuring that the filtration systems are removing them as they pass through in the water flow. Removal of freshwater Ich *Ichthyophthirius multifiliis*, an infectious ciliate, in channel catfish in raceways was shown by Bodensteiner et al. (2011) due to the flushing of tomites, the swarmer stage of Ich,

by the continuous water flow. Amylo has a similar life cycle and should be treatable in the same manner. A drum screen filter with 40-60 µm screen should remove the dinospores, reducing but not eliminating the instances of infection. This was anecdotally apparent in culture of Red Drum in RAS culture by the authors while rearing juveniles. Lawler (1977b) proposes the disinfection of the incoming water (e.g., by UV) to reduce the risk of infective stages in the culture system.

NOTE: Biosecurity is paramount in running a successful aquaculture program. Please ensure that the cause of the disease has not been initiated by operator negligence. Always use sterile techniques when moving, sampling, and/or feeding fish. Do not cross-contaminate supplies. Have nets assigned to individual tanks or systems and use 70% alcohol (ethanol of isopropyl) on hands whenever necessary. Initiate a complete biosecurity plan for the farm and do not deviate from it. It is beyond the scope of this manual to discuss biosecurity planning as much of the process is very specific to a given farm. For a thorough treatment of biosecurity concepts and planning, see reviews by Yanong and Erlacher-Reid 2012, Yanong 2012, Yanong 2013 (SRAC Publications 4707, 4708 and 4712), Prider 2004, Scarfe, et al. 2006, and Scarfe and Palić 2020, among others.

Table 5. Taxonomic List of reported pathogens of farmed Florida pompano *Trachinotus carolinus* and/or its congeners (partially adapted from Weirich et al. 2021).

Pathogen	**Host/s**	**Distribution**	**Ref.**
VIRUSES			
Family Iridoviridae			
Genus *Megalocytivirus*			
Red Seabream Iridovirus (RSIV)			
	Florida pompano, *Trachinotus carolinus* L.	Central America	López-Porras et al. 2018
Family Nodaviridae			
Genus *Betanodavirus*			

Nervous Necrosis Virus (NNV)		
Golden pompano, *Trachinotus ovatus* L.	China (experimental)	Su et al. 2015
Snubnose pompano, *Trachinotus blochii* (Lacepède)	Philippines	Pakingking et al. 2011
	Malaysia	Ransangan et al. 2011
BACTERIA		
Aeromonas hydrophila Beaz-Hidalgo et al. 2013 Syn. *A. liquefaciens*		
Florida pompano, *Trachinotus carolinus* L.	Alabama (USA)	Hawke 1976
Phylum Proteobacteria Garrity et al. 2005 **Class Gammaproteobacteria** Garrity et al. 2005 **Family Psedomonaceae** Winslow et al. 1917		
Pseudomonas sp. Migula 1894		
Pseudomonas alcaligenes Monias 1928		
Florida pompano, *Trachinotus carolinus* L.	Alabama (USA)	Hawke 1976
Golden pompano, *Trachinotus ovatus* L.	China	Zhou et al. 2002 Zhao et al. 2007 Huang et al. 2008 Wang et al. 2009 Xia et al. 2015
Pseudomonas maltophilia Syn. *Stenotrophomonas maltophilia* Palleroni and Bradbury 1993 (accepted name)		
Golden pompano, *Trachinotus ovatus* L.	China	Zhou et al. 2002 Zhao et al. 2007 Huang et al. 2008 Wang et al. 2009 Xia et al. 2015
Family Vibrionaceae Veron 1965		
Vibrio anguillarum Bergeman 1909 Syn. *Listonella anguillarum*, *Listonella anguillara, Vibrio piscium, Bacillus anguillarum*		
Florida pompano, *Trachinotus carolinus* L.; Florida pompano, *Trachinotus carolinus* L.; Snubnose pompano, *T. blochii* (Lacepède)	Alabama (USA)	Hawke 1976 FAO 2009a
Vibrio harveyi Baumann et al. 1981		

Syn. *V. carchariae, V. trachuri*		
Snubnose pompano, *Trachinotus blochii* (Lacepède)	Indonesia (experimental)	Fransiska et al. 2019
Vibrio ponticus Macian et al. 2005		
Palometa, *Trachinotus ovatus* L.	China	Xia et al. 2012 Zhao et al. 2007 Tu et al. 2017
Vibrio vulnificus West et al. 1986		
Palometa, *Trachinotus goodei* (Jordan & Evermann)	Madrid (Spain) (aquarium)	Gibello et al. 2019
Golden pompano, *Trachinotus ovatus* L.	China	Zhou et al. 2002 Zhao et al. 2007 Huang et al. 2008 Wang et al. 2009 Xia et al. 2015 Su et al. 2012 Zhu et al. 2018
Photobacterium damselae subsp. *piscicida* Gauthier et al. 1995 Syn. *Vibrio damsela, Listonella damsela, Pasteurella piscicida, Photobacterium damselae*		
Golden pompano, *Trachinotus ovatus* L.	China	Zhou et al. 2002 Zhao et al. 2007 Huang et al. 2008 Wang et al. 2009 Xia et al. 2015 Su et al. 2012
Phylum Bacteroidetes Krieg et al. 2012 **Class Flavobacteriia** Bernardet 2012 **Family Flavobacteriaceae** Reichenbach et al. 1992		
Tenacibaculum maritimum Suzuki et al. 2001 Syn. *Flexibacter maritimus, Flexibacter marinum, Cytophaga marina*		
Florida pompano, *Trachinotus carolinus* L.	Alabama (USA)	Hawke 1976
Florida pompano, *Trachinotus carolinus* L.; Snubnose pompano, *T. blochii* (Lacepède)		FAO 2009a
Phylum Actinobacteria		

Order Corynebacteriales			
Family Mycobacteriaceae			
Mycobacterium marinum Aronson, 1926 Syn. *M. platypoecilus, M. balnei*			
	Florida pompano, *Trachinotus carolinus* L.	Philadelphia (USA) (aquarium)	Aronson 1926
	Florida pompano, *Trachinotus carolinus* L.	Florida (USA)	Yanong et al. 2010
Family Nocardiaceae			
Nocardia seriolae Kudo et al. 1988 Syn. *N. kampachi*			
	Golden pompano, *Trachinotus ovatus* L.	China	Zhou et al. 2002 Zhao et al. 2007 Huang et al. 2008 Wang et al. 2009 Xia et al. 2015
PROTOZOAN PARASITES			
Phylum Ciliophora *incertae sedis*			
Class Prostomatea			
Order Prorodontida			
Family Holophryidae Perty 1852			
Cryptocaryon irritans Brown 1951			
	Florida pompano, *Trachinotus carolinus* L.	Florida (USA)	Sinderman 1977a
	Florida pompano, *Trachinotus carolinus* L.; Snubnose pompano, *T. blochii* (Lacepède)		FAO 2009a
	Florida pompano, *Trachinotus carolinus* L.; Palometa, *T.* goodei (Jordan & Evermann); Permit, *T. falcatus*	Venezuela (experimental)	Gómez-Gaspar 1987
	Golden pompano, *Trachinotus ovatus* L.	China (experimental)	Dan et al. 2006 Luo et al. 2008 Wang et al. 2016 Hu et al. 2017 Zhong et al. 2020
Phylum Ciliophora *incertae sedis*			
Class Oligohymenophorea de Puytorac et al. 1974			

Subclass Peritrichia			
Fam. Scyphidiidae Kahl, 1933			
Scyphidia sp. Dujardin, 1841 Syn. *Riboscyphidia* sp.			
	Florida pompano, *Trachinotus carolinus* L.	Alabama (USA)	Williams 1974a
Fam. Trichodinidae Claus 1874			
Trichodina sp. Ehrenbeg 1830 Syn. *Cyclochaeta* sp.			
	Florida pompano, *Trachinotus carolinus* L.	Alabama (USA)	Williams 1974a
Phylum Myzozoa Cavalier-Smith & Chao **Class Dinophyceae** Fritsch 1927 **Order Thoracosphaerales** **Family Thoracosphaeraceae** Schiller 1930			
Amyloodinium ocellatum Brown & Hovasse 1946			
	Florida pompano, *Trachinotus carolinus* L.	Alabama (USA)	Williams 1974
	Florida pompano, *Trachinotus carolinus* L.; Snubnose pompano (*T. blochii*)		FAO 2009a
	Florida pompano, *T.* carolinus L.; Palometa, *T. goodei*; Permit, *T. falcatus*	Venezuela (experimental)	Gómez-Gaspar 1987

Status of Genetics and Selective Breeding (L.E. King, D.J. Bradshaw, and P.S. Wills)

To date there is no known directed program for selective breeding of the pompano. The extent of known breeding is via nondirected breeding that occurs within the context of individual farms' breeding programs. These often use a mix of fish collected from the wild and/or F1 or later generations that may or may not have been selected based on growth rates. Regardless, we know of no records of heritability estimates or even pedigree tracking that may have occurred. Pedigree tracking is very important in a breeding program and has been used in salmon breeding to minimize inbreeding and to record traits such as disease resistance and environmental tolerance that are not easily measured. In general, farms currently are not even likely to maintain a tagging program that would facilitate the collection of data necessary to determine the required information for selective breeding. Due to the extensive use of parents collected directly from the wild it is not a stretch to state that pompano to date are not at the point of domestication although breeding under controlled conditions is well established.

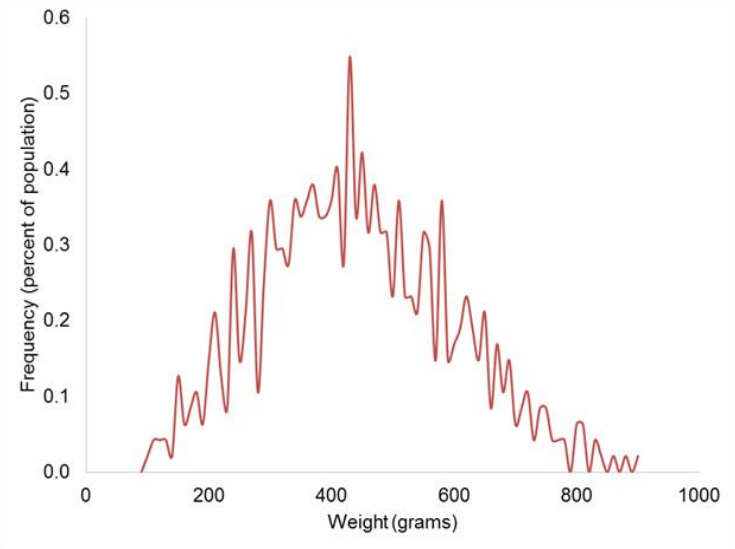

Figure 62. Frequency distribution of weights (g) from a subsample (n=689) of Pompano taken randomly during the final harvest of fish from a commercial demonstration system at HBOI, Fort Pierce, FL on April 8, 2014.

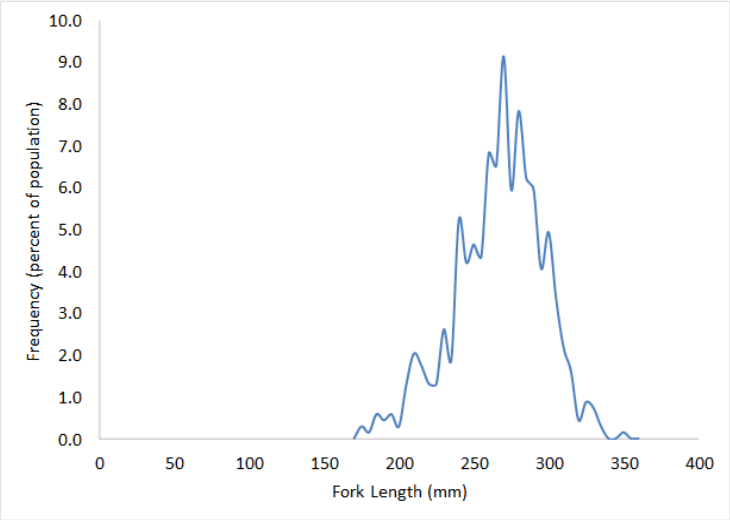

Figure 63. Frequency distribution of fork lengths (mm) from a subsample (n=689) of Pompano taken randomly during the final harvest of fish from a commercial demonstration system at HBOI, Fort Pierce, FL on April 8, 2014.

The establishment of traits of interest for selection is a primary step in beginning a selective breeding program with any species. For pompano, based on observations in commercial scale research production systems, a trait of utmost importance is improved growth rates after 250 g with concomitant reduction in growth variability. Being that the breeding populations available today are primarily of wild origin the offspring show very high growth variability in both weight and length with fish in a commercial demonstration project conducted at HBOI ranging from 99.5 g (0.22 lbs; Fork Length, FL=175 mm, 6.9 inches) to 897.5 g (1.98 lbs; FL=350 mm, 13.8 inches).

Other traits that should be selected for immediately include resistance to specific diseases (e.g., Vibriosis and Marine velvet disease), reduced stress response at culture densities, improved digestion of terrestrial proteins (e.g., soy in feeds), increased tolerance to low salinities, improved feed conversion efficiency, higher dress out percentage, among others.

Research is being conducted at HBOI to help facilitate the establishment of a marker-assisted selective (MAS) breeding program for pompano. The lack of genetic information available for this species is a hurdle in accomplishing this task. To this end, the whole genome of the pompano was assembled (733.5 megabase pairs) and annotated with a set of protein-coding

genes (26,891). The next step was identifying the genetic variability occurring in the wild population that can be selected for in a breeding program. A population genetics study was conducted to determine the genetic variation found in the wild population of pompano caught off Florida's east and west coasts. These two groups were found to be part of one population with genetic flow occurring between the groups. A total of 7,804 high-quality SNPs (single nucleotide polymorphisms) were also identified between these two groups, which were predicted as having possible effects on traits such as growth, reproduction, and immune response. Finally, a comparative genomic analysis study was conducted on the genomes of the pompano, Permit (*T. falcatus*), and Palometa (*T. goodei*) to identify a genetic component for the differing sizes of these three species. A greater number of growth-related genes were identified in the Permit and Palometa than in the pompano (N = 318; N= 318; N=189, respectively). Species-specific growth-related genes were also identified in all three species. Further research needs to be conducted to investigate what roles these species-specific genes have in the differing sizes of these species.

This genetic research lays the groundwork for establishing a MAS breeding program for the pompano. A genome with protein-coding genes and high-quality SNPs have now been identified, but what type of effects these SNPs have still needs to be investigated to be able to select the highest-quality broodstock. In combination with further sequencing, these SNP can be used to develop solid-state SNP arrays which are small pieces of silicon glass (chips) that have DNA probes attached to their surface at specific spots. The DNA probes are fragments of DNA that will bond to SNPs in a DNA sample from the individual fish that is being tested. The spots on the array that have SNP DNA bonded to them will change color or fluorescent intensity. This allows the geneticists to rapidly determine the genotype of an individual broodfish based on 1,000s to 1,000,000s of SNPS simultaneously (Regan et al. 2021). Once developed, SNP arrays

are cheaper, have simpler sample processing and downstream analysis, and have faster turnaround time than genotyping-by-sequencing approaches and thus can be more readily applied for aquaculture breeding programs (Yanez et al. 2023).

These arrays, or similar marker detection techniques, can then be used to detect specific genetic markers for quantitative trait loci (QTL), (a.k.a., QTL mapping). QTLs are regions (i.e., genes or gene polymorphism) of the genome associated with variation in quantitative phenotypes (e.g., rapid growth, better dress-out percentage, etc.) in a population that can be used in MAS program to assess the odds that an individual will have a particular trait (Regen et al. 2021). QTLs for survival, immunity, body length, sex determination, and other important production traits have been identified in other aquaculture species and used in selective breeding programs (Yanez et al. 2023). QTL mapping has two phases: linkage mapping and association mapping. Linkage mapping tracks QTLs and the coinheritance or separation of markers in full-sib or half-sib families. Association mapping assesses linkage disequilibrium, or the nonrandom association of alleles at different loci, in an unrelated group of individuals or a natural population (You et al. 2020). For more complex genetic traits, genomic selection (GS) will be needed to expand upon the capabilities of MAS by using SNPs to predict genomic breeding values for selection candidates based on the combined effects of all markers (Regan et al. 2021). GS is based on the theory that most QTLs will be in strong linkage disequilibrium with at least one marker with enough loci across the genome (Regan et al. 2021). While MAS is cheaper and effective with traits affected by a few loci with major effects, GS is more useful for traits controlled by many genes or loci, each having a minor effect on the trait (Regan et al. 2021).

Marketing and Business Planning Considerations (M. Davis, and P.S. Wills)

Economic Modeling:

An economic model of pompano production was run based on the pompano Economic MSExcel™ Model hosted on the HBOI education workshop web page located at: https://www.fau.edu/hboi/research/aquaculture-innovation/center-for-marine-and-warm-water-aquaculture/education/pompano-culture-workshop-2013/ (last access March 6, 2023). Assumptions of the model were updated to 2023 figures where possible and based on the average and best-case metrics measured during the Sea Grant funded project US Department of Commerce (DOC), NOAA Sea Grant project award number NA18OAR4170345 titled "11.417: Wills - Final Steps Toward Commercialization of pompano Aquaculture." The updated MSExcel™ model is located at the web page: https://www.fau.edu/hboi/research/aquaculture-innovation/center-for-marine-and-warm-water-aquaculture/education/pompano-aquaculture-industry-workshop-2023/ (Last access 6/21/2023). Both versions of the pompano farm model are presented as is for informative purposes but should not be used in and of themselves or in their current form for business decisions. The assumptions made in developing these models likely do not apply to the situation of a given farm as they are derived from the demonstration and experimental system studies conducted at HBOI-FAU. Use at your own risk there are no guarantees made or implied from use of the model.

Business Planning:

Business Plan Template (outline):

I. Executive Summary

This section should provide an overview of the Florida pompano aquaculture business plan, including the purpose of the plan, a summary of the individual business concept, and the key financial and operational objectives hoped to be achieved. Keep in mind that this should be a one-page summary of the entire plan and is typically written after the rest of the plan is completed.

- Briefly introduce the business and the product.
- Provide a summary of the market opportunity.
- Explain the competitive advantages of the business.
- Outline the financial projections.

II. Business Overview

This section should provide an overview of the business, including the vision and mission, the target market, and competitive advantage(s).

- Explain the company's vision and mission.
- Describe the legal structure of the business.
- Identify the key personnel and their roles.
- Provide an overview of the production process.

III. Product Description

This section should provide a detailed description of the pompano product, including its unique selling points, production process, processed forms (e.g., whole fresh, skin on fillets), pricing strategy, and distribution channels.

IV. Market Analysis

This section should provide an in-depth analysis of the market for Florida pompano, including the size and growth potential of the market, the demand for the product, the competitive landscape, and the regulatory environment.

- Identify the target market for the product.
- Analyze the size of the market and its growth potential.
- Describe the competition and their market share.
- Analyze the trends in the seafood industry.

V. Marketing and Sales Strategy

This section should describe the marketing and sales strategies that you will use to promote and sell product, including the target market, the marketing channels, and the promotional tactics.

- Define the marketing and sales goals.
- Describe the marketing channels and tactics.
- Identify the pricing strategy.
- Explain the sales strategy and sales cycle.

VI. Production Plan

This section should describe the day-to-day operations of the business, including the production process, equipment and technology required, staffing needs, and suppliers and vendors the operation will work with.

- Identify sources of raw materials.
- Explain the production process and quality control measures.
- Describe the equipment and facilities needed.
- Provide a production schedule.

VII. Management and Operations

- Describe the management team's qualifications.
- Outline the organizational structure.
- Identify the key operational processes and procedures.
- Explain the risk management plan.

VIII. Financial Plan

This section should provide a detailed financial plan for the business, including startup costs, revenue and profit projections, cash flow projections, and funding sources used to finance the business.

- Provide an overview of financial projections.
- Outline start-up costs and capital requirements.
- Explain revenue and expense projections.
- Provide realistic cash flow and profit and loss statements.

IX. Risk Analysis

This section should identify the potential risks and challenges that the business may face and describe strategies that will use to mitigate risks and overcome challenges.

X. Conclusion

This section should summarize the key points of the business plan and emphasize why the Florida pompano business is a viable and profitable venture.

- Summarize the business plan and key takeaways.
- Provide a call-to-action and next steps.
- Mention the future growth potential of the business.

Marketing (M. Davis)

There are five steps to building an aquaculture business and they include the following:

1. Research and Training: This step is a time when you collect knowledge about the species, the systems and potential for aquaculture. It is a time to determine if the species is in the experimental, technologically feasible or commercial stage of development. This knowledge can be acquired from research organizations such as FAU Harbor Branch, from publications and articles, manuals, aquaculture workshops and conferences and hands-on experiences. This is also a time to determine if you will lead the aquaculture effort or if there is a need to hire experts that can assist in the preliminary stages and/or day-to-day operations.
2. Evaluation of Expectations – It is important to evaluate your expectations, as well as those of your family and business partners in regard to running an aquaculture business. Some things to consider are that aquaculture is a 24 hours-per-day / 7

days-a-week operation, it takes time (up to a year or more after growout systems are complete) for the first product to be ready for the market and, depending on the size of the business, the return on investment can take many years to be realized, and the upfront costs for capital and operations can be high.

3. Planning: Market, Production and Business - These three planning stages are critically important to the success of the aquaculture operation. They need to be done prior to investing in a business, gathering capital funds, and breaking ground. These are essentially the road map for the business.
4. Testing: Demonstration-Scale – A demonstration-scale aquaculture operation can provide the business with an opportunity to begin the operation at a smaller scale to test the systems, train the employees and establish markets.
5. Commercial-Scale Production – This is the scale that will provide the aquaculture company with the operational funds to cover costs through the sale of product. The quantity of the product will need to be high enough to cover the costs of the operations with surplus to pay back capital costs over time, have funds for unexpected needs such as maintenance, replacement equipment, mortality events, and storms, and pay investors dividends (if the farm is capitalized via this means).

Market Analysis

Building a marketing plan can be difficult because selling your product can be a long way off. For example, you need time to build the infrastructure and then grow the product. This could be 18–36 months before the product is ready for market. You cannot wait until the product is

ready for market to gauge your market and determine your revenue stream. Therefore, there is a simple method to begin your market investigation and it is called the 'Four Ps of Marketing':

1. Product
2. Price
3. Place
4. Promotion

Each of these will now be explored using pompano as the example:

Product

There are several forms in which a fish can be sold, including: whole, gutted (head on or off) or filleted (skin on or off), and fresh never frozen or frozen. The product can be packaged in heavy waxed boxes, vacuum-packed, IGF, and labeled. All types of processing will require processing permits and HACCP protocols* (Note: check local laws). Typically, pompano is sold either whole on ice (1–2 pounds) or as skin on fillets with the pin bones removed (4-6 or 5-7 ounces) (Figure 64). One advantage of the pompano is that it does not need to be scaled as the scales are very small and not noticeable when cooked. The fish are available for market at about

Figure 64. On the left is a whole Florida Pompano ready for market and on the right are Pompano fish fillets.

1-year-old. To establish steady market demand, the aquaculture business will need to estimate sales frequency and amount of product available.

*NOTE on HACCP: In December 1995, the FDA issued seafood regulations based on "Hazard Analysis and Critical Control Points" or HACCP. It is a preventive system to ensure safer foods and is designed to identify hazards, establish controls, and monitor those controls. Each step of the process from growing to harvesting to processing needs to be included in HACCP. Resources available to learn more include the Cooperative Extension Service or Sea Grant Marine Advisory Service, seafood trade organizations, regional offices of the Food and Drug Administration or National Marine Fisheries Service. Courses are offered around the country on preparation of HACCP plans for your operation and it is highly recommended that a representative of your farm who will be responsible for any onsite processing attend one of these courses (see https://www.flseagrant.org/seafood-safety/seafood-haccp-training-and-education/).

Price

The farm gate price is equal to the cost it takes to grow the product and should be below the market price, either wholesale or retail depending on market targeted. The aquaculture operation needs to be able to sell the product to cover the farm gate price and processing and delivery to market. Most of the seafood, 60–80%, is sold through restaurants as a deboned fillet of fish (4–6 or 5–7 ounces). The dress out for pompano is 56–59%. Here is an example for retail pricing of pompano (see Table 6).

The equation is: *Farm Gate Price/Yield = Product Value*

Assuming that farm gate price is $13.99 per pound for whole fish prior to processing the fillets will be valued at $13.99 / 0.56 yield = $24.98 per pound for deboned fillet. This

does not include the processing, packaging, and delivery. In this example, it will be necessary that the farm gate price to grow the fish be below $13.99 per pound.

Table 6. Florida pompano retail market prices (January 2023).

Product	**Crab-e-Bills (Sebastian, Florida)**	**All Fresh Seafood (Online)**
Whole Fish	$13.99	$13.99
Fillets	$28.99	$24.99

There are ways for the farm to bring in higher revenue for their product if the buyer sees a higher value for the product. This could include marketing advantages such as emphasizing locally grown, a natural product and/or a convenient delivery method. Typically, new products in short supply may also demand a higher price and there are always direct, niche, or value-added products that provide marketing opportunities. If the operation has smaller sale volumes, direct marketing may be necessary. Direct marketing means that the aquaculture business is selling directly to the consumer. The larger the operation the more likely that marketing will target a commodity market.

Here are some direct marketing ideas:

- Local Customer Base
- Roadside Market
- Fish Fry Fund-raiser
- Fairs and Festivals
- Office Building Markets
- Restaurants
- Retail Stores
- Value-added Market

It is also critical to know what is important to your consumer and buyer such as:

- Health
- Nutrition
- Convenience
- Variety
- Source – Wild, Farmed
- Value
- Sustainability
- Safety and Quality

Place

Most aquaculture farms are located on a site that is optimal for the biological and environmental factors necessary for growing the species and for the operation. Access to markets is another variable that should be considered when siting a farm. For instance, distance from processing plants, transportation (trucking, airport, highways), regulatory inspection, and storage (freezer) should be taken into consideration when choosing a site. This can be a competitive edge for the business when the processing and sales can be delivered at the least cost.

Promotion

Questions that should be asked when introducing a new aquaculture species such as the pompano to the marketplace include:

1. Will the market be local and national scale?
2. Who is the target audience and where are they located?

3. How can the audience be reached and what will be used to trigger an action?
4. What is the cost of each marketing option (magazine, social media, flyers, TV) and what demographics will be reached and how to call to action?

There are many advantages of farmed fish products, in general, that can be used to assist with marketing the products. These include:

1. Harvest times – consistency in harvest
2. Fresh never frozen product
3. Processing to market specifications
4. Size is uniform
5. Flavor is reliable
6. Personal touches
7. Meeting the customer's needs

Florida Atlantic University's College of Business, Consumer and Buyer Behavior Class' Marketing Surveys

Florida Atlantic University Harbor Branch Oceanographic Institute partnered with the FAU College of Business Instructor Eileen Acello, a faculty member within the Marketing Department. Her areas of expertise are food marketing such as branding, consumer behavior, and retail advertising. In Fall 2019, students in Acello's course 'Consumer and Buyer Behavior' worked with FAU Harbor Branch on the following project goal:

- The student groups' challenge was to pitch seafood as a healthy protein choice (product idea) to be consumed two times per week with the goal to increase seafood consumption in the US -- to a group of venture capitalists (the classmates).

In this project the students used secondary sources and primary sources, e.g., interviews, surveys, and/or focus groups to collect their data. At the beginning of the project the students were provided with a presentation from Megan Davis, Ph.D., FAU Harbor Branch Research Professor, Aquaculture and Stock Enhancement Program. This presentation gave the students background on the seafood industry and information relevant for their class project. There was a question-and-answer period and Megan also provided follow up materials throughout the project.

The students were divided into two groups for each generation: Gen Z (born after 1996), Gen Y (born between 1981-96), Gen X (born between 1965-1980), Baby Boomer (born between 1946-64). Therefore, there were eight groups total. The common questions asked of the participants during the interviews and surveys:

1. Do they eat seafood?
2. How often?
3. Where do you eat seafood?

4. What do they know about aquaculture?
5. Do they prefer aquaculture vs wild caught?
6. What is their reason to eat seafood?
7. What is their reason not to eat seafood?

Tables 7 and 8 show a summary of the results compiled by Megan Davis and FAU Harbor Branch volunteer Obby Tapley. These summary tables were compiled from findings and information that the student groups presented in their reports and PowerPoints. Table 7 is a compilation of data that answered the questions shown above. Table 8 is the strengths, weaknesses, opportunities, and threats (SWOT) analysis.

Table 7: Summary findings from surveys and interviews categorized by the generational groups by the FAU College of Business Consumer and Buyer Behavior course.

Survey Question Category	**Gen Z (1)**	**Gen Z (2)**	**Gen Y (1) Millennials**	**Gen Y (2) Millennials**	**Gen X (1)**	**Gen X (2)**	**Baby Boomers (1)**	**Baby Boomers (2)**
Number Surveyed	50	Many on social media	50	54	30+	41	43	94
Number Interviewed		8					11	8
Consume seafood (%)	98	76	92	85	93	98	95	92
Consume seafood 2x/wk or greater (%)	36	29 (1/wk)	20	11 (1-2/wk) 45 (3-4 /wk)	46	N/A	approx. 42	20 (3-5 wk)
Consume seafood less than once per week (%) (they eat seafood)	32 (1-3/mo)	30	42	44	46 (at least once per week)	50 (at least once/wk) 50 (< 2 / mo)	58 (1-5/mo)	69 (1-2 /wk)
Reason to eat seafood **See notes***	Health Taste	Health Taste	Health	Health Taste	Health	Health Taste	Health Taste	Health Variety Taste
Reason not to eat seafood	Smell Taste Texture Allergy Contaminants	Smell Taste Texture Unfamiliar	Price Taste	Taste	Price No cooking confidence Taste Smell	Price	Price	Taste Smell Allergies Price
Knew about aqua (%) (Y/N)	N		62 N 38 Y	48 N 52 Y	25 Y 75 N Inconsistent data	N		82 Y 18 N
Prefer wild capture (W) or aquaculture (A) (%) **See notes****	64W	No preference Need info to know more	42W	54 W 42 No preference 4A	75 Fresh		86 Fresh	75W 25A

% Place of consumption	Home Restaurant	Home	Home 46 Restaurant 42	Home 50 Restaurant 50	Home preference b/c price	Home preference b/c budget	Restaurants Home	Home 56 Restaurant 44
Source of knowledge about seafood, recipes	Family friends Online ed. Online sales. Social media. Store kiosk. Word of mouth.	Family. Friends. Info on packaging. Meal kits.	42% internet 27% social media. 12% TV 2% print 17 other	22% friends Cell phone ads on seafood prep. Specialty store with info.	Grocery store	Social media. Google. Yelp. Email.	TV. Print media. Packaging info. Online.	Word of mouth. Video ads. Facebook. Cooking show. Restaurants.
Unmet needs	Easy recipes. Info on nutrition. Demo shows. How to clean and cook.	Recipes on package. Cooking video. Fresh, healthy, not GMO seafood.	Health info. Nutritional benefit info. Attractive price.	How to prepare and cook. What are species choices. Targeted online ads. Recipes at store.	Green marketing. Prepared meals in store with food label and directions. Aquaculture is ecofriendly.	How to cook. More nutrition info. Knowledge of aquaculture.	Restaurant partnerships. Knowledge of aquaculture. Meal prep info. Recipes. Source of seafood.	Videos on seafood facts. Recipe guides.

Notes:

1. *Health as a reason to consume includes non-GMO, organic, low fat, protein, low calorie, fatty acids, vitamins, and minerals.
2. **Prefer wild caught or aquaculture. Many prefer wild but said if aquaculture is sustainable, it is good consume. Some said they needed more information on clean aquaculture. People are confused between fresh, wild caught and aquaculture. Some are probably eating fresh aquaculture products and think they are eating wild. In the surveys fresh is compared with frozen, canned, etc.

Table 8**:** Summary findings from surveys and interviews expressed as a "SWOT" analysis for each generational category as compiled by the FAU College of Business Consumer and Buyer Behavior course students.

SWOT Categories	**Gen Z (1)**	**Gen Z (2)**	**Gen Y (1) Millennials**	**Gen Y (2) Millennials**	**Gen X (1)**	**Gen X (2)**	**Baby Boomers (1)**	**Baby Boomers (2)**
Strengths	Healthy protein. Easy to prepare, cook. Sustainable aquaculture.	Nutrition. Low carbon footprint.	Protein. Healthy fat and minerals.	Has become more popular.	Healthy sustainable US seafood. Support domestic supply to help fishermen and ocean.	Nutrition. Aquaculture is reliable, consistent, sustainable.	More disposable income. Most eat seafood. Understand healthy protein. Large demogr.	1.2 million jobs in US. Protein. Recommended by American Health Org.
Weaknesses	Lack public awareness. Taste, smell. Lack education on health benefits and easy preparation.	Price. Disease. Contamination.	Price. Taste. Little promotion of seafood.	With COVID19 restaurant closures.	Lack awareness of quality, source of supply and whether aquaculture is safe or not.	Do not know how to cook, prepare. Lack understanding of aquaculture. Food quality concerns.	Do not consume enough. Lack understanding of aquaculture.	Price. Mercury confusion. Do not know how to prepare, cook.
Opportunities	With COVID-19 internet sale and digital advertising campaign. Provide meal, recipe ideas.	More seafood restaurants. Easy access to recipes, cooking help. Provide more health benefits information. Willing to spend.	Want to be healthier. Seafood info online. Cheaper using aquaculture.	Online delivery. New supply chains.	Support domestic fisheries, local sourcing. Provide cooking instructions. Use green marketing.	Already consume. Cooking shows. Expand aquaculture in US. Powerful consumers.	Aquaculture info on facebook, online, Dr. office, free seminars. Tell farm name. Restaurant partnerships. Free samples.	Health. Sustainability. Cardiovascular health. Price incentives.
Threats	Lack awareness of benefits of aquaculture. Traditional fish markets. Other protein sources. Mercury poisoning concern.	COVID 19. People eating less meat.	No cooking knowledge. Negative view of aquaculture. Value protein in poultry, meat.	With COVID19 take out only at restaurants. Collapse export market.	Lack demand. Price due to aquaculture expense. Hesitation to try new seafood.	Myths and misinformation on aquaculture. Other protein rich foods.	Price. Lack of aquaculture presence.	Rising import duties. Misinformation. Water pollution in oceans, rivers, lakes. Hesitant to try new seafood.

In Spring 2020, Eileen's students worked off the findings from the prior course and applied them in the 'Principles of Advertising' course. The students worked with FAU Harbor Branch on the following project questions:

- How to reach the consumer about seafood?
- How to make the consumer feel comfortable trying seafood?
- How to build consumer confidence in buying and cooking seafood?
- How to educate the consumer that…
    - Seafood is healthy to eat.
    - Seafood is easy to cook at home.
    - Aquacultured (i.e., farmed) seafood is safe and healthy to eat.
    - Consuming seafood 2 times per week is recommended for a healthy diet.

Similar to the Consumer and Buyer Behavior course the students in Principles of Advertising also used secondary sources and primary sources, e.g., interviews, surveys, and/or focus groups to collect their data. At the beginning of the course the students were provided with a presentation from Megan Davis, Ph.D., FAU Harbor Branch Research Professor, Aquaculture and Stock Enhancement Program. This presentation gave the students background on the seafood industry and information relevant for their class project and a summary of the findings from the Consumer and Buyer Behavior course. There was a question-and-answer period and Megan also provided follow up materials throughout the project.

The students were divided into two groups for each generation: Gen Z (born after 1996), Gen Y (born between 1981-96), Gen X (born between 1965-1980), Baby Boomer (born between 1946-64). Therefore, there were eight groups total and their finding are summarized in Table 9.

Table 9: Summary findings from surveys and interviews categorized by generational groups for the FAU College of Business Principles in Advertising course. Overall comments were that the consumer was interested in the health benefits of eating seafood.

Topics	Gen Z	Gen Y	Gen X	Boomers
Social Media Preference	Instagram and Snapchat	Instagram and Facebook	Facebook and Friends	Facebook and In Stores
How Information is Gathered	Through Videos and Online	Foodie Influences	Online Reviews, TV Advertisements, Videos	Online and Print Advertisement
Where is Seafood Eaten	Variety of Places	Need of Learn How to Feel Comfortable Cooking	Home	Home
Type of Seafood Eaten	Organic and Natural	Preference to Salmon, but Will Eat Variety	Variety	Wild, Fresh

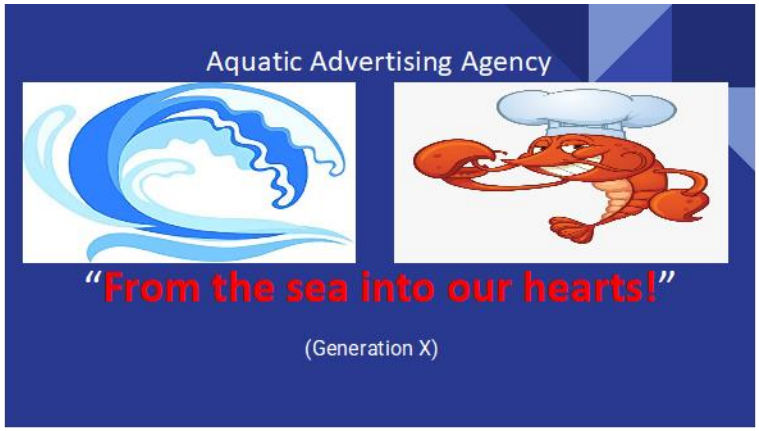

Figure 65. Catchy Marketing presentation slides produced by the FAU Principles in Advertising course students.

Marketing Resources:

1. Seafood Markets, restaurants, online sales
2. Seafood Nutrition Partnership https://www.seafoodnutrition.org/
3. Seafood Source https://www.seafoodsource.com/
4. NOAA Fisheries Fishwatch https://www.fishwatch.gov/
5. Shamshak et al. 2019. US Seafood consumption JWAS
6. USAS webinar Seafood in the Diet: Consumption Patterns at Retail and Food Service https://youtu.be/PhWnTZgpi44
7. David Love et al. 2020. Food Sources and Expenditures for Seafood in the United States. Nutritional Value of Seafood https://doi.org/10.3390/nu12061810

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Appendix A: Conversions and Equations for Aquaculture

Weight and Mass:

1 ounce (oz) = 28.35 grams (g)

1 oz = 28,349.5 milligrams (mg)

1 pound (lb) = 16 oz

1 lb = 453.6 g

1 lb = 0.4536 kilogram (kg)

1 ton (US, short ton) = 2,000 lbs

1 ton (US, short ton) = 907.2 kg

1 g = 0.035 oz

1 kg = 35.27 oz

1 kg = 2.2 lbs

1 g = 1,000 mg

1 g = 0.001 kg

1 kg = 1,000 g

1 ton (long ton or metric ton) = 2,240 lbs

1 ton (long ton or metric ton) = 1,016 kg

Length:

1 inch (in or “) = 2.54 centimeters (cm)

1 foot (ft or ‘) = 12 in

1 ft = 30.48 cm

Length (cont):

1 yard (yd)= 3 ft

1 yd = 36 in

1 yd = 0.914 meters (m)

1 cm = 10 millimeters (mm)

1 m = 100 cm

1 m = 1,000 mm

1 kilometer (km) = 1,000 m

1 km = 1,093.6 yd

1 km = 0.62 miles (statute)

1 mile = 1.61 km

1 mile = 0.869 nautical miles

1 nautical miles = 1.15 miles

Volume:

1 gallon (gal) = 4 quarts (qt)

1 gal = 128 fluid ounces (oz)

1 gal = 3.785 liters (l)

1 qt = 32 oz

1 qt = 2 pints

1 pints = 16 oz

Volume (cont.):

1 cup = 8 oz

1 cubic foot (cu ft) = 7.48 gal

1 cubic yard (cu yd) = 27 cu ft

1 cu yd = 0.765 cubic meters (m^3)

1 m^3 = 1.31 cu yd

1 m^3 = 1,000 liters (l)

1 l = 1,000 milliliters (ml) or cm^3 or cc

Concentration:

1 mg/l = 1 part per million (ppm)

1 g/l = 1 part per thousand (ppt or ‰)

1 kg/ m^3 = 1 ppt

3.785 mg/gal = 1 ppm

8.34 lbs per million gallons = 1 ppm

2.72 lbs/acre-foot = 1 ppm

1,233 g/ acre-foot = 1 ppm

Temperature:

°F = ((9/5) x °C) + 32

°C = (°F - 32) x (5/9)

Appendix B: Example Data Sheets

Broodstock Inventory and Data Sheet

Tank #	PIT TAG #	Sex:	Wt. (Kg)	Egg/Milt Sample	Injection	Comments
1A	43000170420	F	0.745			
	226001019180	F	0.867			
	226001018965	M	0.808			
	226001019128	M	0.855			
	226001019179	F?	0.914			
	43000170491	F	0.962			
	43000170450	M	0.719			
	43000170460	M	0.582			
	43000170451	M	0.727			
1B	43000170468	F	0.798			
	43000170432	F	0.848			
	226001018938	F	1.016			
	226001018995	F?	1.116			
	226001019186	M	0.68			
	43000170492	F	0.816			
	43000170403	F	1.097			
	43000170414	M	0.767			
	43000170352	M	0.617			
	43000170412	M	0.555			
2A	43000170494	F	0.934			
	43000170406	F	0.965			
	43000170416	F	0.857			
	226001018981	M	0.457			
	226001018936	F?	0.867			
	226001018948	M	0.635			
	43000170458	F	0.926			
	43000170437	M	0.678			
	43000170427	M	0.711			
	43000170417	M	0.657			
2B	43000170402	F	1.154			
	43000170459	F	1.054			
	43000170449	F	0.829			
	226001018911	F?	0.945			
	226001019197	M	0.823			
	43000170439	F	0.978			
	43000170478	F	0.767			
	43000170448	M?	0.804			
	43000170467	M	0.771			
	43000170457	M	0.584			

Conditioning Cycle Data Sheet

Tank Identification:			**Species-Pompano**	
			Date started:	
			# of fish in the group:	
Week	**Light**	**Temperature (°C)**	**Comments**	**Light/Dark**
1	12.5	25		12.5/11.5
2	13.0	26		13.0/11.0
3	13.5	27		13.5/10.5
4	13.5	28		13.5/10.5
5	14.0	29		14.0/10.0
6	14.0	29		14.0/10.0
7	13.5	28		13.5/10.5
8	13.5	27		13.5/10.5
9	13.0	26		13.0/11.0
10	12.5	25		12.5/11.5
11	12.0	24		12.0/12.0
12	11.5	23		11.5/12.5
13	11.0	22		11.0/13.0
14	10.5	21		10.5/13.5
15	10.0	20		10.0/14.0
16	9.5	19		9.5/14.5
17	9.0	18		9.0/15.0
18	9.0	18		9.0/15.0
19	9.0	18		9.0/15.0
20	9.0	18		9.0/15.0
21	9.5	19		9.5/14.5
22	10.0	20		10.0/14.0
23	10.4	21		10.5/13.5
24	11.0	22		11.0/13.0
25	11.5	23		11.5/12.5
26	12.0	24		12.0/12.0
27	12.0	24	Hold for Spawning (up to 6-8 months)	12.0/12.0
*26 weeks of conditioning (roughly 6.5 months)				
**24-32 weeks of spawning (roughly 6-8 months)				

Rotifer Counts:

Date:	Day:	Date:	Day:	Date:	Day:	Date:	Day:
Tank:	Count:	Tank:	Count:	Tank:	Count:	Tank:	Count:
1		1		1		1	
2		2		2		2	
3		3		3		3	
4		4		4		4	
5		5		5		5	
6		6		6		6	
7		7		7		7	
8		8		8		8	

Date:	Day:	Date:	Day:	Date:	Day:	Date:	Day:
Tank:	Count:	Tank:	Count:	Tank:	Count:	Tank:	Count:
1		1		1		1	
2		2		2		2	
3		3		3		3	
4		4		4		4	
5		5		5		5	
6		6		6		6	
7		7		7		7	
8		8		8		8	

Date:	Day:	Date:	Day:	Date:	Day:	Date:	Day:
Tank:	Count:	Tank:	Count:	Tank:	Count:	Tank:	Count:
1		1		1		1	
2		2		2		2	
3		3		3		3	
4		4		4		4	
5		5		5		5	
6		6		6		6	
7		7		7		7	
8		8		8		8	

Date:	Day:	Date:	Day:	Date:	Day:	Date:	Day:
Tank:	Count:	Tank:	Count:	Tank:	Count:	Tank:	Count:
1		1		1		1	
2		2		2		2	
3		3		3		3	
4		4		4		4	
5		5		5		5	
6		6		6		6	
7		7		7		7	
8		8		8		8	

Rotifer Daily Water Parameters:

	Date:	Day:	Date:	Day:	Date:	Day:
Tank:	D.O./Temp.	Sal/pH	D.O./Temp.	Sal/pH	D.O./Temp.	Sal/pH
1						
2						
3						
4						
5						
6						
7						
8						

	Date:	Day:	Date:	Day:	Date:	Day:
Tank:	D.O./Temp.	Sal/pH	D.O./Temp.	Sal/pH	D.O./Temp.	Sal/pH
1						
2						
3						
4						
5						
6						
7						
8						

	Date:	Day:	Date:	Day:	Date:	Day:
Tank:	D.O./Temp.	Sal/pH	D.O./Temp.	Sal/pH	D.O./Temp.	Sal/pH
1						
2						
3						
4						
5						
6						
7						
8						

	Date:	Day:	Date:	Day:	Date:	Day:
Tank:	D.O./Temp.	Sal/pH	D.O./Temp.	Sal/pH	D.O./Temp.	Sal/pH
1						
2						
3						
4						
5						
6						
7						
8						

General System Record Sheet

DATE												
		DO		Temp	Salinity							
System	Tank	am	pm	am / pm	am / pm	Feed amnt				Morts	Notes	
2A	1											
	2											
	3											
	4											
2B	1											
	2											
	3											
	4											
2C	1											
	2											
	3											
	4											
2D	1											
	2											
	3											
	4											

WQ	2A	2B	2C	2D	BF Pressure		In	Out	BW secs	Purge time (min) / filter comments
Alk					2A	1				
pH						2				
TAN					2B	1				
NO_2						2				
Cu					2C	1				
Ca						2				
Additions					2D	1				
Bicarb						2				
								Notes:		

Water Meters	Salt	Fresh	Drum Filter
2A			
2B			
2C			
2D			

Daily/Weekly/Monthly Task Checklist

STARR H6 Monthly Animal Care Checklist — Month _____

Daily Tasks ☑	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
A.M.Walk through,✓ reservoirs																															
A.M. DO,Sal,Temp																															
Record Filter Pressures																															
Check/Remove Morts																															
Wipe O2 probes																															
Purge swirl Seps																															
Replenish Foot Baths																															
Run Water Quality/Chem																															
Enter Data																															
Lunch (12-1)																															
Load Feeders (after 12:00)																															
Backwash Filter																															
Check Filter Valves																															
Replenish Foot Baths																															
Purge all Valves																															
Add Bicarb, Soda Ash																															
P.M. DO, Sal, Temp 4:30																															
Spray down floor drains N+S																															
Wipe O2 probes																															
P.M. Walk through																															
Initials when complete																															
Weekly Tasks (initl box)	M	T	W	TH	F	S	S	M	T	W	TH	F	S	S	M	T	W	TH	F	S	S	M	T	W	TH	F	S	S	M	T	W
Date																															
Brush Inside Tanks (N & S)																															
Clean Static Beds																															
Clean Side Boxes+diverter box																															
Clean FW (3) bag filters H6																															
Service Drum Filters(*)																															
Empty Trash Cans																															
Check/Fill Chemical Containers																															
Clean Inside System Sumps																															
Hose Down Outside Sump																															
Change Chlorine/Thio (^)																															
Clean/Exchange Diffusers																															
Disease Check																															
O2 Probe Maintenance																															
Organize Work Areas																															
Rotate Pumps(number running)																															
Clean Up Quadrants	NW							NE							SW							SE									

(*) (SD)sprayed down inside DF,edges+discharge hose

(#) pick up unused equipment and store,brush down spider webs

(^)change cl/thio dips sooner if needed

(L)lubricate drum rollers with white grease, top bearing w/lithium grease

hose down floors, supply lines and anything else that collects dust

(SP)check spray nozzles

(PS)pressure sprayed screens

wipe down feeders(you have all week to do this)

Supplemental Materials

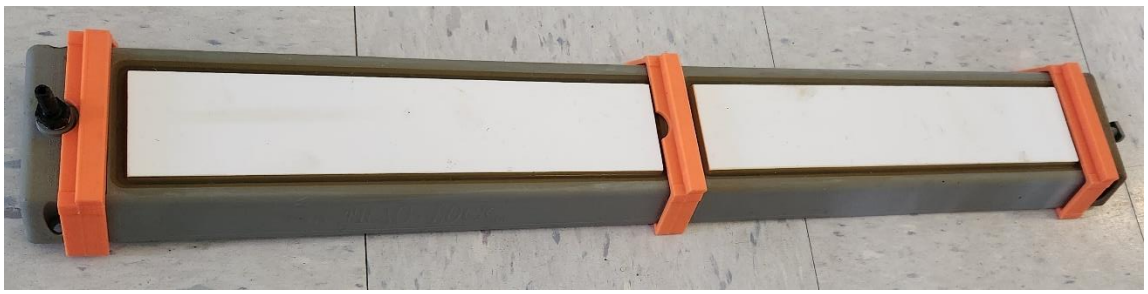
Two 3D printer files are available on the HBOI Website and on thingiverse.com (links provided below) for you to print and use. NOTE: Use these at your own risk as printing quality and other factors will have a profound effect on usability and we have no control over these factors. These are:

1) The Flow Sensor we use at HBOI

https://www.thingiverse.com/thing:6097124/files

And,

2) A Safety Clip we use for PointFour™ Diffusers (Pentair Aquatic Ecosystems, Apopka, FL)



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CULTURE MANUAL FOR THE FLORIDA POMPANO *Trachinotus carolinus* (Linnaeus, 1766)
Florida Atlantic University's Harbor Branch Oceanographic Institute
2023