Semi-Annual Progress Report
To National Marine Fisheries Service, NOAA, DoC
Reporting on Activities in the Unallied Science Project Titled:
Science Consortium for Ocean Replenishment and Enhancement (SCORE)
Multi-Year Award Number NA4NMF4720434

For the period 1 July through 31 December 2004

Submitted by:

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A. Brief Project Overview:

The Science Consortium For Ocean Replenishment And Enhancement (SCORE) is a science-based approach to stocking hatchery-reared marine organisms to help rebuild depleted marine fisheries (marine fisheries enhancement). SCORE scientists are conducting research to resolve critical uncertainties about the effectiveness of culture-based marine enhancement as a fishery management tool. It is anticipated that significant progress will be made by SCORE scientists, leading to greater and greater success from marine enhancement programs in the U.S.

As scientific gains are made in understanding the potential, SCORE scientists are partnering with NMFS and regional fishery-management agencies to develop policy and apply fishery-enhancement science to rebuilding depleted coastal stocks. Linkages with local fishing communities provide the cadre of citizens needed to support and expand enhancement as a fishery management strategy. Much of the enhancement technology developed here will be supported by funds generated by contributions and license fees paid by stakeholders and user groups. To fully embrace and use the marine enhancement concept, demonstrated success stories are needed in a few key states. SCORE research is planned and coordinated to achieve such successes. Built around the principles of a responsible approach to marine stock enhancement (Blankenship and Leber; and see Leber, 2002), SCORE scientists are conducting key experiments to resolve critical uncertainties about how to control the biological, ecological, and economic effectiveness of marine fisheries enhancement.

SCORE is an R&D initiative conducted by a consortium of national partners. It is a powerful alliance of scientists and fishery managers currently working in the field of marine stock enhancement in the U.S.A., which encourages improved utilization of their expertise and resources. Bringing these scientists and managers together through SCORE allows synergisms to develop that would not occur otherwise.

Multi-Year Contract Period and Relation to this Reporting Period

This Multi-Year contract commenced on July 1, 2004 for the 5-year period ending June 30, 2009. The funding period for this phase of the Multi-Year contract is July 1, 2004 through June 30, 2005. This interim report covers progress made during the period July 1, 2004 through December 31, 2004.

B. Project Accomplishments:

Mote Marine Laboratory Progress – July through December 2004

Aquaculture Research to Develop Rearing Technology for SCORE Species:

Wild Strip-Spawning Efforts with Common Snook

The 2004 snook spawning season to produce juvenile snook for stock enhancement research began in late June. These spawning efforts were conducted to provide fertilized eggs to Florida Fish and Wildlife Conservation Commission (FWC) biologists at their hatchery facility in Port Manatee, FL. The FWC is a collaborator on this project. Of the nine wild strip-spawning attempts completed in July and August 2004 for Mote's snook production, only one provided enough eggs (170ml's) to stock a single production tank. There were many possible reasons for the low snook production numbers in 2004. However, the most likely reason appears to have been the late start in the spawning season. Lack of tank availability and the need for labor devoted to construction, kept us from being able to bring eggs into the hatchery in the earlier months of the spawning season (May and June). This delay in spawning efforts was further compounded by the fact that less fish were captured in areas that historically had been good sites for capture of spawning snook. Another hindrance to the spawning success was the high frequency of captured females that had already spawned. These results lead us to believe that the spawning season in 2004 occurred earlier in late spring/early summer and that we missed the prime spawning period. The late start also hampered environmental conditions. Spawning attempts are typically conducted near the passes on an outgoing tide. As spawning attempts are conducted later in the season, the tidal changes occur later in the evening, which results in difficult collection conditions due to darkness. One other problem that greatly affected our spawning success was that two of our three best spawning sites (New Pass and Longboat Pass) had undergone drastic topographical changes from 2003 to 2004. This change made this site unworkable and a safety hazard for operating the gear for collecting the snook broodstock. Although fish were seen in these areas, the nets could not be dropped in the locations needed to capture them.

Development of a year round captive spawning protocol for common snook

In February, we began transferring snook broodstock from Mote's main campus to the Mote Aquaculture Park's (MAP) new broodstock maturation facility. By late April, a total of twenty-four adult snook were transported and established in two independent tanks (54,315 L/tank). The fish were held in freshwater until November when salinity was increased from 0 ppt to 10 ppt. Construction of the final stage of salt water sludge treatment system was completed at the end of December, which now allows us to increase the salinity in those systems to full strength seawater. Water clarity and water chemistry is very good in these new systems. The snook broodstock are monitored for egg development and growth every 4 months. The latest examination of the fish in October 2004 showed no signs of spawning conditions, but we do not expect to induce spawning until the spring of 2005.

Determination of an optimal diet for common snook broodstock

The captive broodstock at MAP continued to be maintained on a fresh cut broodstock diet of shrimp, kapelin, squid, herring, and mackerel. Modifications in the diet are being considered for the fish being maintained in full strength seawater.

Larval Rearing Production Results

Of the 9 spawning attempts dedicated to Mote's snook production in 2004, only one provided enough eggs (170ml's) to stock a single production tank. At first grading (40 days-after-hatch [DAH]), a total of 3,190 fish were harvested from the larval rearing tank, which were later size graded and stocked into three nursery culture tanks. This number was slightly lower (2,025 juvenile snook) at the second size grading at 91DAH. At 133 DAH, there were 1,646 juvenile snook left in culture. The fish ranged in size from 109.6-132.6 mm. Only 12 fish were observed to have lordosis at the 133 DAH size grading. The recorded percentages of lordosis recorded after the first three size grading were 0%, 0.01%, and 0.01%, respectively. The low occurrence of lordosis in the 2004 production season, compared to the high percentages experienced in previous years, may be due to the low numbers of eggs stocked this year. The lower numbers of larvae present at first feeding, may have benefited the larval population by allowing more larvae the opportunity to acquire the needed amount of food organisms to fulfill the larvae's nutritional needs for normal development. Another hypothesis to account for the low percentage of lordosis is related to rotifer production in 2004. The increased fecundity and production of the rotifers in the new recirculating rotifer system allowed for more rotifers bearing multiple eggs to be harvested. This added nutrition of the eggs on the harvested rotifers may have attributed to the passing of nutrients to the growing snook larvae and, therefore, preventing lordosis among the population. This fecundity of the rotifers may have led also to a lower percentage of lordosis in an indirect manner. The increase of rotifers bearing multiple eggs at harvest would have allowed more rotifer eggs to enter the larvae culture systems. Once hatched, these eggs would result in smaller rotifers present in the larval tanks. These smaller sized rotifers may have enabled the larvae to more easily capture and handle their prey during the period of early larval feeding.

Development of nursery culture methods for juvenile queen conch

Mote completed the first phase of the calcium feed study with Harbor Branch Oceanographic Institution (HBOI). All length and weight data, along with test animal shells, were sent to HBOI for comparison with their results. The remainder of the Turks and Caicos' experimental conch were sent to Mote's Key West facilities where the diet study is continuing. Another 200 Florida Queen conch are being reared at Mote Aquaculture Park for eventual stock enhancement research trials in the Florida Keys. These animals are approximately 35 mm in length and are being grown to approximately 65 mm for the release study.

Studies To Assess The Effectiveness And Potential Impact Of Stock Enhancement:

Test of Density-Dependency Effects with Hatchery-Reared Juvenile Snook Released in Critical Nursery Habitats

A primary concern with stock enhancement programs is whether stocked fish contribute to abundance or simply displace wild individuals. Especially during juvenile stages, density-dependent mechanisms and habitat limitations can have a strong effect on the abundance of juvenile populations. Aside from ecosystem-wide effects, the release of a piscivorous and cannibalistic species into a natural nursery system can have deleterious effects on the wild cohort and/or on the released fish. Responsible stock enhancement programs must investigate the extent of these potentially harmful and costly effects.

The release of hatchery-reared juvenile fish allows for manipulation of recruitment in juvenile fishes. Used in a controlled manner, we are able to manipulate population levels in different habitats.

In 2002, we conducted an experiment to evaluate the effects of density dependent mortality on juvenile snook populations in our study area. In this study we manipulated recruitment levels in different nursery habitats through supplementation of hatchery released juvenile snook. In two habitats (treatment - Bowlees Creek and Whitaker Bayou) we attempted to double the existing wild age-0 snook population, while in two others (control – North Creek and South Creek) we attempted to only increase the age-0 population by 10%. We monitored the population for up to 10 months after the releases to determine changes in wild and hatchery snook populations. In essence we found the following notable results:

- Our estimates of the age-0 snook population size were within an order of magnitude. In the treatment creeks population levels initially increased by 126% and 38%. In the control creeks population levels initially increased by 31% and 6%.

- From 1 through 10 months after the releases, no significant differences in wild fish abundance were found between control and treatment study sites.

- There was an indication of slight decline in the hatchery-released population by 10 months after release however.

- In all creeks, age-0 abundance dropped significantly during the fall season. Abundance increased again in the winter after the first cold snap of the season.

- Overall, the treatment habitats showed an increase in total age-0 abundance, while the control habitats did not. This result could be confounded by intrinsic differences in the habitats and demands further studies using crossover designs with treatment and controls assignments. Both treatment creeks had been

historically subjected to intensive anthropogenic alterations such as dredging and channelization. Control creeks, however were subjected to less historical anthropogenic alterations and represent a more pristine condition of juvenile snook habitat.

2004 Experimental Design

We repeated the abovementioned experiment in 2004 by releasing hatchery-reared juvenile snook to manipulate recruitment levels in the nursery habitats. In 2004, we stocked approximately 250-300% of the abundance of wild juvenile snook in the treatment creeks and only stocked about 10% of the abundance of wild juvenile snook in the control creeks. Pre- and post-release sampling designs were identical to the 2002 study. Standardized sampling occurred prior to the release in March-April 2004, and after the releases in June- July, 2004. We plan to conduct a standardized sampling effort again in January- March, 2005.

To further evaluate the abundance and distribution of juvenile snook before and after the releases, we used acoustic telemetry technology in the juvenile fishes. Specifically the goals of the acoustic tag component of this study were to evaluate habitat use, survival, and interaction between the wild and hatchery reared individuals. We tagged wild and hatchery juvenile snook with sonic transmitters (Vemco, V8SC1L 24mm tags) prior to the releases in both control and treatment creeks. A total of 36 hatchery (9 per creek) and 36 wild snook (9 per creek) were tagged and released with these tags. Within each creek, movements were continuously recorded with semi-permanently moored acoustic receivers and active manual tracking activities using a hydrophone. Manual tracking was conducted over 24 hour periods, and diurnal habitat usage was specifically recorded. Acoustically tagged snook will be tracked throughout the fall and winter (V8SC1L battery life ~ 300 d).

In April 2004, 7,737 juvenile hatchery-reared snook were tagged for this experiment (Table 1) and in June 2004, they were released. Tagging occurred at the MML Aquaculture facility. Kerry Mesner and Cindy Armstrong (FWC Stock Enhancement Research Facility [SERF] biologists from Port Manatee) assisted Mote staff during the tagging. Mark IV coded-wire tag machines and detectors on loan from SERF were used to tag the fish. All fish were additionally tagged with red VIE marks in the caudal fin as an external identification of a hatchery fish. Subsamples of snook from each system were subjected to health exams by an independent agency and were considered "healthy" for release.

In November 2004 we implanted 43 sonic tags in juvenile snook (200mm-300mm FL). These fish were captured and released in Sarasota and Manatee Counties in nearshore estuarine systems. These tags have a 300 day battery life and should function until next summer. Tagging these additional fish will further our understanding of wild, age-1 snook migrations.

To date we have 34 submersible acoustic receivers (Vemco VR2s) distributed throughout the study area. Twelve of these were purchased as part of another project examining the influence of fishing mortality and emigration on adult snook populations in Sarasota Bay. The acoustic receivers are downloaded every month and this will continue until the end of the tag battery life. Manual acoustic tracking is also being performed twice a week to supplement the tracking capabilities of the VR2s.

Refining Tag Technology with the Common Snook and Red Drum

Adapting Tag Technology toward Stock Enhancement of the Common Snook

Previous studies at Mote Marine Laboratory investigating VIE retention in juvenile snook reported promising VIE retention in the caudal fin rays, while poor retention was reported in the head and jaw areas of the juvenile snook. A manuscript (Brennan, *et al.*) entitled "An evaluation of tag performance with juvenile common snook under field and laboratory conditions" is currently in press with the North American Journal of Fisheries Management. This manuscript describes the results primarily obtained from field recaptures and lab studies detailing tag experimentation work from 1997-2002 (see, 2001-2002 Final Report - MML Technical Report 761). Because VIE tags essentially become obscure after 1 year --even in the caudal fin rays (our highest retention site tested)–we are investigating the potential use of this tag in the highly visible cornea tissue of juvenile snook. Preliminary results from laboratory studies indicate an absence of negative effects on tissue health, and high retention and visibility.

In June 2004, researchers at SERF and MML collaborated to implement the "VIE-cornea" study using red drum and common snook. Approximately 2000 red drum and 600 juvenile snook were used in this study. Both treatment and control groups were set aside to monitor tag retention and tag induced mortality. This study is planned to continue into the winter 2004/2005.

We are also developing the capability for the use of PIT (passive integrated transponder) tags in ecological studies with juvenile snook. Many studies show PIT tags to be extremely useful in ecological studies requiring multiple recaptures, individual information, and long-term data collection needs. We are injecting PIT tags into wild juveniles in the nursery system according to size, capture location, and date. These activities are allowing us to look at individual growth rates and migration patterns, short term survival estimates and ontogenetic habitat shifts of wild snook. We are also looking into the potential for developing and employing benign monitoring systems with these tags. We have also injected PIT tags in adult snook to determine snook spawning behaviors and periodicity. See below for details.

We have begun to investigate the use of acoustic transmitters (Vemco, V8SC1L, 24 mm long) with juvenile (age-0) snook. We plan to implant both hatchery and wild snook with transmitters and monitor movement patterns of both groups as our releases this spring transpire as described above. We will report on the progress of this study as it matures.

Adapting Coded-wire Tags to "Phase-I" Red Drum

A successful tagging system is needed to implement a large scale economically viable stock enhancement program with red drum. In response we have been working collaboratively with SERF personnel to refine the coded-wire tagging system for red drum. Previously "Phase-I" (25-60 mm SL) red drum were considered too small to tag with the current tagging technology. Work centered around adapting the coded-wire tag to Phase-I red drum. Initial tests with CWT's in Phase-I red drum revealed that CWT retention in 25-40 mm SL red drum (where CWT were targeted in the anterior dorsal muscle) was very high: 100% 5 weeks after tagging (N=91). These fish were tagged "free-hand" without the use of head molds. Following this, several size-dependent head molds were fabricated to increase the efficiency and streamline and the tagging process.

The success of these experiments could greatly improve the ability to test comparative survival rates of different size classes of red drum released into the wild. Although it is most cost effective to release small sized fish, survival rates of smaller fish may not be as high as those of larger, more expensive fish. A tagging system that will allow a quantitative evaluation of survival rates of differently sized fish in the wild will rapidly progress the science of stock enhancement of red drum in Florida.

A long-term experiment is in progress with phase I head molds. Juvenile red drum (20-60 mm SL) were tagged in 2 size groups (25-35 mm SL) to determine the smallest size that red drum can be tagged with CWT's. A manuscript that describes these activities is currently being written.

Fishery Independent Assessment of Adult Habitat

Identify Recruitment of Hatchery Snook to the Adult Populations

Survival of organisms in their environment is closely associated with habitat quality, because habitat provides many essential factors including suitable environmental conditions, food, and protection from predation. With large variations in habitat (structure, prey abundance, refuge) and even intrinsic mechanisms such as density dependence operating, habitat quality can have a large influence on survival and growth of a species.

To evaluate the effect of microhabitat on post-release survival and growth, we released tagged juvenile snook into four different microhabitats in 1998 and 1999. Since then we have performed standardized random sampling in the release microhabitats conducted on generally a monthly basis. As follow-up work to this work, we need to collect information from the adult fishery, which gives us information on ultimate survival and growth rates of our released fish. Fish recovered from these habitats represent long-term data and are unbiased geographically because the spawning habitats are miles from the juvenile habitats and fish recruited to these habitats originate from a collective assortment of juvenile habitat. We are in the process of producing a publication entitled "Effects of

release microhabitat on survival and growth of hatchery-released snook in a Florida estuary" that describes this study.

Assessment of snook spawning distribution and frequency

A protracted spawning season leads to multiple spawning efforts of individuals, energy allocation over a spawning season, changes in egg quality over the spawning season, and spawning site preferences. In testing the feasibility of a stock enhancement program for the common snook, development of a sound aquaculture system for snook is necessary and knowledge of snook spawning biology is an important aspect of this.

Eggs derived from different terms of the season may vary in hatching success and larval quality. An understanding of the ecological ramifications of this is important for responsibly producing progeny for year class supplementation.

Wild caught female snook were tagged with Passive Integrated Transponder (PIT) tags throughout the spawning season of summer 2004. Each female was scanned for previous tags, measured, aged (from scale annuli, aging only first time tagged fish), given an index of maturation, and the stage at which eggs are harvested. We categorized female maturation stages as follows:

Maturation Stage	Code
Unsure of sex	0
Female, but not ready	1
Female, almost ready	2
Eggs flowing	3
Spawned out	4

The stage at which eggs are harvested from mature females are assigned to codes as follows:

Eggs Harvested	Code
No Eggs	0
Eggs harvested 1st try	1
Eggs harvested 2nd try	2
Eggs harvested 3rd try	3

Snook were collected during spawning cycles on the new moon and full moon phases from June through August. Collections were conducted at spawning sites from Longboat Pass to New Pass. Snook were captured with seines and trammel nets. All tagged fish were released after processing.

During the summer of 2004, 394 adult snook were captured in spawning habitats (Table 2), three were hatchery recaptures and seven were wild snook recaptures. We tagged 273 snook with PIT tags. To date, six snook with PIT tags have been recaptured at the spawning sites and of these all were tagged and recaptured at the same site.

Fishery Dependent Sampling of Snook Populations in Sarasota Bay

7TH ANNUAL "SNOOK SHINDIG"

The specific goals of the Snook Shindig were the same as the previous years tournaments and were to:

- To promote angler awareness and enthusiasm of the stock enhancement program, and the Snook Foundation's research activities,
- allow stock enhancement researchers to further develop a working relationship with local fishermen,
- allow researchers to collect important dispersal information on the tagged and released snook in Sarasota Bay and its surrounding waters,
- allow researchers to collect important angling CPUE data of the hatchery snook,
- allow researchers to collect contribution rates of hatchery snook from different areas of Sarasota Bay and its surrounding waters,
- allow researchers to collect growth and condition data from both wild and hatchery snook, and
- attain a relative contribution effect of hatchery-released snook to wild snook populations among years.

The 7th annual Snook Shindig was held on November 12, 2004 and was a great success. The Captain's Meeting started at 5:30 pm on November 12, 2004, with Chris Malkin (Snook Foundation Board Member) welcoming the participants to the tournament and explaining the tournament rules and point system. Dr. Ken Leber from MML was introduced and discussed the importance of stock enhancement and the data collected in the tournament. Bill Halstead from FWC/SERF followed with information on the special permits issued to each angler. "Lines in the water" started at 7:30 pm November 12, 2004 and the tournament was officially over on November 13, 2004 at 1:00pm.

Special Authorization Permits

Special authorization permits were issued among the 80 anglers who attended the captains meeting. One permit was issued to each fishing "group" and allowed participating anglers to hold snook outside of the window limit until an official checked the catch. This permit was only valid for the duration of the tournament.

Weigh-in Stations

The following weigh-in stations were set up for landing and tag checks. These "stations" were mobile units that approached anglers when requested.

Location	Volunteers
Sarasota Bay roamer	Brett Blackburn, Gina Russo, Carrie Mesner
Bowlees Creek	Nate Brennan

Sarasota Bay	Johnny Walker
Sarasota Bay	Ken Leber, Reko Salt
North Creek	Bill Pine
Phillippi Creek to StickneyPoint	Pete Simmons, Nick Parnell
N. Albee Bridge	Roger DeBruler, Howard Wells
S. Albee Bridge to Venice Inlet	Meaghan Darcy, Dave Medici
South Creek/Blackburn Point	Jason Bennett, April
South Longboat Key	Dave Wilson
North Longboat Key	Jim Michaels
Big Pass	Dean Doherty

All stations were active for the entire tournament. Landed snook were checked for the presence of tags and all pertinent information recorded. Three hundred nine snook were caught, 19 (6.15%) of which were hatchery raised.

In the adult category, the winners were: 1st place, Capt. Merilee Dunn; 2nd place, Dave Robinson; 3rd place, Geoff Smith. The winners in the under-16 youth division were: 1st place, Eli Weiss; 2nd place, Eric White; 3rd place, Dan Trentalange. Weiss and Robinson also won an invitation to the IGFA tournament scheduled for the Florida Keys. (As a professional fishing guide, Dunn was not eligible for an invitation to the amateur-only IGFA event.) The award ceremony was held at the Keating Center at MML and catered by Bonefish Grille.

An evaluation of cannibalism risk in juvenile snook

Cannibalism in snook has been well documented in artificial rearing situations (unpublished data, Mote Aquaculture) and is also documented to occur in the wild (Florida Fish and Wildlife Conservation Commission, unpublished data, MML, unpublished data). However, as a top predator in estuaries, the degree that inter-cohort and intra-cohort cannibalism influences abundance of juveniles has never been quantified and remains unclear. In artificial rearing situations, cannibalistic behavior has been observed to be especially intense during early life stages and this early life cannibalistic tendency (if it exists) may serve as strong evidence for density dependent mechanisms operating in juvenile snook populations. To responsibly test the feasibility of stock enhancement of snook, understanding the extent and intensity of cannibalism in juvenile snook, (similar to the sizes and ages of snook we are stocking) is necessary. If snook are highly cannibalistic during juvenile phases then stocking could have detrimental ramifications, such as hatchery juveniles significantly cannibalizing wild juveniles, wild juveniles cannibalizing hatchery released juveniles, or combinations of both. In any of the above cases, environmental systems will have a limitation on the amount of snook supported, and stocking activities may not be an effective means for replenishing a population.

We continued to quantify allometric cannibalistic tendencies of age-0 and age-1 snook in the laboratory during fall 2004. Aquaria were monitored until cannibalism occurred at which point the fish were harvested from the aquaria. Additional trials ensued and will

Sarasota Bay	Johnny Walker
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be continued into the next interim report. Details of this study will be reported in the next report.

To determine cannibalism incidence and frequency in the wild, stomach contents of all captured snook were collected. Stomachs were pumped with creek water and contents preserved in 10% buffered formalin. This component of the study will continue over the next year and will include season and time of day in the design.

Testing the capability of rural snook fisheries

We are investigating the capability of using the common snook in urban fishery environments. Forty snook (300-450 mm SL; two age classes) were tagged with individual PIT tags and VIE marks and stocked into a freshwater pond at the Mote Aquaculture Farm. The pond is designed for snook to incorporate thermal refuge, vegetative habitat, structure, and various prey species. An automatic feeder supplements snook feeding activities. The submersed temperature loggers continue to monitor surface and bottom temperatures in the pond. During the hurricanes there were lapses in recordings due to storm interference. We will continue to download this data seasonally to understand seasonal trends. Hook and line catches show that the fish have grown and body condition is good.

Assist the Florida Fish and Wildlife Conservation Commission (FWC) with Strategic Planning for their Marine Stock Enhancement Program

In line with the short and long-term objectives of strategic planning for the Fish and Wildlife Conservation Commission's marine stock enhancement program, several steps have been made toward (1) improving the effectiveness of FWC's marine stock enhancement program, (2) adapting and refining the aspects of a "Responsible Approach to Marine Stock Enhancement" (Blankenship and Leber, 1995) that have not yet been fully implemented in Florida, and (3) identifying and prioritizing potential marine fish species for stock enhancement in Florida.

- Dr. Ken Leber (Mote Marine Laboratory) has been working closely with the Florida Fish and Wildlife Conservation Commission's Stock Enhancement program to further our partnership in stock enhancement.

- Dr. Leber has continued to work closely in developing and implementing the pilot releases and nursery sampling of the juvenile red drum released in Tampa Bay.

In addition to managing collaborative aspects of this project at Mote Marine Laboratory, Dr. Leber participated in several planning meetings with FWC staff during this grant period. These included a Project Tampa Bay meeting on July 14 at the FWC Stock Enhancement Research Facility in Port Manatee, a planning meeting with Bill Halstead on August 25, and meetings at FWC's Fish and Wildlife Research Institute (FWRI) on 26 August and again on 1 October to discuss economics issues involved in evaluating stock

enhancement effectiveness. Dr. Leber also assisted FWRI in October in the planning and conduct of a semi-annual meeting with the Florida Marine Stock Enhancement Advisory Board, a stakeholder group organized by FWC to assist in planning future goals, objectives and activities of the FWC Marine Stock Enhancement Program.

UNH Progress – July through December 2004

The overall goal of our winter flounder stock enhancement program is to accelerate recovery of the fishery by increasing spawning stock biomass. To meet this goal, we have developed a multidimensional research program designed to produce large numbers of high quality juveniles, to optimize release strategies, and to test the feasibility of winter flounder stock enhancement. Elements of the program addressed in this reporting period have included:

Rearing, tagging and releasing winter flounder juveniles

Because of size at release is an important variable, and because we have released relatively small fish (5-6cm) in the past, we chose to hold some of the 2003 fish in the laboratory over the winter until they reached a larger size. In late June 2004, these cultured fish (n=562, mean size=10.9 cm, age=14 months) were tagged with a mixture of tags including, Visible Implant Elastomer Tags, Decimal Coded Wire Tags™, and Visible Implant Alphanumeric Tags, and released in the Hampton-Seabrook estuary. The release was done by transporting the fish, via live tanks on a boat, to the release site. Here, divers transferred them into acclimation cages located on the bottom of the estuary. Forty-eight hours later, divers released the fish from the acclimation cages into the wild. Weekly sampling for these fish, at the 5 sampling stations, continued through October 2004. None of these fish were recaptured.

In addition to the fish described above, in the spring of 2004 we produced approximately 2,000 winter flounder juveniles for a second set of 2004 releases. These fish (mean size=5.9 cm, age=6 months) were tagged in September with Decimal Coded Wire Tags™, acclimated as described above, and released into the Hampton-Seabrook Estuary in late September. In addition to these cultured fish, 64 wild juvenile winter flounder were caught at the release site in the preceding days, tagged with Visible Implant Alphanumeric Tags, and released. Because of our lack of success in recapturing fish from the earlier release, we changed our sampling protocol. We hypothesized, based upon recent results from a European study with a similar species, that our fish were not dispersing away from the release location, and that our sampling program, with five sampling locations spread over about 15 km^2, was too large. Thus we established a grid 300m long by 100m wide (3,000 m^2), divided into 50m intervals in both directions. The release location was at the center of this grid. Observations of the released fish began immediately. This was facilitated by 15 of the fish being tagged with large, red streamer tags that were clearly visible to the divers who released the fish from the acclimation cage. They observed that the released fish moved only a few meters from the release site. Sampling for released fish began the day of release, and continued over the next seven days. Replicate beam trawls were made at every intersection of the grid (21 stations).

On the day of release, beam trawl tows were made at each grid intersection every 3 hours, beginning one hour after release. Each grid intersection was repeatedly sampled over the following 6 days. Fourteen cultured winter flounder were caught through this effort. Of notable importance, all cultured fish were recaptured within meters of their release location while wild juveniles were recaptured at all locations within the 3000m^2 sampling grid. This confirmed our hypothesis that the released, cultured fish do not quickly disperse away from the release site, and indicated that the scale at which we has been sampling in the past was probably too large. The low numbers recaptured may indicate that mortality of released fish is higher than we previously believed. Because the divers observed a large aggregation of green crabs around, and presumably attracted by, the acclimation cages, we believe the source of mortality may be green crab predation. To alleviate this, we will move the acclimation cages away from any crab aggregation in all subsequent releases. Gut contents of a sample of wild juveniles were compared to the gut contents of all recaptured cultured fish. Results indicated that the wild juveniles had more in their stomachs, and had a more diverse diet than cultured fish. One of the greatest differences was that the cultured fish contained small mussels, which, because of their size and color, resemble the formulated diets the cultured fish had been, provide. This suggests that cultured fish differ from wild fish in their feeding ecology, at least in the short term, and that this difference may be due to their feeding history.

Characterization of the Wild Winter Flounder Population in the Estuary

Because relatively little is known about the winter flounder population within the Hampton/Seabrook Estuary, we have established a sampling regime to determine the seasonality of: 1) the abundance of winter flounder in the estuary; 2) the size class distribution of winter flounder; and 3) the spatial use of the estuary by different size classes of flounder.

Five sampling stations were selected throughout the estuary based on their physical and geographical parameters. At each station, a fixed submersible DST CTD data logger (Star-Oddi ^{TM}) was anchored to a cinder block to record temperature and salinity at hourly intervals. Surveys of these stations began in June and continued through October 2004. In addition to these abiotic measurements, 3 types of collecting gear were used at each station on each weekly sampling occasion. In shallow water (<1.5m) we employed a 17m x 2m beach seine. Three replicate samples, each with an approximate swept area of 550m^2, were taken near low tide. In mid-depth areas (1.5-3m) we employed a 1m beam trawl. Three replicate 50m long tows were taken within the 1.5-3m depth interval, each parallel to the shore. In the deeper areas (>3m) we used a 4.8m otter trawl with 25mm mesh in the body and 6mm mesh in the cod end. As with the beam trawl, three replicate tows were taken within the depth interval, each parallel to the shore, and each approximately 100m length. The catch from all sampling has been identified, enumerated, and measured. All winter flounder caught were checked for tags. Abundance was estimated as catch-per-unit-effort (CPUE), given as number caught per m^2 sampled.

To characterize the benthic community in the estuary and, therefore, winter flounder prey availability, we took a bi-weekly series of 6 replicate benthic cores (0.0079m^2 to a depth

of 10cm) at each station. Cores were stored in Zip-Lock™ bags, placed in ice, and returned to the laboratory where they were sieved through a 1mm mesh sieve. All prey taxa are stained with Rose Bengal and preserved in 10% buffered formalin. All will be identified, counted, and weighed in the next several weeks.

Sexual differentiation

Sexual differentiation of winter flounder has never been investigated. Because the sex ratio of cultured winter flounder, and the factors that may influence it, are completely unknown, and because the sex ratio of stocked fish is fundamentally important, we have been studying sexual differentiation and sex ratio of cultured fish as part of this study. Starting at metamorphosis, a total of 376 cultured fish were sampled from the general hatchery population at approximately 10 mm total length (TL) intervals at 110, 124, 160, 231, and 323 days post-hatch. On each sampling occasion, randomly collected fish were euthanized by an overdose of anesthesia (MS-222), measured, and weighed. Fish were fixed and stored in modified Davidson's fixative for at least 48 h. Prior to histological processing, the samples were washed in freshwater and stored in 70% alcohol. Histological processing involved embedding the fish in paraffin, sagittal sectioning (5 μm), and staining with hematoxylin and eosin. Slides were numerically coded and examined by three viewers in a blind test to determine fish sex based on structures and cells associated with gonadal tissue. Fish were scored male, female, unknown (gonadal tissue visible but unidentifiable to sex), or missed (no gonadal tissue present on slide). Sex ratio data were calculated and analyzed using Chi-square goodness of fit. Those fish that were scored as "unknowns" or "missed" were excluded from the statistical analyses. In addition, to determine if fish age or size affected the sex ratio, the data were sorted accordingly and reanalyzed using Chi-square goodness of fit tests. Results suggest that sexual differentiation in winter flounder occurs at a size < 41 mm TL. A total of 65 females and 162 males were identified from the 41-110 mm TL cultured flounder population yielding a sex ratio that was significantly skewed towards male (χ^2=44.7, df=1, p<0.001). This trend held true when cultured fish were sorted by age and size, with the exception of those fish 61-70 mm TL; an aberration probably due to a small sample size.

Stress Physiology

Because hatchery reared fish are handled as they are tagged and transported to the release site, it is important to understand how these practices stress the fish, and what impact this has, if any, on their survival and performance after release. A series of stress experiments have quantified the physiological effects of tagging, transportation and density on juvenile winter flounder through whole body measurements of cortisol concentrations. For the tagging experiment, juvenile winter flounder (42 mm mean total length ± 0.1 SEM) were marked with either a visible coded wire (VIE) or decimal coded wire (DCWT) tag and allowed to recover in a separate holding tank. In order to determine the time period needed for cortisol to return to baseline values after tagging, each tagging experiment was divided into seven time trials. After the initial tagging (time 0), flounder were sampled every three hours for 12 hours (i.e. at 3, 6, 9, and 12 hours) after the initial tagging occurred. Two additional samples were taken at 24 and 48 hours. The results

from the tagging experiment indicate that VIE tags provoke a stronger and more prolonged ($p<0.05$) increase in cortisol levels when compared to decimal coded wire tags (DCWT). For the transport and density studies, the effects of stress at five different stocking densities (100%, 200%, 300%, 400%, and 600%) were investigated during transport at four different transport intervals (5 minutes, 45 minutes, 90 minutes and 48 hours). Transport produced a stress response at all densities 45 minutes and 90 minutes after transport. However, only fish stocked at a density of 600% sustained cortisol levels that were significantly different than control levels after 48 hours. The results of this study suggest that regardless of transport density or tag type, a minimum of 48 hours are needed for the juvenile flounder cortisol levels to recover to baseline/control levels.

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Larval Nutrition and Microparticulate Diet Research

The real time instrument method to determine leaching rates from microparticles is operational. The measurements are reproducible and fit models that characterize the physical changes of leaching and mass loss for different feed formulations and particle sizes. A draft publication on this method is in preparation and should be ready for internal review by the end of November.

The microparticulate diets made from trypsin digested fish muscle perform as well as or better than other materials we have measured in the leaching tests. Limited use (due to the difficulty of scaling up the production) with clownfish larvae indicate that the particles can support development, growth and survival though more testing is needed to determine at what stage larvae can be weaned. A growth trial with cod larvae is planned for this winter. Improvements in processing techniques and starting material have resulted in improved yield. The impact of factors such as drying and digest time on leaching have been evaluated. Yttrium has been incorporated into the diet for digestibility studies.

A digestibility study was conducted in April. Pacific cod larvae were fed either rotifers, *Artemia* or the microparticulate diet described above. Fed larvae were placed in scintillation vials overnight for evacuation. We did this four times over three weeks to look at changes in digestibility during ontogeny. Feces samples (for week one) were analyzed for yttrium (marker) and protein. Initial analysis gave highly variable values among replicates, but the minute amounts of marker and protein detected in the samples were above the limits of detection. We are working on honing detection limits further prior to analysis of the remaining samples, however we may redo the feeding trial to generate larger samples.

A digestibility study using four different markers, lanthanum, dysprosium, yttrium, and ytterbium oxide was conducted to see if there would be differences in apparent digestibility coefficients computed from the four markers. *Artemia* were marked and fed

to pacific cod larvae and samples were collected as above. These samples have yet to analyzed.

All the data generated for the digestibility marker (lanthanum, dysprosium, yttrium, and ytterbium oxide) uptake and depletion from *Artemia* study has been analyzed and a draft manuscript is ready for internal review.

Lingcod Enhancement Study

14 adult lingcod (7 male and 7 female) produced in our hatchery were surgically tagged with acoustic tags (Vemco Corp.) and released at two sites in southern Puget Sound on Friday August 13th, 2004. Stationary hydrophones from the two release sites, Toliva shoals and Zees reef, and others in southern Puget Sound (see map of hydrophone placement from the proposal), were checked a month after the release and then again at the end of October. They will be retrieved several more times during the winter of 2005. Equipment to conduct active hydrophone surveys was acquired at the end of October 2004 and active surveys of the release areas will be conducted. The location of male fish identified during the active acoustic survey in February will be confirmed by use of a video equipped ROV to determine if they are guarding eggs. Results to date indicate that the vast majority of the fish stay near the release site.

Pacific Cod Enhancement Study

Approximately 300 Pacific Cod juveniles were produced from adults spawned in February and March 2004. During the reporting period these fish were being grown to a size large enough to tag. Most will be used for lab studies however, tagging and release of a small number of tagged fish (as above) will occur in 2005 per the proposal.

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