MARINE AQUACULTURE AND
STOCK ENHANCEMENT IN FLORIDA:
RESEARCH AND DEVELOPMENT

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A. INTRODUCTION
Progress made on the deliverables included in the Mote Marine Laboratory (MML) Florida Fish and Wildlife (FWC) Grant No.13029 titled “Marine Aquaculture and Stock Enhancement in Florida: Research and Development” for the period of 16 July 2013 through 30 June 2014 are presented in this Final Report. The purpose of this grant was to conduct research to develop and refine maturation and spawning technology, increase survival during larval rearing, advance juvenile production strategies to further mass-production aquaculture technology for common snook, *Centropomus undecimalis*, and report grant findings through publications, meetings and presentations. This work was primarily conducted at Mote Aquaculture Research Park (MAP).

B. BACKGROUND
Common snook is an ideal candidate for demonstrating ecologically and economically successful stock enhancement in marine and estuarine environments; in part because of their high site fidelity and strong tie to estuaries throughout most of the life cycle, coupled with good survival of stocked hatchery fish and their relatively high economic value. The results from Mote field experiments (Brennan et al. 2005, 2006, 2008) suggest a huge opportunity exists to demonstrate in Florida one of the first examples in the world to quantitatively document economically-effective enhancement of a marine fish population resulting from stocking marine hatchery fish. However, more advances in snook aquaculture technology are needed to provide the capability to scale-up release magnitude to production levels that can significantly enhance fishery landings.

Since 1987, FWC and MML have partnered to develop snook culture technologies and to rear snook in their finfish hatchery facilities to provide the fingerlings needed for pilot-release experiments. There has been substantial progress at MAP with the development of maturation and spawning methods for captive wild broodstock using environmental conditioning (Main et al., 2007; Yanes-Roca et al., 2009; Rhody et al., 2010, 2013, 2014) to produce common snook at a small scale. Research conducted at MAP during this grant period fulfilled the requirements of the four required research deliverables. In addition, the minimum requirements for the fifth deliverable were exceeded. The research objectives for each deliverable focused on improving spawning and culture methods that will lead to successful production of common snook. The deliverables for this grant period included:

1. Maintain and condition common snook broodstock at MAP for maturation and spawning research and to produce larvae and juvenile snook for experimental trials.
2. Evaluate the effect of environmental conditioning associated with tidal shift to induce volitional spawning in mature captive adult snook broodstock.
3. Investigate the effects of photoperiod, different live foods, and probiotics on survival and growth in common snook larvae.
4. Assess vulnerability of fingerling snook to lordosis resulting from exposure to water currents.
5. Increase awareness of research results and assist the commission with strategic planning.
C. SUPPORT OF COMMISSION MISSION
The FWC has dedicated its marine stock enhancement program to following the recommendations of Blankenship and Leber (1995) for a “responsible approach to marine stock enhancement”. The Commission is interested in using stock enhancement technologies to help resolve critical uncertainties about growth, survival, habitat utilization, and essential fish habitat of important recreational fishes. In response, MML’s Directorate of Fisheries and Aquaculture continued to assist FWC in developing aquaculture and marine fisheries enhancement technologies, testing new fisheries enhancement technology for common snook, and assisting in planning of FWC’s general approach to all marine fisheries enhancement in Florida.

D. DELIVERABLES
1. Maintain and condition common snook broodstock at MAP for maturation and spawning research and to produce larvae and juvenile snook for experimental trials.
   i. Introduction – To ensure quality eggs and larvae for common snook research and future stock enhancement practices, MML continued to maintain captive broodstock populations. The practice in the past was to obtain fertilized eggs by strip spawning wild snook at the peak of their maturation (Yanes-Roca et al., 2009, 2012). This was problematic because there were issues with the quality of the eggs obtained and the spawns could only be obtained seasonally (May-August). To meet the year-round demand for eggs for research productive captive broodstock are required. By using photothermal manipulation we can condition adult snook to reach a level of maturation where they can be induced to spawn through hormone manipulation (Main et al., 2007; Yanes-Roca et al., 2009; Rhody et al., 2010, 2013, 2014). During this grant period it was our goal to obtain a new population of production-based broodstock and to use photothermal manipulation and hormone therapy to induce spawning.
   
   ii. Methods – At MAP broodstock common snook were maintained in a 48.0 m³ tank (20-2) that was 6.1 m diameter and 1.83 m deep. The system included a 0.085 m³ drop filter (Aquaculture Systems Technologies, L.L.C, New Orleans, LA) for solids removal, a 900 L moving bed reactor containing 0.283 m³ plastic extruded floating media (AMBTM media®, EEC, Blue Bell, PA) for biofiltration, a protein skimmer, and two 150 W High Output SMART HO UV® units (Emperor Aquatics, Inc®, Pottstown, PA).

To ensure the health of the fish for maturation, water chemistry and water quality was recorded and monitored closely to provide optimum conditions. Water quality parameters were measured daily and included: dissolved oxygen (≥5 and ≤9ppm), temperature (±1°C of the desired temperature), salinity (35±1ppt), and pH (≥7.5 and ≤8.4). Water chemistry was monitored twice a week and was used to monitor the levels of nitrogenous waste in the water. The parameters measured for tank system 20-2 included: total ammonia nitrogen (<0.5ppm) (Figure 1A), total nitrite nitrogen (<1.0ppm) (Figure 1B), and total nitrate nitrogen (<50ppm) (Figure 1C). If ammonia or nitrite parameters were high, a water exchange was performed to alleviate the problem. Although the target maximum nitrate nitrogen concentration was 50 ppm, limitations on availability of low-nitrate water and biosecurity concerns prevented us from being able...
to exchange water on a regular basis. In June 2014, we linked the snook broodstock tank to a denitification filter and will use this filter to keep the nitrate concentrations below 50 ppm whenever possible.

Photothermal conditioning protocols established at MAP (Yanes-Roca and Main, 2012) were used to condition the fish. Once adult common snook were harvested, they were placed in tank 20-2 where they underwent a quarantine phase during which the photothermal cycle was maintained at 28°C and 15 hr light: 9 hr dark. After the quarantine was completed, the fish were transitioned to their maturation temperature of 30°C and held there until 11 March 2014. At this time, they were transitioned into their winter/resting phase of 22°C and 11 hr light: 13 hr dark. Following a two-month resting cycle, the snook were brought up to spring conditions for a month (26°C, 13 hr light: 11 hr dark) and then moved to summer/spawning conditions on 10 June 2014 (Appendix, Figure 2).

The snook broodstock were fed a diet of fresh thread herring and shrimp to more closely match the fatty acid profile of eggs produced by wild snook (Yanes et al., 2009). On scheduled feeding days, food was prepared and divided into at least two feedings per day. During feeding events, observations were made on general fish condition and feeding.

To sample fish, two gates made from plastic mesh secured to a polyvinyl chloride (PVC) pipe frame were positioned in a tank to corral all of the fish in a small wedge. Fish were moved individually from the wedge using a soft mesh net and were placed into a floating oval polyvinyl tank of seawater where they were anesthetized using Tricaine methanesulphonate (MS-222™) at 300 ppm, which was pH buffered with 600 ppm sodium bicarbonate. The fish were scanned to obtain their individual passive integrated tag (PIT) number, weighed (kg), measured for fork length (mm), and evaluated for gonadal maturation (Neidig et al., 2000; Rhody et al., 2013). To assess male snook maturation, gentle pressure was applied lengthwise to the abdomen below the testes to observe if milt was flowing. If no milt was obtained, a cannulation biopsy was performed, using a 5 French gauge system (fr) premature infant feeding tube, 1.0 mm inside Ø on the 5fr and 2.7 mm on the 8fr. to check for milt and to determine if the male had sex shifted to a female. No hormone therapy was used for males.

To assess female oocyte maturation, a cannulation biopsy was performed with an 8fr premature infant silastic feeding tube. An oocyte sample was used to assess the stage and step of maturation using light microscopy (Neidig et al., 2000; Rhody et al., 2013). If females were in late secondary growth (SGl) or were secondary growth full grown (SGfg), they were implanted with a dose of 25µg/kg Gonadotropin-releasing hormone analog (GnRHa) and were returned to the tank. Volitional spawns were approximately 24hr post implantation and two to three spawns were generally observed.

iii. Results and Discussion – The initial sampling to assess the size and sex of common snook fish occurred on 31 July 2013. After sampling and redistribution of some of these fish to other systems, there were 40 snook broodstock maintained for maturation and spawning production (Tank 20-2). Of those fish, 27 were male, 13
females, and one fish of unknown sex. One mortality of a snook of unknown sex occurred on 25 August 2013; however, the small size of this fish was indicative of being a male snook. It appeared that a larger snook had attempted to consume the fish. The 39 fish remaining in the population represented a ratio of $2\delta:1\varphi$. On 15 August 2013, water temperature was increased to summer/spawning temperature conditions ($30 \pm 1^\circ C$) and continued light conditions conducive to spawning. The broodstock were maintained under spawning photothermal conditions though 11 March 2014 to allow 4 sampling/spawning events.

The first sampling to assess reproductive condition was on 17 October 2013. Though we started with 39 common snook adults on 31 July 2014, there were 32 fish at the time of sampling. The loss of six fish was attributed to cannibalism. A few days following the sampling effort, vertebra and jawbones larger than the herring fed to the fish were found in our tank system filter baskets. Cannibalism of broodstock size fish had not been encountered previously at our facility. We do not believe that this behavior was caused by under feeding, because the fish were provided with 4.0% of their biomass in feed daily. This rate was much higher than our usual snook broodstock feeding, which was an average of 2.5% of their biomass every other day. The losses were all smaller males, shifting the sex ratio from our target $2\delta:1\varphi$. At sampling, one male was moved to tank 15-2 to replace a male mortality in that tank, leaving a population of 15 males and 17 females.

At the 17 October 2013 sampling event, 3 of 17 females were identified as mature and implanted. Though our goal was to have a higher ratio of mature females in this population of fish, it was not unexpected from a group of new broodstock. Past snook broodstock populations at MAP have taken up to a year to acclimate to the point of becoming reproductively viable. From this event, two spawns were collected on 18 and 19 October and are presented in Appendix, Table 1, resulting in 688,500 eggs collected with a mean fertilization rate of 64.4% and a mean hatch rate of 47.9%. Fertilized eggs from the first spawn were used to complete a larval photoperiod experiment (See Deliverable 4).

On 8 November 2013, one fish was found at the surface of tank 20-2, unable to regulate its swim bladder. Attempts were made to capture the fish to deflate the swim bladder, but it could not be caught. Within 24 hrs the fish was at the bottom of the tank, unable to right itself and was lethargic. The fish was captured, euthanized, and a necropsy was performed that revealed an over inflated swim bladder and a fishhook that had passed through the gut and was lodged in the peritoneum. The MAP staff was already in contact with our aquatic veterinarian, Roy Yanong, D.V.M., University of Florida, Tropical Aquaculture Laboratory, concerning mortalities that had occurred with other common snook at MAP with similar symptoms; he was contacted concerning this mortality and a request for recommendations was made. R. Yanong, D.V.M. stated that these were symptoms possibly resulting from something mechanical in nature (e.g. super saturation of nitrogen) and was not a possible pathogen.
It was found that system 20-2 had nitrogen saturation levels above 100% and steps were immediately taken to correct the problem and eliminate nitrogen super saturation as a possible cause of the mortalities. As a cautionary measure, in case a possible pathogen or parasite caused the mortalities, an even stricter quarantine policy was followed. The quarantine included limited entry to the rooms by only two staff members who were required to wear a lab coat upon entry that was kept in the room. In addition, routine practices, such as thoroughly dipping the YSI meter probe and cable in dilute bleach and then rinsing with a freshwater dip, and using hand sanitizer and footbaths before entering or exiting were followed.

The second sampling of this group of broodstock occurred on 5 December 2013. At the sampling, 3 of the 15 females had SG1 oocytes, indicating they were mature to implant. After implantation, two spawning events occurred on 6 and 7 December (Appendix, Table 1). A total of 1,807,940 eggs were collected, with a mean fertilization rate of 20.4% and no hatching. There is no definitive explanation as to why these spawning events provided poor results. Water quality parameter ranges were within targeted acceptable ranges including dissolved oxygen ≥5 and ≤8, pH ≥7.5 and ≤8.4, salinity 35±1.0 ppt, and temperature 30±1.0°C. In addition, these were proven systems for spawning broodstock. There were several possible reasons for a lack of male contributions, including that the fish were too far out of phase, they were not cycled long enough to break the infradian rhythm, or the cause of mortalities in November 2013 contributed to a reproductive dysfunction. Lack of male contributions was unlikely, even though this tank did not hold our target sex ratio of 2♂: 1♀, of the 15 males, only two did not express milt. It should also be noted that even though our ratio of males to females is not ideal, we maintained a 1:1 ratio from 2006-2010 with many successful spawning events. Our two predominate theories for the poor spawn events were that the fish were too far out of phase (6 months from peak) and/or the fish were not held in captivity long enough to alter their infradian rhythm. Common snook are primarily summer spawning fish, and we emulate these spawning conditions, but their infradian rhythm may play a large role in their reproductive success. This attempted spawn was the furthest out of phase we have ever attempted. Further studies are needed to observe how far out of phase common snook can be maintained to be reproductively viable, and to assess if wild snook in captivity can be manipulated enough to cause an artificial infradian rhythm.

The third sampling event was on 16 January 2014. No broodstock mortalities occurred between this sampling and the previous sampling in December 2013. The tank population remained at 32 total fish (15♂ and 17♀). At the sampling, 11 of the 17 females were mature and implanted, which was promising since only three of the females from the sampling in December 2013 were found to have oocytes mature enough to be implanted to produce spawns. Two females were observed with oocytes maturing past SG into oocyte maturation (OM), and identified as oocyte maturation eccentric germinal vesicle (OMegv). From this event two subsequent spawns were collected on 17 and 18 January (Appendix, Table 1). The total number of eggs collected from both spawns was 2,250,700, with a mean fertilization rate of 51% and mean hatch rate of 82.4%. Fertilized eggs were hatched and used to stock microcosms for genotyping (See
Deliverable 3) and to stock a larval temperature experiment (See Deliverable 4).

On 1 February 2014, one fish in tank 20-2 was found in the morning swimming erratically at the surface exhibiting obvious buoyancy control issues. It was removed and euthanized with MS-222 (300ppm) buffered with sodium bicarbonate. Tissue samples including eye, brain, heart, anterior kidney, posterior kidney, swim bladder, gill with arch, stomach, liver, intestine, spleen, were excised and prepared for histology and virology evaluations, as instructed by R. Yanong, D.V.M. The only remarkable observation made during our on-site necropsy was that the fish had an over inflated swim bladder. As stated previously, we were in constant contact with R. Yanong, D.V.M. concerning mortalities of common snook at MAP exhibiting similar symptoms throughout the grant period. On 23 January 2014, a telephone conference call with MAP staff, R. Yanong, D.V.M. and six other aquatic veterinarians was made to discuss the mortality issue, including potential causes and solutions.

Two additional fish were found in tank 20-2 on 5 and 25 February 2014 exhibiting the same symptoms. These fish were removed from the system, euthanized, and a necropsy performed. Tissue samples (as described above) were removed and preserved for possible histology and virology testing. R. Yanong, D.V.M., again stressed that the symptoms appeared to be caused by something mechanical, and was not caused by a pathogen. Our focus at MAP had focused on eliminating super saturation of nitrogen as a possible cause of mortalities. Though the system did have nitrogen saturation above 100% in the past; steps were taken to resolve the problem. We did not have the equipment to continuously monitor saturation levels, which was recommended as a next step.

On 6 March 2014, the 20-2 broodstock were sampled again for maturation status, with 8 of the 14 females having SGfg oocytes. These fish were implanted and resulted in two spawning events on 7 and 8 March 2014 (Appendix, Table 1). The total number of eggs collected from both spawns was 427,539, with a mean fertilization rate of 68.6% and mean hatch rate of 55.1%. The spawn size was below expectation. Other than the unknown cause of the mortalities in this system, another possible explanation could be that March 2014 was our fourth spawn during this simulated summer maturation period. Previous results suggest that common snook could be reliably spawned three times during the summer maturation period, followed by a resting/winter phase. We have previously observed a lack of reproductive success when we sample more than three times before cycling fish out of reproductive phase. However, we made a decision to attempt fourth spawn during the grant period to carry out one more larval experiment before phase shifting the fish and allowing them to rest. The fertilized eggs were stocked for a larval feed experiment (see Deliverable 4).

On 8 March 2014, another snook in this system was observed displaying the same health problems. A necropsy was not performed on this fish because it presented during the time spawning was anticipated, and it was decided that it was in the best interest of obtaining spawns to not alter the behavior or stress the other fish by trying to capture the sick fish. This fish died and was removed on 10 March 2014.
From the initial 32 fish in this population, eight succumbed. These mortalities occurred from 9 November 2013 to 27 March 2014. As discussed previously, we have looked at possible system mechanical issues, as well as examination of premortem fish tissue samples using histology, bacteriology, and virology (Betanodavirus) to try to identify the underlying cause of mortalities. From analysis of these samples there has been no conclusive identification of a potential pathogen or mechanical problem. It does appear that whatever the cause, no fish mortalities occurred in the broodstock systems since 1 April 2014, which was two weeks following the shift into their winter phase.

On 14 May 2014, a second conference call was held between MAP staff, Chris Young (Grant Manager, Florida Fish and Wildlife Conservation Commission-Stock Enhancement Research Facility [FWC-SERF]), R. Yanong, D.V.M., and aquatic veterinarians from Sea World, Florida State Aquarium, the University of Florida, and MML to discuss our next course of action. Because of the negative results on every test performed, we focused on less common issues found to cause similar symptoms in finfish. After much discussion, a thiamine (B1) deficiency was identified as a potential problem that may of lead to the fish mortalities. Thiamine deficiencies have been observed in adult Baltic salmon (Amcoff et al., 1998), Coho salmon, steelhead, and lake trout (Brown et al., 2005; Fitzsimons et al., 2005). Symptoms for these fish were reported to be lethargy, problems with equilibrium, and in severe cases, death. To resolve this possible deficiency, a commercial thiamine supplement was purchased and fed to the fish by including it in the muscle of thread herring at 40mg/kg. This product will be used until we develop a more complete vitamin enrichment to include in the common snook diet.

iv. Minimum Level of Performance - We completed the four outlined tasks for this deliverable, which included:

• Broodstock will be maintained and reared using photothermal conditioning protocols for spawning trials;
• Broodstock will be sampled up to four times to determine state of maturation and to induce mature fish to spawn;
• Data related to spawning trials will be summarized and reported; and
• If fertilized eggs are produced, they will be provided for larval experimental trials.

Broodstock were maintained and reared under a photothermal conditioning regime and were successfully induced to spawn during each of the four sampling events. Fertilized eggs were provided for genetic work in Deliverable 3 and for larval experimental trials described in Deliverable 4.

2. Evaluate the effect of environmental conditioning associated with tidal shift to induce volitional spawning in mature captive adult snook broodstock.

i. Introduction - At MAP and in many aquaculture settings, hormone therapies are often used to stimulate spawns, but that method can be costly and requires repeated handling of broodstock. Many fishes require additional environmental cues either to complete gonadal maturation or stimulate mating and spawning in captivity (Mylonas et al., 2010). In captivity, some species spawn readily and some are synchronous with specific
environmental cues (e.g. moon phases, tidal shifts), while others species are not. Because there is a paucity of information, an integrated concept of the impact of environmental factors on the reproductive process of fishes has not yet emerged (Sundararaj, 1981). Nevertheless, it is known that fishes integrate their physiological functions with environmental cycles. We investigated the effect of mock tidal shifts combined with photothermal conditioning to mature fish to vitellogenesis and trigger volitional spawns. Our study goal was to achieve volitional spawns with captive broodstock using a combination of environmental cues to reduce cost and handling stress.

**ii. Methods** - Populations of snook broodstock were held in two (15-2 and 15-4) 28 m³ tanks, each equipped with recirculating filtration systems. Both tanks started with 15 fish with a sex ratio of 2♂: 1♀. The fish were cycled using photothermal protocols established at MAP (Main et al., 2007; Yanes-Roca and Main, 2012). The experimental broodstock populations in Tanks 15-2 and 15-4 were fed our “standard” diet of fresh frozen herring and shrimp, which has been shown to be sufficient for broodstock to mature and produce fertilized eggs. Water quality and chemistry (Appendix, Figure 3A, B, C and Figure 4A, B, C) measurements were taken as described in *Deliverable 1*.

These snook populations were designated for evaluating the effects of an artificial tidal shift as an environmental cue to induce maturation and volitional spawning. The tidal shift was produced by a flow direction change in the experimental tank (15-4). Controlling the directional flow there were two solenoid valves connected to the main recirculating line, with each valve controlling the flow through diffuser bars facing opposite directions (Appendix, Figures 5 and 6). Simulated tidal shifts were scheduled to occur every 11.5 hr, with a 30 min slack tide between each directional change. Photothermal conditioning was maintained for these systems as described in *Deliverable 1* (Appendix, Figure 2). While fish were exposed to photothermal conditions for maturation and spawning an egg collector was set inline on each system on 17 August 2013, and is checked twice daily (~0800 and ~1700) for eggs. If spawns were obtained, it was planned that the eggs would be assessed for volume, fertilization rate, and hatch rate following methods presented in Neidig et al., 2000.

The fish in systems 15-2 and 15-4, were scheduled to be evaluated four times for maturation. Because of a mortality event, only three samplings occurred. Sampling the fish in these systems was conducted using the same techniques as described in *Deliverable 1*, except for application of hormone therapy. To determine if there was a difference in female maturation between the fish in the two tanks we used the wet mount technique and histological preparations as described in Neidig et al., 2000 and Rhody et al., 2013.

**iii. Results and Discussion** – No volitional spawns were collected from either system. Early sampling results, discussed below, were promising, but it is believed that the unknown cause of the mortalities in these systems interfered with maturation and possible spawns.
Beginning on 15 August 2013, the broodstock systems were adjusted to spawning photothermal conditions; they were held under those conditions for the duration of the sampling timeline. The first sampling occurred on 16 October 2013. Both systems were sampled to determine the maturation status of each population. In the control tank, one female had oocytes in secondary growth (SG) and was staged at secondary growth full grown (SGfg), the other four females were in primary growth (PG), and were staged at primary growth perinucleolar (PGpn). In the experimental tank, two fish were immature with PGpn oocytes, and three fish presented with SGfg and oocyte maturation germinal vesicle migration (OMgvm) stages. These results were promising with two out of five fish having oocytes as advanced as OMgvm. At MAP, broodstock common snook have been observed in these late stages of maturation, but it has been uncommon. Over the past four years of evaluating oocyte stages of our broodstock, we have only found fish that were mature in two of 30 samplings, over four different populations and from over 40 different female common snook. Even though no fully volitional spawns occurred, the results of using flow in a conditioning tank could be beneficial in common snook maturation, and possibly lead to increased reproductive success from hormone therapies.

As observed in 20-2, snook mortalities expressing similar symptoms (buoyancy issues and lethargy, and as seen in the necropsies, an over inflated swim bladder) occurred in broodstock tank systems 15-2 and 15-4. The first mortality was on 15 October 2013 in 15-2, followed by two more on 24 and 31 October 2013. On 17 November 2013, a fourth snook mortality occurred in tank 15-4. Necropsies were performed on all of the fish, and the carcass of the fish that expired on 31 October 2013 was sent to R. Yanong, D.V.M. for diagnostics. In January 2014, the diagnostic team provided the same information as reported for the 20-2 mortalities; that the cause of the mortalities in these systems were most likely mechanical and possibly due to super saturation of nitrogen, because there were no positive results for the pathological tests that were run. The report by R. Yanong “Fish Disease Diagnostic Lab Diagnostic Summary for Common Snook Cases 13TAL250 (1 fish), 13TAL274 (1 fish), and blood sampling (5 fish) January 13, 2014” was provided to MML. Tissue samples from the fish on 17 November 2014 were sent for histology evaluation. For tank systems 15-2 and 15-4, steps were taken to alleviate nitrogen saturation levels above 100%, as they were in system 20-2. As a precautionary measure, in case mortalities could have been caused by a pathogen or parasite, strict quarantine procedures as described for tank 20-2 in Deliverable 1 were followed.

The second sampling of fish in these tanks was conducted on 4 December 2013 at which time no differences were observed among the fish in the control or treatment tanks. At this sampling, there were no fish in either treatment with oocytes staged further than early secondary growth (SGe), which was considered immature by our implant standards. It was expected that the fish would be in later stages of maturation, at least late secondary growth (SGl) and SGfg. The lack of an advance stage of maturation could be attributed to one of any number of factors, including that the fish being sampled were too far out of phase, their natural infradian rhythm, and/or the cause of the mortalities created reproductive dysfunction. Our theories were that the
fish were either sampled too far out of phase and/or they were not in captivity long enough to create an artificial infradian rhythm, if it is even possible. Further studies are needed to observe environmentally manipulated common snook broodstock sampled at different periods of the year to determine how far they can be phase shifted while obtaining successful spawning events. Other studies should include identifying whether an artificial infradian rhythm can be programmed in wild caught captive broodstock.

Following the second sampling we continued to experience fish mortalities in tanks 15-2 and 15-4, with similar symptoms. Both tanks started with 15 (10♂ and 5♀) fish at the beginning of the study. Before the final sampling in March 2014, our control tank (15-2) had decreased to 12 (8♂ and 4♀) fish, and our experimental system (15-4) included 6 (3♂ and 3♀) fish. As stated in Deliverable 1, symptomatic fish were euthanized and tissue samples were saved for histology and virology as requested by R. Yanong, D.V.M. These included brain and eye from seven fish for virology to examine them specifically for Betanodavirus; test results were negative for the virus in all of the fish. At this time, it was still the belief that the mortalities were caused by a mechanical problem and that super saturation was the most probable cause. Though steps were taken to alleviate nitrogen saturation levels above 100%, equipment was not available to monitor saturation levels continuously.

On 5 March 2014, both systems (15-2 and 15-4) were sampled for the third and last time to determine maturation of each population. In the control tank (15-2), only one female had oocytes in secondary growth (SG) and was staged at secondary growth full grown (SGfg), the other two were in primary growth (PG) and were staged at primary growth perinucleolar (PGpn). At that time, two small males were removed from the system due to poor health, and another fish succumbed two days post sampling. In the experimental tank (15-4), all fish were immature with PGpn oocytes.

After the last sampling, the fish were shifted into a resting phase for two months, and were brought into spring conditions as of 13 May 2014. We continued to lose fish following this final sampling. From the initial loss of fish on 15 October 2013 to 21 May 2014 we lost 9 fish in 15-2 and 10 from 15-4. There was a marked decline in mortalities after the fish were shifted into their winter phase, with only one fish lost during this time.

There were no volitional spawns observed in the fish held in the experimental system and based on biopsy evaluations their oocytes were regressed at the last sampling. Even with these results, we believe that this study has merit based on the initial sampling when fish were observed to have advanced oocytes in the experimental system, which was prior to any mortality events. Oocyte regression and lack of volitional spawning could be attributed to the condition that resulted in the loss of fish in these systems. The snook in the flow tank were affected the most with nine mortalities and six in the control, three of which occurred at or after the last sampling. It is our belief that the extenuating variables inhibited maturation.
As stated in *Deliverable 1*, during a phone conference on 14 May 2014, the veterinarian team agreed that a possible thiamine deficiency could have been the cause of fish mortalities. The veterinarian team and C. Young, FWC-SERF Grant Manager, agreed that it would be in our best interest to combine all common snook broodstock at MAP into one production based population. The 20-2 tank system was chosen because it was the largest of three broodstock tanks to accommodate the large biomass, and the fish in the system had the lowest mortality rate at 25.0%, compared to 50.5% in 15-2 and 66.7% in 15-4. The remaining broodstock were combined at MAP in tank room 20-2 on 21 May 2014, to be used as a single group for production. Consolidating these fish would allow MAP staff to focus on one population of broodstock in hopes of identifying the cause of the mortalities, reduce possible contamination with other systems, and provide us with an opportunity to thoroughly clean the other two systems for rebuilding the spawning populations of common snook at MAP.

**iv. Minimum Level of Performance** - We completed the four outlined tasks for this deliverable, except that three samplings for maturation status were performed instead of four as described above:

- Broodstock will be maintained and reared using photothermal and with or without tidal conditioning protocols;
- Broodstock will be sampled up to four times to determine state of maturation and egg collectors will be monitored for volitional spawning;
- Data related to spawning trials will be summarized and reported; and
- If fertilized eggs are produced, they will be provided for larval trials.

Broodstock were maintained and reared under a photothermal conditioning regime with and without tidal conditioning protocols, the fish were sampled three times to determine state of maturation with mature females being observed in the Quarter 2. These results were promising with two out of five fish having oocytes as advanced as OMgvm, although no eggs were found in the egg collectors, which were monitored through the summer/maturation period. The trial was terminated because of fish mortalities.

**3. Use a molecular based approach to improve broodstock management strategies and provide a better understanding of captive common snook broodstock spawning characteristics.**

**i. Introduction** – In the present study, a molecular based assessment was used to investigate mating patterns, reproductive success and spawning periodicity of captive common snook broodstock. The data is intended to provide a better understanding of individual spawning performance in captive common snook held under controlled environmental conditions.

**ii. Methods**

*Larval sampling* - Larvae from five of the eight mass spawning events were genotyped during the study (Appendix, Table 2). Broodstock typically spawned during early to late evening where a spawning event or ‘spawn’ was defined as the sum of eggs or offspring.
produced during a single evening. Following each nightly spawning event, eggs were transferred from the broodstock tank to 100 L hatching tanks. At 4 - 6 hr post fertilization (blastula stage), aeration was removed and non-viable (sinking) eggs were discarded. At 17 - 20 hr post fertilization, approximately 250 newly hatched larvae (volumetrically measured) were stocked into individual microcosms. Accuracy of initial stocking ranged from 221 to 254 larvae per microcosm. The microcosms (25 for each spawn), made of PVC (2.6 cm diameter), were held in a shallow rectangular recirculating raceway tank system equipped with ultraviolet sterilization. The base of each microcosm was covered with a 200 µm nylon mesh to prevent the escape of larvae while allowing circulation of the water. Temperature in the raceway tank was maintained at 28 ± 1 °C.

To assess daily larval survival for each spawn, the total number of live larvae from each of five microcosms were counted on 1, 2 and 3 days post-hatch (dph) (total of 15). An additional 150, 3 dph snook larvae were randomly sampled from the remaining ten microcosms (20 larvae per microcosm) and individually stored in 95% ethanol until they could be genotyped.

**DNA extraction** – Genomic DNA was extracted from all snook broodstock (fin clip biopsy) within the three spawning tanks and approximately 1,000 3 dph snook larvae (whole animal) using the PureGene DNA Extraction kit (Qiagen, USA) according to the manufacturer’s instructions. Each sample was digested with 300 µl of cell lysis buffer, 2 µl of proteinase K (20 mg/µl), and incubated overnight at 55°C. To increase DNA yield, 5 µl of glycogen (5 mg/ml) was added to the precipitation step and the DNA resuspended with 30 µl of hydration solution. DNA was quantified using a Nanodrop 1000 Spectrophotometer (Thermo Scientific, USA). Adults and larvae yielded an average of 500 ng/µl and 10 ng/µl of pure DNA respectively. All samples were stored at 4°C prior to PCR amplification.

**DNA microsatellites and PCR amplification** – Eight polymorphic microsatellite markers, Cun01, Cun08, Cun19, Cun10A, Cun18, Cun11, Cun14 and Cun16 (Seyoum et al., 2005) were assayed in three robust PCR multiplexes (detailed in Appendix, Table 2). Each 12.5 µl PCR reaction consisted of 0.3 U of GoTaq (Promega, Madison, WI, USA), 2.5 µl 5 x GoTaq Buffer, 0.2 mM each dNTP, 3 mM MgCl₂, 1.25 mg/ml BSA, 0.8 µM each primer, and 5 to 20 ng DNA template (150 ng/µl for adults). Thermal cycling parameters for all amplifications were: 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C 30 s, and a final extension of 7 min at 72°C. PCR reaction products were stored at 4°C until genotyped.

**Genotyping and parentage analyses** – Each PCR multiplex was screened with 1 µl of each PCR product being added to 12 µl Hi-Di formamide containing 0.86 µl Home Made ROX DNA size standard (De Woody et al., 2004). Microsatellite alleles were detected and sized on an ABI 3130xl genetic analyzer (Applied Biosystems, Carlsbad, CA, USA) and scored with GeneMapper v4.0 (Applied Biosystems, Carlsbad, CA, USA). The genotyping data were interpreted using the exclusion-based program Probmax (Danzmann, 1997).
iii. Results and Discussion

Genotyping – Of 1,000 offspring that were initially screened for 8 loci, complete genetic profiles were obtained for 936 individuals. Of these 78% assigned to at least one family under the stringent exclusion model (i.e. assuming no genotyping error at all) – with 22% not assigning to any expected family. Using a more realistic model for practical genotyping, i.e. allowing up to one allele mismatch across the 8 loci composite genotype per progeny, 92% of individuals were assigned to families; 85% unambiguously to a single family. Thus, actual assignment rates were in line with predicted expectations.

Spawn performance - From October 2013 to March 2014, larvae were genotyped from five of eight spawning events in tank 20-2. Successive spawning over a maximum of two consecutive days was observed in three female and two male broodstock with five female and nine males contributing in both years 2013 and 2014 (Appendix, Table 3). Overall, spawning performance was highly variable in terms of the total number of eggs produced (ranged from 196,633 to 1,346,300 eggs/spawn), fertilization (from 11.4 to 82.7%) and hatch rate (from 48.9 to 82.7%) (Appendix, Table 1). The mean number of eggs produced per spawn was 646,835 ± 152,662 (SEM). Three day larval survival ranged from approximately 71.0 to 92.9% (Appendix, Table 1).

Parental contribution – Offspring were identified from four (25%) of the total sixteen female broodstock in 2013 and from two to five (20 to 36%) in 2014 (Appendix, Table 3). Approximately 7.5% of males had a detectable contribution in 2013 whereas, 26 to 58% contributed in 2014 (Appendix, Table 3). Female contribution ranged from 19 to 38% throughout the study period. Overall, parental contributions were highly skewed as seen in previous studies (Rhody et al., 2014). A single dam was responsible for 73.0% of the offspring (146 out of 200 sampled) accounted for in 2013 (Appendix, Table 4a). Similarly, in 2014, a total of 90.6% (725 out of 800 sampled) were assigned to three dams. Neither of the two females that were contributors in year one contributed in year two. Patterns of parentage assignment were similar among male broodstock. A single male sired up to 82.5% of assigned fry in year one (165 out of 200) and the same male sired 63.5% (508 out of 800) in year two (Appendix, Table 4b).

iv. Minimum Level of Performance - We completed the two outlined tasks for this deliverable:

- Obtain samples for DNA analysis
- Genotype adult and larval samples to determine parental contribution for individual broodstock

Samples were collected for DNA analysis from the January and March 2014 spawns, adults and larvae were genotyped. The data set was compiled and is presented in this report and the following manuscript resulted from this work: Rhody, N.R., C. Puchulutegui, J.B. Taggart, K.L. Main, and H. Migaud. 2014. Parental contribution and spawning performance in captive common snook *Centropomus undecimalis* broodstock. Aquaculture, 432, 144-153.
4. Investigate the effects of photoperiod, different live foods, and probiotics on survival and growth in common snook larvae.

i. Introduction – Mass production of common snook through the post-larval stage remains a major bottleneck in aquaculture technology for snook (Yanes-Roca et al., 2012). Rearing constraints include the availability of high-quality eggs (high deformity rates, limited yolk reserves, early yolk absorption, poor fertilization and hatch rates); inconsistent larval survival due to difficulties in transitioning from endogenous to exogenous feeding and from live food to micro-diets; availability of a diverse array of nutritious live foods; and an incomplete understanding of optimal environmental conditions for snook larvae. Larval rearing research at MAP during this grant period has focused on studies to investigate live lighting regimes, prey feeding strategies, and probiotics on improved larval growth and survival.

4.1 Optimization of larval growth and survival to 15 dph using three photoperiod regimes.

i. Introduction – It is essential to identify the optimal environmental conditions required to improve larval fish survival during the incubation phase (Blaxter, 1969). The effect of photoperiod on larval survival is one of these factors. Photoperiod has been observed to influence larval development and survival in a wide range of fish species (Barahona-Fernandes, 1979; Blaxter, 1980, 1986, 1988; Boeuf and Le Bail, 1999; Duray and Kohno, 1988), though no studies have been reported for common snook.

At MAP, focus was placed on evaluating if an extended period of light might help to overcome the first feeding bottleneck in common snook larval culture. This was the second experiment testing the influence of photoperiod, but for a longer period of time. The previous experiment was conducted with larvae to 8 dph as compared to this experiment, which evaluated larvae to 15 dph.

ii. Specific Project Deliverables and Methods - The experiment was conducted in 100 L conical black tanks at a stable temperature of 28°C and a salinity of 35 ppt. Each tank was stocked with 55 larvae per L. Three rows of four tanks were used and each row had its own water recirculation system and light regime. All tanks were provided with mild aeration and the larvae were fed enriched rotifers commencing on 2 dph. The trial duration was 15 days, where three photoperiods were tested (12 hr light/12 hr dark, 18 hr light/6 dark and 24 hr light). Sampling of 50 larvae per treatment took place on 3, 5, 10 and 15 dph. Total length (TL) and myomere height was recorded for each individual in the sample and from 100 larvae that survived to 15 dph. Analysis of variance (ANOVA) single factor was used to compare differences among experimental treatment effects for growth parameters and overall survival. Data normality was tested using the Shapiro and Wilk test (Shapiro and Wilk, 1965), all data was normal.

iii. Results and Discussion - In the photoperiod trial, there was a significant difference between the light treatments and their effect on growth performance with the larval held to 15 dph (Appendix, Figure 7). At 3, 5, and 10 dph, there was a no significant difference among treatments. There was no significant difference in TL between the 12, 18 and 24 hr treatments (Appendix, Figure 7). Mean myomere heights were not significantly
different among treatments at 3 dph, although at 5 and 8 dph a significant difference was observed between the 12/12 treatment and the other two treatments; no significant difference in myomere height was found between the 18 hr and the 24 hr light treatments.

In terms of survival, after 15 dph the larvae exposed to a 12/12 photoperiod had an average survival rate per tank of 1.78%; 2.39% in the 18/6 photoperiod regime and 9.54% for those exposed to continuous light (24 hr) (Appendix, Figure 8). A treatment significant difference was found (p <0.05). A multiple comparison post-hoc Duncan procedure revealed that the 24 hr treatment was significantly different from the 12 and 18 hr treatments (p <0.05), but no differences were detected between the 12 hr and 18 hr treatments. No significant difference between the 12 hr and the 18 hr treatment was attributed to the complete loss of larvae in Tank A, from unknown circumstances. The lack of survival in Tank A greatly affected the standard deviation and significance. Overall, based on growth and survival results, larvae exposed to the 24 hr light treatment performed best, followed by the 18/6 photoperiod regime.

4.2 Optimization of larval growth and survival using live feed.

i. Introduction – One of the key restrictions in larval rearing is first feeding at early stages of development. This is a major bottleneck for larval culture, due primarily to their small size and often poorly developed digestive system (Person Le Ruyet et al., 1993). The suitability of copepods as live prey for marine fish larvae is now well established, but their use in aquaculture remains sporadic. Although rotifers are of lower nutritional value, the relative ease of production of rotifers (Brachionus spp.) and Artemia nauplii continues to ensure their predominance as a live feed for larval rearing. Studies have highlighted differences in the levels and ratios of fatty acids, lipid classes, and pigments between copepods and traditional live prey used in hatcheries. Such differences are important for fish larval nutrition. The consequences of poor nutrition during fish larval development may be obvious, for example deformities or malpigmentation, but often the effects may be obscure, as in effects on temperature tolerance or growth during later life stages (Støttrup, 2000). Copepods are also valued as a live feed in aquaculture applications due to their small body size (e.g. 40-120 µm). Rotifers cultured at MAP were approximately 130 to 180 µm in length, and these are still slightly larger than what the average snook larvae can capture during the early rearing stages (Yanes et al., 2012). Therefore, the goal of this study was to compare experimental treatments of copepods and rotifers on growth and survival of early-stage common snook larvae.

ii. Material and Methods -Three experimental systems were used to run larval feed trials to a target of 15 dph. These included:

1) Small Experimental - Consisted of 100 L conical black polyvinyl tanks maintained at a stable temperature of 28°C and a salinity of 35 ppt. Each tank was stocked with 100 larvae per L. Three rows of four tanks (A – L) were used and each row of tanks had its own recirculation system with a 24 h light regime maintained at 1000 LUX. Larvae in each tank were fed starting at 2 dph.

Both treatments consisted of six tanks. Treatment 1 was fed copepods (Parvocalanus sp.) from 2 to 7 dph, after 7 dph the snook larvae were weaned onto enriched rotifers.
Size range was 40 to 120 µm and feeding densities used varied from 2 to 10 copepods per mL. Copepods for this trial were provided by Erik Stenn, AlgaGen®, at no cost (other than shipping). Treatment 2 was fed enriched rotifers throughout the trial using the same feeding density as in Treatment 1.

2) **Beaker Experimental** - The same methodology as in the small experimental system but instead of 100 L tanks, 1 L glass beakers were used with light aeration.

3) **Microcosm Experimental** - The last ”system” used was a set of 12 (6 copepod treatment reps, 6 rotifer treatment reps) small 2.54 cm open PVC pipes with 500 µm mesh bottoms to keep larvae inside. With a volume of 250 mL, the microcosms were maintained in a water table with continuous gentle aeration. The same methods described for the previous two systems were followed, in terms of water quality, photoperiod, feeding regime and prey density. At 7 dph larvae were harvested from each microcosm to collect growth and survival data.

### iii. Results and Discussion -

1) **Small Experimental** - At day two after stocking, only 5 tanks contained live larvae, which was attributed to low oxygen levels due to a leak in the oxygen supply hose. Of the 5 tanks remaining, one of the tanks (J) had less than 20 larvae. The other four tanks had more larvae, although numbers were still lower than expected. Due to the low survival and the loss of 7 tanks from day 2, no comparison between treatments was possible. At day 7, larvae in the 4 remaining tanks were harvested to collect survival and growth data.

Survival data was as follows:

- Tank A, B, C, D, E, H, G = 0 larvae,
- Tank F = 23 larvae, I= 38, J= 157, K=173 and L= 251

Average survival between all tanks was 2.6% with tank (L) having the highest survival of 5% (Appendix, Table 6). Larvae TL ranged from 2.6 mm to 3.5 mm with an average length of 2.9 mm. A total of 95% of the larvae (25 per tank) had copepods present in their guts.

2) **Beaker Trial** – Due to the low amount of eggs produced and their quality, this system was not used, since there were not sufficient larvae to adequately stock the number of necessary beakers needed to run the trial.

4) **Microcosm Trial** - This trial was run to 7 dph, when larvae were enumerated and measured. At termination there were live larvae in 7 microcosms (4 from those provided copepods [COPE] and 3 from those exposed to rotifers [ROT]) (Appendix, Table 5). Overall, the larvae in the rotifer treatment had 20% survival versus 46.6% survival in the copepod treatment. This was a significant difference in survival (p < 0.005). In terms of TL, there was a significant difference (p < 0.005) between larvae in each treatment, 2.81 mm and 3.1 mm, in the rotifer and copepod treatments, respectively.
The results for this trial should be considered to be preliminary, because the trial duration was for a short period of time (7 days) and it was performed with a small number of larvae. Further trials are needed, preferably in larger systems where water quality would not as easily influence survival, to obtain more reliable results.

4.3. Effects of probiotics on survival and growth in larval common snook.

i. Introduction – Several studies on probiotics have been published during the last decade. However, the methodological and ethical limitations of animal studies make it difficult to understand the mechanisms of action of probiotics, and only partial explanations are available. Nevertheless, some possible benefits linked to the administering of probiotics have been suggested as: (i) competitive exclusion of pathogenic bacteria, (ii) source of nutrients and enzymatic contribution to digestion, (iii) direct uptake of dissolved organic material mediated by bacteria; and others are still being investigated as: (iv) enhancement of the immune response against pathogenic microorganisms and antiviral effects.

Material and Methods – A seven-day trial was conducted to investigate the effects of probiotics (Bacillus sp.) on survival and growth of common snook larvae. During the trial, 200 larvae were stocked into beakers each containing 1 L of sterile seawater. Trials included a control (n = 20) and treatment (n = 20). The treatment consisted of the addition of activated Bacillus added at a concentration of 1 g / 100 L prior to inoculation with larvae (day 0) and again on days 2, 4 and 6 of the trial. Samples were taken daily to quantify Vibrio. To do so, 100µL of beaker water was sampled and dispensed into a 9.9 mL dilution buffer tube (10^2 dilution) and the tube was shaken vigorously for a minimum of 15 seconds. A 100µL of this solution was dispensed (10^3 dilution) onto a thiosulfate-citrate-bile salts-sucrose agar (TCBS) plate to isolate Vibrio spp. Preparation of the sample for culture included using a sterile bent glass rod to spread the liquid evenly around the plate (one rod per plate) and incubating the plate at 30°C for 24 hours. Colony counts (yellow or green) were used to determine the mean value. The product of this mean and the reciprocal of the plate’s dilution was the concentration of the Vibrio organisms in colony-forming units per milliliter.

Results and Discussion – No significant difference was shown between the treatment and the control in terms of survival and growth for common snook larvae. Irrespective of treatment all larvae reared in the trial did not survive past day 5. Because of the high variability in survival of common snook, we recommend that additional trials be conducted to evaluate the potential benefits of probiotics on snook survival.

iv. Minimum Level of Performance - We completed the three outlined tasks for this deliverable:

- Completion of at least one probiotic/no probiotic larval rearing trial (up to 7 dph)
- Completion of at least one lighting regime larval rearing trials (up to 10 dph)
- Completion of at least one live prey feeding strategy trial (up to 7 dph)

In the probiotic trial, no significant difference was observed between survival and growth of snook larvae up to 5 dph. It was recommend that to better assess the potential benefits
of probiotics that additional trials were needed. In the photoperiod trials, larvae exposed to the 24 hr light treatment had the best growth and survival, followed by the 18/6 photoperiod regime. The integration of copepods in larval snook diets was promising, though the results were considered to be preliminary.

5. Assess vulnerability of fingerling snook (40 dph) to lordosis resulting from exposure to water currents.

i. Introduction — Malformity of the vertebral column, in reared fish, decreases the quality of aquacultured fish by negatively affecting their external morphology, growth, and survival (Andrades et al., 1996; Kihara et al., 2002). Skeletal malformities can be attributed to nutritional deficiencies (Cahu et al., 2003, Fraser and De Nys, 2011, Izquierdo et al., 2010), genetics (Karahan et al., 2013, Afonso et al. 2000, Gjerde et al., 2005, Kause et al., 2007), spawn quality (Cerdá et. al., 1994, Clagett-Dame and DeLuca, 2002; Fernández and Gisbert, 2011), and environmental conditions during rearing (Castro et al., 2008, Battaglene et al. 1994, Andrades et al. 1996, Divanach et al. 1997, Afonso et al. 2000, Kranenbrag et al. 2005, Sfakianakis et al. 2006). The effects of environmental conditions on malformities in reared fish can be the result of knowledge gaps and inappropriate rearing conditions during these early life stages.

Earlier work at MAP has shown that spinal malformity can be induced by subjecting juvenile snook to water currents (Brennan et al., unpublished data). This work showed that 70 dph snook juveniles exposed to continuous water currents (at about 100-110 mm/s) for 20 days exhibited a 38% spinal malformation rate compared to 6% in control non-current tanks and by study day 56 the malformation incidence in the flow treatment tanks increased slightly to 42%.

The present study was performed to determine susceptibility of earlier-stage juvenile common snook to spinal malformities reared under experimental water flow rates. Snook are known to contain ossified vertebral tissue by 31 dph (Wittenrich et al., 2009), but because ossification is a gradual process and bones strengthen with increasing age, juvenile snook are thought to be most vulnerable to spinal malformation at the youngest stages. Furthermore, in the hatchery, when reared in high densities, snook are known to engage in schooling behavior with rapid swimming as early as 25 dph (Brennan et al., unpublished data) and this may induce spinal malformations, even in systems without obvious directional water flow. It is thought that this swimming behavior is motivated by cannibalism predation threat, which intensifies through the weaning-to-dry-diet stage. Therefore, an experiment was conducted at MAP using a younger stage of snook, to examine their susceptibility to skeletal malformation when exposed to different water velocities to identify appropriate rearing environments at this younger stage. The MML staff collaborated with the FWRI-SERF staff on the spinal malformity study. Collaborative work included the use of a digital water flow meter to measure actual in-tank water flow in the “flow” and “no flow” treatments at the MAP facility and discussions on study design.

ii. Specific Project Deliverables and Methods – Spinal malformation may be present in the production cohort due to a variety of ontogenetic developmental issues mentioned
above. Among these could include density effects, which can induce high speed swimming (or schooling) behavior. This is typically observed to begin around 25 dph in the 8-2 production tank system when snook are capable of cannibalistic predation. Therefore, a subsample of snook \((n=120, 14-16 \text{ mm TL})\) were removed from the production cohort-rearing tank and placed in a separate 38 L aquarium to serve as a time-control for the study where the high-speed swimming did not occur. The subsample time-control fish were maintained in a 38 L rectangular aquarium containing refuge and maintained with similar feed and recirculating supply water as the production cohort. At 40 dph the aquaria snook were moved to an isolated 900L circular fiberglass tank (1.54 m diameter x 60 cm deep) that was situated similarly to the control tanks of the primary water flow study (below) and maintained throughout the study.

Experimental Flow Study: At 40 dph, a subsample \((n=106)\) of snook, representing an unsorted sample from the 8-2B population, was harvested and preserved in 10\% buffered formalin for 48 h, then stored in 95\% ethanol for use in measuring the incidence of vertebral malformity at the onset of the study. On the same day, snook fry \((0.2-0.6 \text{ g wet weight, 30-40 mm TL})\) were harvested from the 8-2B production tank, of which 200 were stocked into each of eight 900L circular fiberglass tanks \((1.54 \text{ m diameter x 60 cm deep})\). Four of the tanks \((\text{flow treatment})\) were supplied with directional water flow via a PVC pipe \((5.1 \text{ cm [2"] x 50 cm long with 5 mm holes spaced every 10 cm})\) situated horizontally above the water surface to create an in-tank current effect. These tanks also contained a 36 cm diameter cylindrical spacer situated in the center of the tank to maintain snook in the treatment current, away from the center mounted stand pipe drain where water flows were less. The remaining four tanks \((\text{controls})\) received a similar water in-flow rate that cascaded directly onto the tank water surface and did not cause any obvious directional currents in the tank. Control tanks were also situated with plastic aquarium plants to provide additional cover and minimize cannibalism-induced schooling.

All tanks were fitted with automatic belt feeders and fish were fed daily with the same-pelleted feed and feed allotment schedule that was used in the production tanks. High salinity water was used to recirculate through the experimental system and water temperature was controlled by ambient air temperature. Daily measurements averaged 25\(^\circ\text{C}\) throughout the study. Dissolved oxygen was maintained at a minimum of 5 mg/l and was controlled by continuous aeration in each tank and supplemental oxygen when necessary. Water chemistry parameters \((\text{ammonia, nitrite, and nitrate})\) were tested twice weekly and water exchanges and system filter back flushing occurred as necessary based on water chemistry results \((\text{water changes occurred if: NH3-N} >0.5 \text{ mg/L; NO2-N} >0.5 \text{ mg/L; NO3-N} >30 \text{ mg/L})\). System water continuously re-circulated through a filtration system including a UV sterilizer, a biofilter, and a bead filter. All replicate treatment tanks received water inflow at a rate of approximately 30-60 L/min.

Water currents in each experimental tank were measured using a digital flow meter \((\text{Onset, OTT MF PRO, model IP167; loaned from FWC SERF staff})\), and measurements were taken at the surface and bottom at 4-8 locations \((\text{from a top view perspective})\) within each tank. Flow rates were adjusted so water currents in the treatment tanks were...
approximately 110 mm/s (about 2-4 body lengths/s) and control tanks had no obvious currents (0±12 mm/s). Water flow measurements were taken at study week 0, 1, and 8.

Visual examination of the study fish occurred at 1, 2, 3, 4 and 8 weeks after study onset. For examinations that occurred on weeks 1 and 2, weights, lengths and visual deformity estimates were taken from each of 25 randomly-selected juvenile snook from each experimental replicate tanks. The same procedure occurred at week 3 and 4, except that data were collected from 100 randomly selected snook from each tank. For each sampled fish, spinal malformities were categorized as lordotic, kyphotic, or scoliotic, and enumerated in an anterior-to-posterior order of occurrence, they were then returned to their respective experimental tank. On week eight, all fish from each tank (about 1,600 total fish) were harvested, anesthetized with MS-222 (90 mg/l) then individually weighed and examined for spinal deformities. Each fish was also photographed using a digital Cannon EOS Rebel© with a 35-70 mm zoom lens fixed at 50 mm. The camera was mounted to a standardized camera mount with standardized metric rulers, and the photographs were taken using a remote controlled trigger. Fish were photographed on their sides and were aligned along a mounted ruler on a standardized mount. The body lengths of the first 100 snook were measured manually (TL, SL) to calibrate the digitized lengths. All fish in the 25 dph control group were also harvested, weighed, visually examined for deformities, and individually photographed as described above.

The status of the snook from the above study was as follows:
- Severely deformed individuals (n=) from the 900 L tanks were euthanized and preserved for analysis of the spinal deformities with radiology and possibly mammography.
- Non-deformed and mildly deformed individuals from the 900L tanks (n=) were reallocated to the 8-1 system where the remainder of the production cohort continues to be maintained.
- The 25 dph control group continues to be maintained in a separate 900 L tank.

The status of the production cohort was as follows:
On 26 November 2013 the cohort maintained in 8-1was sorted by size category for the second time and “small” fish were reallocated into tank 8-1B. The “medium” fish were reallocated to 8-1A, and “large” fish were reallocated into a 900 L tank in the 5-1 system.

iii. Results and Discussion - 2013 Water Flow Study. The study was completed successfully as described above. A complete analysis of the results is in progress for publication in a scientific peer-reviewed journal. A summary of the experimental results follows:

Overall, after 64 days of flow treatment, juvenile snook held in the experimental system showed highly significant results. Juveniles held in the “flow” treatments showed about 130% increase in occurrence of spinal malformities (treatment mean= 81.8% ± 10.1 SD) compared to those held in the “no flow” (or control) experimental treatments (control
mean =34.5% ± 14.9 SD) (T-Test, P=0.001, DF=6, T=5.23). Actual mean in-tank water flow rates for “flow” treatment tanks were 111.8 mm/s while control “no flow” tanks averaged 1.2 mm/s and in-tank flow rates were highly correlated with the proportion of snook developing spinal malformities (Pearson’s correlation coefficient = 0.92, n=8 tanks).

Weekly measurements indicated the highest rate of increase in spinal malformities occurred in the flow treatment tanks during the study days 22-29, when mean malformity rates almost doubled from 35% - 59%. This sharp increase in observed spinal malformity rates might be in part due to increased detectability during the visual deformity checks. During the visual checks for malformity, the actual severity of the observed malformity was not measured but data were simply recorded as an occurrence of malformity. As the study progressed, however, the severity, or angle of the vertebral “buckling”, increased causing the detectability of a given spinal malformity to become more obvious. Therefore, actual quantification of the vulnerability of snook to spinal malformities is difficult because (1) initial damage is subtle and difficult to detect, and (2) continual exposure to the water flow treatment further exacerbated and magnified the spinal deformities. Effects of a given water flow treatment, scaled appropriately to body length and tail beat frequency, for a specific duration should be examined across ontogenetic developmental stages to more accurately determine actual stage-specific vulnerability to spinal malformity.

The final measurement of spinal malformity occurred on study day 64 (7 November 2013). It is important to note that at that time, mean malformity rates in the “flow” treatments were 82%, and this was approximately twice as high as the observed mean malformity rates in the 2011 study. The 2011 study started with 70 dph snook juveniles and after 56 days of flow treatment mean deformity rates were 42%. Also in the 2011 study, the largest experimental treatment effects occurred early on, within the first three weeks, but further increases in malformity rates did not change much after this (mean malformity rates in the flow treatment tanks at day 21 were 39% and only increased to 42% by day 56).

On study day 64 of the 2013 study, the 25 dph control group (that was removed from the production cohort held in 8-2 system and held in a 38 L aquarium, and later held in a “no flow” 900 L circular tank) was also examined for spinal malformities. Eighty-one juveniles represented this group, and no spinal malformities (0%) were detected. This is unlike the 40 dph experimental “no flow” control fish, which exhibited a mean deformity rate of 34%. This group primarily differed from the 25 dph control group by being held in the 8-2 production system from 25-40 dph and the observed malformity rates may have been caused during this earlier stage, possibly due the intensive schooling behavior observed in the 8-2 system.

At the time of this final report, digital measurements of all experimental snook have been collected. These data are being used to generate statistics on the morphometrics characteristic of specific spinal malformities, as presented in Loy et al., 2000, Sfakianakis et al., 2006, and Arechavala-Lopez et al., 2012. This work will also assist in
quantifying the severity of spinal malformities observed throughout the study. These results are being prepared for publication in a scientific manuscript.

iv. Minimum Level of Performance – We completed the two outlined tasks for this deliverable:

- Complete at least one larval production trial to produce 40 DAH larvae for deformity trial
- Conduct a trial to evaluate incidence of lordosis in 40 DAH snook that are and are not exposed to currents

Accomplishing these tasks makes the third task null “If 40 DAH lordosis trial cannot be completed, due to limited availability of 40 DAH snook, we will analyze data from an earlier trial and submit a manuscript for publication.” Results from this study provided valuable data for a publication in progress on reducing deformities.

6. Increase awareness of research results and assist the commission and its partners with strategic planning.
   i. Specific Project Deliverables and Methods – During the grant period MML scientists agreed to help increase awareness of research results by presenting at eight symposia, meetings, or workshops to inform the fishery and aquaculture scientific community, state and Federal fishery management agencies, conservation groups, and the community of our progress. To this effect, it was also agreed that manuscripts would be published in peer-reviewed scientific journals and copies provided with grant reports. In addition, MML would assist the Commission upon their request with strategic planning for the Commission’s marine enhancement program.

   ii. Minimum Level of Performance – We completed the outlined tasks for this deliverable:

- Presentations will be given at eight symposia, meetings or workshops
- Results from snook aquaculture research will be analyzed, written and submitted for publication
- MML scientists will participate in strategic planning marine enhancement meetings with FWC scientists

The minimum level of performance for this deliverable was exceeded, with a total of twelve presentations at national and international scientific conferences, symposia, meetings, or workshops of a required minimum of eight. An abstract and presentation given in February 2014 received two nationally recognized awards from the American Fisheries Society (AFS).

There were seven peer reviewed scientific publications (two aquaculture related and five pertaining to fisheries and stock enhancement) and one published abstract pertaining to aquaculture produced during this grant period. Several additional manuscripts are in progress by MAP staff. One publication was in collaboration with FWRI staff. Other accomplishments included one magazine article and six online media videos featuring common snook aquaculture. One strategic planning meeting for marine enhancement was conducted with L. Barbieri (FWRI) in May 2014. Presentation, publication and articles are included below.
Presentations - Scientific
Mote staff and/or MAP interns pursuing doctoral degrees were authors on twelve presentations at seven scientific conferences, symposia, and workshops (* indicates presenter). Presentation abstracts will be included in the Final Report.

At the conference P. Caldentey was the recipient of two prestigious awards, including the United States Aquaculture Society/American Fisheries Society-Fish Culture Section (USAS/AFS-FCS) Best Student Abstract Award and the Fish Culture Section of the American Fisheries Society Best Student Abstract Award.


Main, K.* Aquaculture Species Overview: Marine Fish. University of South Florida Graduate Class on Marine Aquaculture. Oral Presentation. 18 February 2014.

Main, K.* Sustaining Future Seafood and Fishery Resources through Aquaculture. University of South Florida Graduate Class on Marine Aquaculture. Presented as part of the Mote Special Lecture Series. Oral Presentation. 3 March 2014.


Publications – Scientific
During the grant period we produced six peer-reviewed manuscripts and one peer-reviewed published abstract. More specifically, four of the manuscripts published concerned stock enhancement and sea ranching, one of which was a collaborative paper with L. Barbieri (FWRI); and three manuscripts and one abstract resulted directly from common snook research conducted at MAP. K. Leber was one of seven editors of the Fourth International Symposium on Stock Enhancement and Sea Ranching Reviews published in Fisheries Science; and the Blankenship and Leber “Responsible Approach to Stock Enhancement” paper was included in the book “Foundations of Fisheries Science.”
Publication titles are included below, with highlighted names indicating MML staff or student interns.


**Publication – Magazine**

One magazine feature article was published.


**Online Media**

McCabe Productions with Captain Blair Wiggins featured six segments focused on snook aquaculture research at MAP and promotion of Project Snook on “YouTube”, at [www.projectsnook.com](http://www.projectsnook.com), and on the television show “Addictive Fishing”, seen on Florida’s Sun Sport Network, the nationwide Sportsman’s Channel, and on World Fishing Network throughout the U.S. and Canada.

**E. OTHER**

Chris Young, Grant Manager (FWRI-SERF) completed three MAP site visits. These visits were on 5 February 2014, to meet with K. Leber and K. Main to discuss progress toward completing grant objectives; 5 March 2014 to observe and assisted MAP staff during a snook broodstock sampling event; and on 14 May 2014 to participate in a phone conference with veterinarians and MAP staff concerning snook health issues, and to meet with MAP staff concerning grant plans for 2014-2015.

**F. LITERATURE CITED**


Kranenbarg, S., J.H. Waarsing, M. Muller, H. Weinans, and J.L. van Leeuwen, 2005. Lordotic vertebrae in sea bass Dicentrarchus labrax are adapted to increased loads. Journal of Biomechanics, 38, 1239-1246.


APPENDIX

Tables

Table 1. Common snook broodstock spawning performance from a single mass spawning tank (20-2) from October 2013–March 2014. Data presented represents number of eggs collected, fertilization rate, hatch rate and larval survival to three days. Larval survival is presented as mean ± SEM (n=3). Lower case letters represent spawns in which larvae were genotyped.

Table 2. Details of the ten polymorphic microsatellite markers used in the captive common snook broodstock spawning characteristics study.

Table 3. Relationship between the timing of hormonal implantation and total female spawn contribution for five mass spawning events observed in a single captive common snook broodstock population.

Table 4. Female (a) and male (b) spawn contribution observed from 2013 to 2014 in a single captive common snook broodstock population (Tank 20-2). Results were determined by exclusion-based parentage using 8 DNA microsatellites for genotyping individual larvae (n = 5) spawns.

Table 5. Survival and total lengths of common snook larvae from two live feed treatments (rotifers [ROT] versus copepods [COPE]) in microcosm trials.

Table 6. Percent survival of common snook larvae in the optimization of larval growth and survival trial using live feed, after harvest at 7 dph.

Figures

Figure 1. Water quality parameters for common snook held in Tank 20-2 from July 2013 to June 2014. Total ammonia nitrogen (A), nitrite (B) and nitrate (C) levels.

Figure 2. Simulated photo-thermal cycle used to mature and spawn captive common snook broodstock at Mote Aquaculture Research Park from July 2014 to May 2014. Imposed photo-thermal cycle used to mature and spawn captive broodstock including day length (light h/day) (-----) and water temperature (°C).

Figure 3. Water quality parameters for common snook held in Tank 15-2 from July 2013 to June 2014. Total ammonia nitrogen (A), nitrite (B) and nitrate (C) levels.

Figure 4. Water quality parameters for common snook held in Tank 15-4 from July 2013 to June 2014. Total nitrate nitrogen (A), nitrite (B) and nitrate (C) levels.
Figure 5. Diagram of 15-2 and 15-4 snook broodstock tanks.

Figure 6. Diagram of the control tank (15-2) and the flow shift device in the experimental system (15-4).

Figure 7. Effect of three photoperiod treatments (12, 18 and 24 hrs of light) on common snook length (SL) to 15 days post-hatch (dph).

Figure 8. Effect of three photoperiod treatments (12, 18 and 24 hrs of light) on common snook survival to 15 days post-hatch (dph).
Table 1. Common snook broodstock spawning performance from a single mass spawning tank (20-2). Data presented represents number of eggs collected, fertilization rate, hatch rate and three day larval survival. Larval survival is presented as mean ± SEM (n=3). Lower case letters represent spawns in which larvae were genotyped.

<table>
<thead>
<tr>
<th>Spawning Event (mm/dd/yyyy)</th>
<th>Spawn Type</th>
<th>Number of eggs collected</th>
<th>Fertilization Rate (%)</th>
<th>Hatch Rate (%)</th>
<th>Survival 1DPH (%)</th>
<th>Survival 2DPH (%)</th>
<th>Survival 3DPH (%)</th>
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<td>10/18/2013 a</td>
<td>Volitional</td>
<td>515,100</td>
<td>82.7</td>
<td>88.8</td>
<td>92.9 ± 1.1</td>
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<td>80.6 ± 1.1</td>
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<td>92.6 ± 2.0</td>
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<td>79.8 ± 1.3</td>
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Table 2. Details of the ten polymorphic microsatellite markers used in the present study.

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<tr>
<th>Locus</th>
<th>Primer sequence (5’ – 3’)</th>
<th>Annealing Temperature (°C)</th>
<th>Repeats</th>
<th>No. of Alleles</th>
<th>Allele Size Range</th>
<th>Multiplex</th>
<th>Primer Label</th>
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<tr>
<td></td>
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Table 3. Relationship between the timing of hormonal implantation and total female spawn contribution for five mass spawning events observed in a single captive common snook broodstock population.

<table>
<thead>
<tr>
<th>Spawning Event (mm/dd/yyyy)</th>
<th>Total No. Males and Females in Tank</th>
<th>No. of Males / Females Spawned</th>
<th>Total Male / Female Spawn Contribution (%)</th>
<th>No. Females Implanted ($n$)</th>
<th>No. Females Spawned ($n$)</th>
<th>Females Implanted (%)</th>
<th>Females Spawned (%)</th>
<th>No. Females Spawned 1 day post-implantation ($n$)</th>
<th>No. Females Spawned 2 days post-implantation ($n$)</th>
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<tr>
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<td>100.0</td>
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<td>81.8</td>
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<td>Mar 8, 2014</td>
<td>12 / 14</td>
<td>7 / 3</td>
<td>58 / 21</td>
<td>11</td>
<td>9</td>
<td>78.5</td>
<td>81.8</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>
Table 4. Female (a) and male (b) spawn contribution observed from 2013 to 2014 in a single captive common snook broodstock population (Tank 20-2). Results were determined by exclusion based parentage using 8 DNA microsatellites for genotyping individual larvae (n = 5) spawns.

A)

<table>
<thead>
<tr>
<th>Date</th>
<th>Oct 18, 2013</th>
<th>Jan 17, 2014</th>
<th>Jan 18, 2014</th>
<th>March 7, 2014</th>
<th>March 8, 2014</th>
<th>Total No. Offspring</th>
<th>Percentage (%)</th>
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B)

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<th>Jan 18, 2014</th>
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<th>March 8, 2014</th>
<th>Total No. Offspring</th>
<th>Percentage (%)</th>
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<tr>
<td><strong>No. of larvae sired by males</strong></td>
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<td>75</td>
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Table 5. Survival and total lengths of common snook larvae from two live feed treatments (rotifers [ROT] versus copepods [COPE]) in microcosm trials.

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<tr>
<th>Diet</th>
<th>Microcosm</th>
<th>Total larvae</th>
<th>Average length</th>
<th>% Survival</th>
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<td>ROT</td>
<td>D</td>
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<td>ROT</td>
<td>E</td>
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<tr>
<td>COPE</td>
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<td>3.12</td>
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Table 6. Percent survival of common snook larvae in the optimization of larval growth and survival trial using live feed, after harvest at 7 dph.

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<th>% Survival</th>
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<tr>
<td>K</td>
<td>3.46</td>
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<tr>
<td>L</td>
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</table>
Figure 1. Water quality parameters for common snook held in Tank 20-2 from July 2013 to June 2014. Total ammonia nitrogen (A), nitrite (B) and nitrate (C) levels.
Figure 2. Simulated photo-thermal cycle used to mature and spawn captive common snook broodstock at Mote Aquaculture Research Park from July 2014 to May 2014. Imposed photo-thermal cycle used to mature and spawn captive broodstock including day length (light h/day) (-----) and water temperature (°C).
Figure 3. Water quality parameters for common snook held in Tank 15-2 from July 2013 to June 2014. Total ammonia nitrogen (A), nitrite (B) and nitrate (C) levels.
Figure 4. Water quality parameters for common snook held in Tank 15-4 from July 2013 to June 2014. Total nitrate nitrogen (A), nitrite (B) and nitrate (C) levels.
Figure 5. Diagram of 15-2 and 15-4 snook broodstock tanks.

Figure 6. Diagram of the control tank (15-2) and the flow shift device in the experimental system (15-4).
Figure 7. Effect of three photoperiod treatments (12, 18 and 24 hrs of light) on common snook length (SL) to 15 days post-hatch (dph). (*Error bars standard error [SE]).

Figure 8. Effect of three photoperiod treatments (12, 18 and 24 hrs of light) on common snook survival to 15 days post-hatch (dph).