SNOOK PROJECT

FDEP Contract No. MR159: MML Project No. 310.622

Final Report

Contract Commenced: September 18, 1996
Report Includes: July 1, 1996 - May 9, 1997

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June 12, 1997

Mote Marine Laboratory Technical Report No. 528

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Mr. Bill Halstead
FL Department of Environmental Protection
Stock Enhancement Research Facility
14495 Harllee Road
Port Manatee, FL 34221


Dear Mr. Halstead:

Please consider this letter and attachments as the Final Report for the above referenced project. The contract (FDEP Contract No. MR159) commenced on September 18, 1996.

This report includes information on collaborative studies conducted by Mote Marine Laboratory’s (MML) Aquaculture/Fish Stock Enhancement Program in cooperation with FDEP from July 1, 1996, through May 9, 1997. Studies were performed at the FDEP Stock Enhancement Research Facility (SERF) and at MML.

Studies performed by MML and SERF included: 1) evaluated methods for determining egg quality; 2) determined upper critical temperature tolerance for hatching embryos and larvae; 3) evaluated human chorionic gonadotrophin hormone (HCG) dose response; 4) assessed gonadotrophin releasing hormone (GnRH) dose response; and, 5) identified which GnRH induces ovulation and produces the best egg quality.

As importantly, though not as visible, areas encompassed were: 6) maintained phytoplankton, *Nannochloropsis oculata*, Tahitian *Isochrysis* sp., and rotifer, *Brachionus plicatilis* cultures; 7) participated in research planning meetings; 8) attended workshops and conferences in support of these studies; 9) provided volunteers and interns to assist with snook project studies; 10) performed literature searches; and 11) prepared data and developed manuscripts for peer-reviewed publications.

A significant amount of data was generated from collaborative snook, performed from May 1996 - September 1997. Computer entry and statistical analysis of data from studies are in progress at MML. Biopsy and tissue samples from studies 3, 4, and 5 require histological processing and light microscopy analysis by FDEP. As processing is completed and data are interpreted, results will be included in reports under the 1997-1998 FDEP/MML contract.
On behalf of Mote Marine Laboratory, appreciation is greatly expressed to the Florida Department of Environmental Protection for supporting this project. In addition, we would like to thank the SERF staff for their warm welcome. We look forward to continuing the successful cooperation between Mote Marine Laboratory and the Florida Marine Research Institute and Stock Enhancement Research Facility.

Sincerely,

Carole L. Neidig
Senior Biologist

Enclosure

cc: Ms. Kelly Boomer-Donnelly (Invoice Enclosed)
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>LIST OF PARTICIPANTS</th>
<th>ii</th>
</tr>
</thead>
<tbody>
<tr>
<td>TABLE OF CONTENTS</td>
<td>ii</td>
</tr>
</tbody>
</table>

## I. INTRODUCTION ................................ 1

## II. STUDIES ........................................... 2

### A. Evaluating Methods for Determining Egg Quality in Snook
- Introduction ........................................... 2
- Methods .................................................. 2
- Results .................................................. 3
- Discussion ............................................. 3

### B. Establish Upper Temperature Tolerance Limit for Hatching Embryos and Larval Snook
- Introduction ............................................ 4
- Methods .................................................. 4
- Results .................................................. 5
- Discussion ............................................. 5

### C. Evaluate the Response of Using Different Doses of Human Chorionic Gonadotrophin (HCG) on Snook in Promoting Ovulation
- Introduction ............................................ 5
- Methods .................................................. 6
- Results .................................................. 6
- Discussion ............................................. 6

### D. Evaluate the Response of Using Different Doses of Gonadotropin Releasing Hormone (GnRH) on Snook in Promoting Ovulation
- Introduction ............................................ 6
- Methods .................................................. 7
- Results .................................................. 7
- Discussion ............................................. 7
E. Identify Which Gonadotropin Releasing Hormone (GnRH) Induces Ovulation In Snook and Produces Best Egg Quality .................................................. 7
   Introduction ............................................. 7
   Methods ................................................... 8
   Results .................................................... 8
   Discussion ................................................ 8

F. Phytoplankton and Rotifer Culture ......................... 8

III. RESEARCH SUPPORT AND ACTIVITIES ..................... 9

A. Research Planning Meetings .............................. 9
B. Scientific Meetings and Conferences .................... 9
C. Miscellaneous ........................................... 10

IV. REFERENCES ............................................. 11

Appendix I  Achievements of the New Snook Project
Appendix II  Proposed Common Snook Research Papers
Appendix III  Poster Presentations
I. INTRODUCTION

The common snook, *Centropomus undecimalis* [Bloch], is a highly prized sport and food fish in south Florida. Declining natural populations resulted in harvest restrictions and stimulated interest in snook as a candidate for aquaculture development and stock enhancement (McCarty et al. 1986).

In 1994, the "New Snook Project", a cooperative research venture between Mote Marine Laboratory (MML) and the Florida Department of Environmental Protection, Stock Enhancement Research Facility (FDEP, SERF) commenced. Over the past three years, researchers involved in the partnership (SERF and MML) encouraged collaborations and networking between institutions to develop reliable methods for hatchery-level production of fingerling snook suitable for effective enhancement of natural stocks. Successful collaborations were established with scientists from Harbor Branch Oceanographic Institute, Whitney Laboratory, North Carolina State University, University of Victoria, Salk Institute and Louisiana State University.

In the last three years, significant advances were made in broodstock handling and spawning of snook, including minimizing broodstock mortalities, obtaining good quality spawns with lower doses of hormone, identifying alternative hormones that were less deleterious to the ovary, and using cryopreserved sperm to fertilize snook eggs which could become a management/genetics tool for stock enhancement. These advances improve the potential for increasing production of snook and developing technology for other marine finfish targeted for stock enhancement or for food production.

This report includes information on research conducted by Mote Marine Laboratory’s (MML) Aquaculture/Fish Stock Enhancement Program in cooperation with the Florida Department of Environmental Protection (FDEP), Stock Enhancement Research Facility (SERF), from July 1, 1996, through May 9, 1997.

Studies performed by MML and FDEP included: 1) evaluated methods for determining egg quality; 2) determined upper critical temperature tolerance for hatching embryos and larvae; 3) evaluated human chorionic gonadotrophin hormone (HCG) dose response; 4) assessed gonadotrophin releasing hormone (GnRH) dose response; and, 5) identified which GnRH induces ovulation and produces the best egg quality.

NOTE: Computer entry and statistical analysis of data from all studies are in progress at MML. Biopsy and tissue samples from studies 3, 4, and 5 require histological processing and light microscopy analysis by FDEP. As processing is completed and data are interpreted, results will be included in reports under the 1997-1998 FDEP/MML contract.
As importantly, though not as visible, areas encompassed were:
6) maintained phytoplankton, *Nannochloropsis oculata*, Tahitian *Isochrysis* sp., and rotifer, *Brachionus plicatilis* cultures; 7) participated in research planning meetings; 8) attended workshops and conferences in support of these studies; 9) provided volunteers and interns to assist with snook project studies; 10) performed literature searches; and 11) prepared data and developed manuscripts for peer-reviewed publications.

II. STUDIES

A status report detailing the accomplishments during the past three years of the new Snook Project, using inhouse expertise and outside collaborations, is presented in Appendix I. A list of proposed and submitted common snook research papers representing the accomplishment of the old (1986-1991) and new (1994-1997) Snook Projects is presented in Appendix II. The list includes contributions through collaborations and networking.

A. Evaluating Methods for Determining Egg Quality in Snook

Introduction

A primary objective in common snook culture, as with other marine finfish, is to provide optimal egg quality for fry production. According to Bromage, et. al. (1994), good quality eggs exhibit low levels of mortality at fertilization, hatch, and up to first-feeding, they should be expected to produce the healthiest and fastest growing fry. However, an obstacle to establishing egg quality is the difficulty in identifying parameters that provide reliable predictions of performance. Preferably, these predictive assessments should be simple to perform and be carried out as soon after ovulation and/or stripping as possible to avoid unnecessary staff time and incubator space used on unproductive batches of eggs. Limited information is available concerning indicators for determining egg quality, especially in marine fishes (Kjørvik et al., 1990).

The goal of this study was to establish reliable and simple to perform procedures for assessing egg quality in snook. The objective was to compare methods of measuring egg quality using eggs produced from natural ovulating females and eggs from hormone-induced spawns. The parameters for determining egg quality were, 1) fertilization rate, 2) egg morphology and diameter, 3) oil droplet diameter, 4) percent hatch; and, 5) larval survival to first feeding.

Methods

Adult snook were obtained by FDEP from southeast Tampa Bay, using a beach seine or trammel net. Fish were collected weekly from May through Mid-September. Females were divided into three groups:
Fish injected with human-chorionic gonadotropin releasing hormone [HCG]; fish implanted with gonadotropin releasing hormone [GnRH]; and natural ovulating females.

Eggs from each group were collected and criteria for snook egg quality determination were compared.

- Percent of fertilized eggs were qualitatively and quantitatively assessed by examining a minimum of 100 eggs and enumerating eggs undergoing symmetrical cleavage.
- Cellular malformations were noted in early cleavage stages.
- Egg/oil ratios were obtained by measuring diameters of a minimum of 50 egg and oil droplets at the blastula stage.
- Hatch success was evaluated by measuring percent of hatched larvae (approx. 20 hours post-fertilization) from a random sample of eggs.
- Survival to first feeding was determined by enumerating larvae surviving to approximately 72 hours post-hatch from three samples of 50 eggs.

Hatch success and survival to first feeding studies were conducted in water tables (located in a SERF environmental room) to maintain a constant temperature (28.0°C). Eggs for hatch success trials were placed in 800ml glass jars containing 400mls filtered seawater. Whereas, eggs tested for survival to first feeding were placed in submerged 400ml glass beakers with fine mesh screen collars, allowing for water circulation but confining eggs and larvae. The seawater in the survival to first feeding system was aerated and circulated through a biofilter and ultraviolet light.

Results
Percent fertilization correlated with percent survival and percent hatch for eggs from GnRH spawned females. Egg diameter correlated with percent hatch for eggs from natural spawns. Egg and oil droplet ratios were closely correlated. Larger eggs had larger oil droplets. Oil droplet diameter correlated with survival of eggs from natural spawns. Percent hatch correlated with percent survival of eggs from GnRH and HCG spawns. Eggs with high percent hatch did not always result in high percent survival to first feeding.

Discussion
Percent fertilization was useful for detecting poor egg quality during early development, although, it did not always correlate with survival of snook larvae to first feeding. Preliminary studies indicate that egg and oil diameters are not a good criteria for egg quality.

Initial observations indicated that percent hatch was a good indicator of egg quality, though. statistical analysis showed that there were no strong...
correlations to support this observation. In addition, the most practical method for determining egg quality should be made before incubation.

Additional parameters measured were:
- Moon phase
- Tide
- Female Length (mm)
- Hormone Dose
- Time of Injection
- Time of Ovulation
- Spawn Volume

Data are being analyzed and results will be reported as available. Because there are no universal egg quality tests available, further investigations are needed to identify specific indicator(s) of snook egg quality for producing good eggs for fry production.

B. Establish Upper Temperature Tolerance Limit for Hatching Embryos and Larval Snook

Introduction
An important consideration in pond production is that of temperature-related stress and mortality of larval snook. Results from experiments performed at MML indicate that prefeeding larvae (two hours post-hatch) and seven days post-hatch snook larvae were intolerant (loss of equilibrium, cessation of feeding, mortality) to changes and/or acclimation to temperatures representative of those found in SERF ponds during summer months. This holds true to observations made at SERF while stocking 12 day post-hatch larvae. As far as can be determined, published data on the upper temperature tolerances of larval snook are nonexistent. To address the upper critical temperature limits of hatching embryos and prefeeding larvae additional temperature tolerance experiments were conducted. Results from these experiments should significantly enhance information needed as criteria for pond stocking.

Methods
Experiments (performed at MML) included four target temperatures (30.0°C, 32.0°C, 33.0°C and 34.0°C), controls (28.0°C) and two salinities (10 ppt and 32 ppt). These temperatures represented realistic values measured in SERF ponds and in shallow water habitats during the months of May through September. Previous experiments at MML have yielded data on emerging embryos, one, seven and nine day post-hatch snook larvae.

Fertilized eggs were obtained from snook collected by FDEP from Tampa Bay. Eight hours post-fertilization, eggs were removed from SERF incubator tanks and transported to MML. At MML, eggs were placed in 1-L beakers at a density of 50 per liter. Beakers contained filtered, UV-light-sterilized seawater
at the spawning temperature of 28.0°C. Beakers (floated using styrofoam rings) were placed in temperature controlled water baths (insulated 20-gallon aquariums), and beaker water temperatures were raised 0.5°C per hour with 300 W submersible heaters until the target temperature was reached (i.e., 30°C = 4 hrs, 32°C = 8 hrs, 33°C = 10 hrs and 34°C = 12 hrs). Four beakers were maintained at each treatment temperature. Target temperatures were maintained for 48 hrs.

Aquarium water was moderately aerated (airstones) and beakers mildly aerated (pasteur pipette secured to airline tubing) to avoid the formation of temperature gradients. Water quality parameters of dissolved oxygen, ammonia, nitrite, and pH were measured immediately prior to, and at termination of a trial. Temperatures (°C) were recorded each 15 minutes (until target temperatures were reached) to the nearest tenth of a degree using a digital thermometer. Recorded observations and comments included: 1) number of unhatched eggs or partially emergent larvae; 2) number of dead larvae; 3) changes in pigmentation; and 4) behavior of larvae (location in water column, movement, loss of equilibrium).

Dead larvae were immediately removed and evaluated. At termination of a trial (48 hrs), larvae (if available) were enumerated and preserved. Larval evaluation included: 1) standard length measurements (mm); 2) presence of bladder crystals; 3) presence and diameter (mm) of oil droplet, and 4) general morphological characteristics.

Results
In 10 ppt, survival was 78 percent for larvae held at 34.0°C. Most mortalities occurred at the time of hatch. At termination 89.6, 83.4 and 86.7 percent of the larvae remained in 28.0°, 30.0° and 32.0°C, respectively. In 32 ppt, survival was 73 percent for larvae held at 34.0°C. At termination 77.2, 74.2 and 76.9 percent of the larvae remained in 28.0°, 30.0° and 32.0°C, respectively.

Discussion
Low mortalities occurred at all target temperatures in both salinities. Previous trials with emerging larvae showed that the acute upper temperature tolerance in 32 ppt salinity should be considered to be between 33.0° and 34.0°C. Recent results demonstrate that there may be additional parameters, such as egg quality and microbial conditions that influence the outcome of these trials.

C. Evaluate the Response of Using Different Doses of Human Chorionic Gonadotrophin (HCG) on Snook in Promoting Ovulation

Introduction
The established procedure to induce spawning in common snook has been to use 1,000 IU/kg HCG. However, the fertility and viability of eggs
produced are variable. An experimental approach toward establishing an optimal/minimal dose to induce ovulation in this fish was initiated by SERF/MML. It was determined that lower doses of HCG were effective in inducing ovulation. Several studies (Ramos, 1986; Lee et al., 1987; Mok, 1985) indicate the lowest doses of hormone that are required give the best egg quality. The goal of this study was to determine the minimum dosage of HCG required to produce reliable ovulation and optimum egg/larval viability.

**Methods**

Snook were collected in Tampa Bay by FDEP. Six groups of five fish were injected with 50, 100, 250, 500, 1,000, 2,000 IU/kg of Human Chorionic Gonadotrophin (HCG). One group of five females were non-injected controls.

Females were placed in individual net pens in indoor rectangular tanks (SERF), monitored for ovulation, and strip spawned. Biopsies were taken 24 hours post-injection, at 30 hours and thereafter as needed to predict time of ovulation. At the end of the experiment, fish were sacrificed and sections of ovaries were removed and fixed for light microscopy investigation (FDEP). Fertilized eggs were placed in incubators (SERF), then made available to Harbor Branch Oceanographic Institute (HBOI), Mote Aquaculture, and were used in MML temperature trials.

**Results**

Doses of 100, 250, 500, 1,000 and 2,000 IU/kg HCG induced ovulation and produced viable eggs. Ovulation did not occur in fish injected with 50 IU/kg HCG. Histology of biopsy and ovary tissue samples have not been completed. Egg quality data is still in the process of being evaluated statistically.

**Discussion**

Preliminary results indicate that the same rate of spawning and egg quality with a much lower dose of HCG. This can substantially reduce the cost of hormones per breeding season. In addition, the dose of hormone may effect egg quality, which may influence larval survival. Tissue and biopsy samples are at FDEP for histological processing and light microscopy analysis by FDEP.

D. Evaluate the Response of Using Different Doses of Gonadotropin Releasing Hormone (GnRH) on Snook in Promoting Ovulation

**Introduction**

The gonadotropin releasing hormone GnRH has been successfully used to spawn a number of fish species, including *Lates calcarifer*, a close relative of snook, in Asia and Australia. At MML (1995), GnRH implants were used for the first time resulting in ovulation of two female snook. Using GnRH implants, a dose/response experiment with doses ranging from 10µg to 150µg was
conducted. The goal was to determine the appropriate dosage of GnRH for inducing ovulation.

Methods
Snook were collected from Tampa Bay by FDEP. Five groups of three fish were implanted with 10, 25, 50, 100, 150µg/kg of mammalian GnRH in the afternoon. One group of three females were non-implanted controls.

Females were placed in individual net pens in indoor rectangular tanks (SERF), monitored for ovulation, and strip spawned. Biopsies were taken 24 hours post-implant, at 30 hours and thereafter as needed to predict time of ovulation. Females were observed for five days post-implant. Fish condition and the occurrence (volume) of eggs in the net pens was recorded. At the end of the experiment, fish were sacrificed and sections of ovaries and pituitary glands were fixed for histological investigation (FDEP). Fertilized eggs were placed in incubators (SERF), then made available to Harbor Branch Oceanographic Institute (HBOI) and Mote Aquaculture.

Results
No females implanted with 10µg/kg of GnRH ovulated. One 20µg/kg implanted female ovulated 72 hrs after being implanted. All 50µg/kg implanted females spawned multiple times over 72 hrs. One 100µg/kg treated female spawned at approx. 26 hrs post-implant. Two of the 100µg/kg treated females had regressed ovaries.

Discussion
Implants of time-released GnRH were effective in inducing ovulation in snook. Preliminary observations show that after 72 hrs ovaries from females treated with GnRH are not damaged as compared to ovaries from snook treated with HCG. Tissue and biopsy samples are at FDEP for histological processing and light microscopy analysis by FDEP.

E. Identify Which Gonadotropin Releasing Hormone (GnRH) Induces Ovulation In Snook and Produces Best Egg Quality

Introduction
Nancy Sherwood, Ph.D. (University of Victoria) and Jean Rivier, Ph.D. (Salk Institute) provided SERF with four different custom-synthesized, time-released GnRH analogs, two identified in snook (A and B), one identified in other perciform fish (C), and one a standard mammalian analog (D). These hormones were pelleted (Innovative Research of America) and implanted in female snook. The natural GnRH analogs were evaluated against the mammalian GnRH to determine their ability to induce ovulation. In addition, spawns were evaluated to determine the GnRH that produces the best egg quality.
Methods
Trials were performed at SERF with fish collected by FDEP from Tampa Bay. Four groups of four females were implanted with four different (A, B, C, and D) GnRH pellets with known release rates and placed in individual net pens (SERF). One group of four females were non-implanted controls. Females were observed for three days post-implant. Observations of fish condition and the occurrence (volume) of eggs in the net pens. At the end of the experiment, fish were sacrificed and sections of ovaries and pituitary glands were fixed for histological investigation (FDEP).

Results
Preliminary observations show that the GnRH identified from another perciform fish (C) did not promote ovulation in snook. The two GnRH analogs identified in snook (A and B) and the standard mammalian analog (D) induced females to ovulate.

Discussion
Preliminary observations identified three analogs that successfully induced snook to ovulate. Two of the analogs have never been used with snook. Tissue and biopsy samples are at FDEP for histological processing and light microscopy analysis by FDEP.

F. Phytoplankton and Rotifer Culture

Phytoplankton and rotifer cultures were maintained at MML. From January-February 1997, MML assisted SERF by culturing phytoplankton and rotifers at SERF for red drum, *Sciaenops ocellatus* larvae studies to be conducted by the University of South Florida.

The phytoplankton, *Nannochloropsis oculata* and Tahitian *Isochrysis* sp. were cultured. *N. oculata* and *Isochrysis* sp., unicellular microalgae, have been widely used in marine fish hatcheries in view of transferring essential fatty acids and other dietary components from the algae via rotifers to fish larvae (James et al., 1986, 1987; Lubzens, 1987).

Culture disks of *Nannochloropsis oculata* cells grown on marine nutrient agar were obtained from Florida Aqua Farms, Dade City, Florida. In addition, *Nannochloropsis oculata* and Tahitian *Isochrysis* sp. in liquid media were obtained from HBOI. Concentrated Micro Algae Grow (MAG), modified from Guilliards' f/2 formulation, was used as a nutrient source. A three stage culture procedure was followed. Culture stages included: 1) Stage I, start culture (2-liter Fernbach flasks); 2) Stage II, second stage culture (20-liter carboys); and 3) Stage III, cylinder cultures (180 liters).

Cultures were maintained at a mean temperature of 27.0°C. Twenty-four hours of illumination was provided. Salinity (ppt), pH and temperature (°C) of Stage III cultures were recorded daily. In addition, inoculations and culture
conditions were noted. Algal cell enumeration was performed as needed, using a dual grid hemacytometer.

Rotifers, *Brachionus plicatilis*, purchased from Florida Aqua Farms were cultured indoors in 20-liter carboys. The adult rotifers averaged 125 microns in length. Culture water was maintained at 19-23 ppt by adding aerated freshwater or filtered seawater.

*Nannochloropsis oculata* was added to the rotifer cultures daily. Phytoplankton amounts were adjusted according to rotifer density and water quality. Temperature (°C), salinity (ppt), pH, culture coloration, volume (liters) and rotifer density (#/ml) were monitored. A record of rotifer inoculations and harvests was kept. As rotifers were enumerated, notations were made on their size, presence of food in gut and the percent of females carrying eggs.

### III. RESEARCH SUPPORT AND ACTIVITIES

#### A. Research Planning Meetings

Staff from SERF and MML held weekly (when possible) research planning meetings at SERF. In addition, MML staff conducted numerous telephone conferences, concerning study plans.

#### B. Scientific Meetings and Conferences

**1996**

- September 3, C.Neidig, R.DeBruler and P.Dufault attended the seminar, "New Developments in the Design and Analysis of Tagging Studies", sponsored by FDEP, St. Petersburg, FL.

- November 15-17, C.Neidig, R.DeBruler and P.Dufault attended the Florida Aquaculture Association (FAA) Conference hosted by FDEP/USF, St. Petersburg, FL.

- November 15, C.Neidig, R.DeBruler and P.Dufault conducted a cooperative (SERF/MML) workshop, "Live Food Culture in Extensive/Intensive Systems" at SERF as part of the FAA conference.

- November 19-20, C.Neidig, R.DeBruler and P.Dufault participated in a Tagging Workshop presented by Northwest Marine Technologies (Seattle, WA) at MML, Sarasota, FL.

- November 21-23, C.Neidig, R.DeBruler and P.Dufault attended the MML/Florida State University (FSU) Stock Enhancement Symposium held at MML, Sarasota, FL.
November 23, C. Neidig chaired a session at the MML/FSU Stock Enhancement Symposium.

1997

February 18, C. Neidig and D. Skapura participated in a World Aquaculture Society sponsored tour of the Hood Canal Shellfish Facilities and Salmon Hatchery, Olympic Peninsula, WA. Note: The tour included large scale-production facilities for oysters, clams, scallops and salmon and processing plants for world-wide distribution.

February 20-23, C. Neidig and D. Skapura attended the Annual International Conference and Exposition of the World Aquaculture Society, Seattle, WA. Note: The following four posters (poster texts are included in Appendix III) were presented displaying information from studies funded by FDEP.

• C. L. Neidig, D. P. Skapura, H. J. Grier, Ph.D. Evaluating egg quality in common snook, Centropomus undecimalis.

• C. L. Neidig and P. C. Dufault. Surface spray -- a method for improving larval common snook, Centropomus undecimalis, survival.

• C. L. Neidig, P. C. Dufault, R. O. DeBruler, Jr. and D. P. Skapura. Methods for maintaining red grouper, Epinephelus morio, (Serranidae) in controlled environment tank systems.


In addition, a poster presented by HBOI resulted from a 1995 cooperative study between HBOI, FDEP and MML.


• March 10, C. Neidig and R. DeBruler presented the four posters listed above at the Mote Marine Laboratory, Monday Night at Mote Series.

C. Miscellaneous

The Mote Marine Laboratory Aquaculture/Fish Stock Enhancement program integrated volunteers and college interns to assist with duties pertaining to cooperative studies performed at SERF. Responsibilities included assisting with snook collections, spawns and related studies.
IV. REFERENCES


APPENDIX I

ACHIEVEMENTS
OF THE
NEW SNOOK PROJECT
ACHIEVEMENTS OF THE NEW SNOOK PROJECT

*Developed new broodstock handling techniques. Application/Conclusion: We can now spawn snook and keep broodstock mortality at an extremely low level, if not achieve 100% survival.

*Conducted successful dose/response studies with human chorionic gonadotropin (HCG). Application/Conclusion: We can now achieve the same rate of spawning and egg quality with a much lower dose of HCG. This can substantially reduce the cost of hormones per breeding season.

*Demonstrated that, as in the old Snook Project, the direct stocking of snook larvae into outdoor ponds has not been successful. Successes with snook have occurred when larvae were head-started in tanks, then transferred to ponds. Pond survival was better than 90%. Application/Conclusion: Our best information with snook is that larvae need to be head-started in tanks and then either grown-out in tanks or transferred to ponds.

*Conducted an international collaboration with Nancy Sherwood (University of Victoria) and Jean Rivier (The Salk Institute) which compared four different custom-synthesized, time-release GnRH analogs (pelleted by Sam Shafie, Innovative Research of America), in their ability to induce ovulation in snook. Application/Conclusion: Use the lowest cost analog in time-release form to spawn snook.

*Spawned snook without hormones, with HCG or GnRH and collected materials to study changes in post ovulatory follicles over time. Application/Conclusion: Fisheries biologists can use this type of information to develop reliable backdating methods which predict the frequency and the time of spawning.

*Conducted successful experiments using gonadotropin releasing hormone (GnRH) to spawn common snook. It was previously stated that this hormone does not work on snook. We determined that single injections of GnRH are not effective for spawning common snook, but that time-released GnRH is superior to HCG because it is less deleterious to the broodstock. Application/Conclusion: While both GnRH and HCG are effective in inducing spawning in snook, time-release GnRH is the hormone of choice.

*Successfully followed post-spawning ovarian regression in snook. While most histology is still not completed, initial evaluation clearly indicates the deleterious effect of HCG on the ovary over five days following spawning. Application/Conclusion: Better information is available, for the first time in any fish species, to make the most rational decision on what hormones can best be used induce spawning of fish.

*While work is still in progress, the germinal epithelium in the ovary of snook has been defined for the first time in any species of fish. Application/Conclusion: The information can be used by fishery biologists to more accurately define gonadal staging criteria.
*Developed the probiotic approach to rearing snook larvae. Survival of snook larvae increased significantly when using probiotic techniques as compared to not using them. These observations need to be studied further. **Application/Conclusion:** Probiotics may make the difference between releasing 6,000 or releasing 60,000 to 80,000 fingerlings. This is a potential major breakthrough in salt water fish aquaculture. Probiotics hold the promise of eliminating outbreaks of disease-causing bacteria in larval rearing systems without the use of antibiotics, thus reducing costs and achieving stock enhancement or commercial level production.

*Documented that tropical storms can interrupt the spawning cycle of common snook. **Application/Conclusion:** Spawning common snook may not be possible after a tropical storm passes over your hatchery and common snook habitat.

*Successfully matured and spawned common snook that were held in SERF ponds. **Application/Conclusion:** Captive snook can be held outdoors and spawned in the spring and summer months. It is not known if more than a single spawning can be achieved.

*Evaluated the use of cryopreserved snook sperm against freshly prepared sperm and developed new methodology for short-term sperm storage. **Application/Conclusion:** Cryopreserved sperm may be used for the fertilization of snook eggs which could become a management/genetics tool for stock enhancement. New short-term sperm storage protocols will increase the genetic diversity of fertilized eggs.

*Developed techniques for the evaluation of egg quality in snook. **Application/Conclusion:** These techniques can be directly applied to other finfish and invertebrates (scallops).

*Presented one paper at the European Aquaculture Society meeting and an additional 6 papers at the annual World Aquaculture Society’s meeting and compiled a listing of 36 potential papers as a result of collaborations between the SERF, Mote Marine Laboratory, Harbor Branch Oceanographic Institution, University of South Florida, North Carolina State University, University of Victoria, and the Salk Institute. **Application/Conclusion:** Research results are pertinent to revising all of SERF’s snook spawning protocols and are probably applicable to other salt water fish species. Although problems remain, the new Snook Project has opened the door to common snook propagation and stock enhancement. We owe this success to our extensive networking and collaborations, as well as to structuring the project to obtain the maximum amount of scientific information from our efforts.
APPENDIX II

PROPOSED COMMON SNOOK RESEARCH PAPERS
PROPOSED COMMON SNOOK RESEARCH PAPERS

The following list of proposed and submitted common snook research papers represents the accomplishment of all years of the old (1986-1991) and new (1994-1997) Snook Projects. Those titles that have been generated within the new Snook Project have their numbers preceded by a star symbol (※). Significant contributions to both Projects have been made through collaborations and networking, particularly in the new Snook Project whose success is derived directly from them. After each paper title or proposed paper title, the collaborator's institutions are indicated: The Stock Enhancement Research Facility (SERF), the Mote Marine Laboratory (MML), the Florida Marine Research Institute (FMRI), the Harbor Branch Oceanographic Institution, Inc. (HBOI), the University of Victoria (UV), the North Carolina State University (NCSU), Louisianna State University (LSU), and the Salk Institute (SI). Four titles have been identified as requiring some additional work.


3. Changes in blood steroids in common snook associated with the annual reproductive cycle. (NCSU, SERF, FMRI)

4. The diel spawning cycle of common snook. (FMRI, SERF)

5. Structure of the common snook pituitary gland and seasonal changes associated with reproduction as revealed by classical stains adapted to plastic sections. (SERF, FMRI)

※6. A comparison of single injection doses of human chorionic gonadotropin and mammalian gonadotropin releasing hormone on the induction of ovulation in common snook. (SERF, MML)

※7. Broodstock handling techniques, oocyte staging, and evaluation of egg quality in common snook. (MML, SERF)

※8: The ovarian germinal epithelium in common snook. (SERF)

1This listing of common snook papers and proposed subjects/titles was revised as of 5/27/97. Please help to update this list by giving additional titles to either Harry Grier or Carole Neidig. A left-justified citation, with author's names in bold print, will replace the centered subject title when a submission is made to either the FMRI editors or a journal. Upon publication, the citation will be right and left justified. Thank you for helping to keep this listing current.
9. Spermatogenesis and spermiogenesis in common snook. (SERF)

*10. Human chorionic gonadotropin dose-response and the timing of injection as they affect ovulation in common snook. (SERF, MML) 
(needs additional work)

*11. The post ovulatory follicle in common snook and estimation of the natural spawning frequency. (SERF, FMRI)

*12. A comparison of four different gonadotropin releasing hormone analogs on ovulation and egg quality in common snook. (SERF, MML, UV, SI)

*13. Sperm cryopreservation and fertilization in common snook. (LSU, MML, SERF)

*14. Post-capture gonadal regression in common snook. (SERF, MML)

*15. HCG-induced ovarian regression in common snook. (SERF, FMRI, MML)

*16. Comparative effects of aldehyde fixatives on qualitative histology of common snook gonads. (SERF, FMRI)

*17. Tag retention and wound healing around plastic tags in common snook. (FMRI, SERF)

18. Sex reversal in common snook. (SERF, FMRI)

19. Photothermal effects on the induction of reproductive readiness in common snook. (SERF)

20. Vitellogenesis in common snook—an ultrastructural study. (SERF)

*21. A qualitative comparison between spawns obtained from hormone-injected and naturally-ovulating common snook. (SERF, MML) 
(needs additional work)

*22. Identification of gonadotropin releasing hormones in common snook. (UV, SERF)

23. Age and growth in common snook. (FMRI, SERF, FMRI)

*24. Temperature effects on larval survival in common snook. (MML) 
(needs additional work)

*25. Pond reproductive maturation and induced ovulation in common snook. (SERF)

26. A comparison of interstitial tissues in the testis of reproductive and
nonreproductive common snook. (SERF, FMRI)

*27. Surface spray - a method for improving larval snook survival. (MML)

*28. A comparison of larval snook growth using different live-food diets. (MML)

*29. Correlation of common snook egg quality with lunar and tidal cycles. (FMRI, SERF, MML)

(needs additional work)

30. The effects of different diets and growth and survival of juvenile common snook. (MML)

*31. Fatty acid profiles from common snook eggs derived from hormone and natural spawned fish. (MML, HBOI, SERF)

*32. Increased production of rotifers treated with Bacillus sp. isolated from common snook (Centropomus undecimalis) larvae. (HBOI)


*35. Wilson, R., Jr., Donaldson, K. (1997). Restriction digest of PCR-amplified mtDNA from fin clips is an assay for sequence genetic “tags” among hundreds of fish in wild populations. submitted: Molecular Marine Biology and Biotechnology. (USF)


NEW SNOOK PROJECT PAPERS PRESENTED AT MEETINGS


Seattle, Washington.


APPENDIX III

POSTER PRESENTATIONS

ANNUAL INTERNATIONAL CONFERENCE AND EXPOSITION OF THE WORLD AQUACULTURE SOCIETY
SEATTLE, WA

FEBRUARY 20-23, 1997
Evaluating egg quality in common snook, *Centropomus undecimalis*

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INTRODUCTION

Common snook, *Centropomus undecimalis*, one of Florida’s most important sport fish species, has been designated for stock enhancement by the state of Florida. However, intensive efforts to achieve hatchery-level production of larval snook has met with limited and inconsistent success.

As with other marine finfishes, a primary objective in snook aquaculture is to provide optimal egg quality for fry production. According to Bromage, et al, (1994), good quality eggs exhibit low levels of mortality at fertilization, hatch, and up to first-feeding; they would be expected to produce the healthiest and fastest growing fry. An obstacle to establishing egg quality is difficulty in identifying parameters that provide reliable predictions of performance. Limited information is available concerning indicators for determining egg quality, especially in marine fishes (Kjørsvik et al. 1990).

The goal of this study was to establish reliable and simple to perform procedures for assessing egg quality in snook. The objective was to compare methods of measuring egg quality using eggs produced from natural ovulating females and eggs from hormone-induced spawns. The parameters for determining egg quality were, 1) fertilization rate, 2) egg morphology & diameter, 3) oil droplet diameter, 4) percent hatch; and, 5) larval survival to first feeding.

METHODS AND MATERIALS

Adult snook were captured by seine net at weekly intervals from May through Mid-September. Females were divided into three groups:

1) Fish injected with human-chorionic gonadotropin releasing hormone (HCG);
2) fish implanted with gonadotropin releasing hormone (GnRH); and
3) natural ovulating females.

Eggs from each group were collected and criteria for snook egg quality determination were compared.

- Percent of fertilized eggs were qualitatively and quantitatively assessed by examining a minimum of 100 eggs and enumerating eggs undergoing symmetrical cleavage.
Cellular malformations were noted in early cleavage stages. Egg/oil ratios were obtained by measuring diameters of a minimum of 50 egg and oil droplets at the blastula stage. Hatch success was evaluated by measuring percent of hatched larvae (approx. 20 hours post-fertilization) from a random sample of eggs. Survival to first feeding was determined by enumerating larvae surviving to approximately 72 hours post-hatch from three samples of 50 eggs.

Hatch success and survival to first feeding studies were conducted in water tables to maintain constant temperature. Eggs for hatch success trials were placed in 800ml glass jars containing 400mls filtered seawater. Whereas eggs tested for survival to first feeding were placed in submerged 400ml glass beakers with fine mesh screen collars, allowing for water circulation but confining eggs and larvae. The seawater in the survival to first feeding system was aerated and circulated through a biofilter and ultraviolet light.

RESULTS
Percent fertilization correlated with percent survival and percent hatch for eggs from GnRH spawned females. Egg diameter correlated with percent hatch for eggs from natural spawns. Egg and oil droplet ratios were closely correlated. Larger eggs had larger oil droplets. Oil droplet diameter correlated with survival of eggs from natural spawns. Percent hatch correlated with percent survival of eggs from GnRH and HCG spawns. Eggs with high percent hatch did not always result in high percent survival to first feeding.

DISCUSSION
Percent fertilization was useful for detecting poor egg quality during early development, although, it did not always correlate with survival of snook larvae to first feeding. Preliminary studies indicate that egg and oil diameters are not a good criteria for egg quality.

Initial observations indicated that percent hatch was a good indicator of egg quality. Statistical analysis showed that there were no strong correlations to support this observation. In addition, the most practical method for determining egg quality should be made before incubation.

Additional parameters measured were:
- Moon phase
- Tide
- Female Length (mm)
- Hormone Dose
- Time of Injection
- Time of Ovulation
- Spawn Volume
- Lipid Analyses
Data are being analyzed and results will be reported as available. Because there are no universal egg quality tests available, further investigations are needed to identify specific indicator(s) of snook egg quality for producing good eggs for fry production.

REFERENCES

INTRODUCTION
Efforts to intensively culture common snook, *Centropomus undecimalis* have met with limited and inconsistent results. Snook larvae inflate their gas bladders within 96 hours of hatching by gulping air at the air-water interface. Because of oil and debris on the water surface from hatched eggs and oil supplements in larval food (zooplankton), larvae may experience difficulties in gas bladder inflation. Larvae with uninflated gas bladders swim poorly and expend extra energy to maintain position in the water column which results in poor survival. A preliminary evaluation of the use of surface spray was undertaken in an attempt to improve culture conditions and reduce early mortalities.

METHODS AND MATERIALS
Numerous methods were tried to eliminate surface oil and debris which included:
- Increased aeration
- Water flow
- Absorbent materials; and
- Surface spray

Circular culture tanks (3,555 L) were equipped with an inflow surface spray directed into the tank with a 360° perimeter nozzle located 80 cm above the surface and over the center of the tank. The outlets were four polyvinyl chloride (pvc) standpipes covered with fine mesh screen positioned quadrilaterally in the tank. Recirculating seawater was passed through a trickle biofilter and ultraviolet light.

RESULTS AND DISCUSSION
Surface spray helped to eliminate surface oils, distribute food items (zooplankton and dry diets), and produce water currents in which larvae could orient and feed. Preliminary observations indicated that use of surface spray improved the culture environment, increased gas bladder inflation in larvae which resulted in greater survival.
INTRODUCTION
A protocol was developed to maintain healthy red grouper, *Epinephelus morio* in indoor tanks for gender reversal and maturation studies. Before this, significant mortalities occurred as a direct or indirect result of poor diet and monogenean trematode infections. Research efforts focused on developing a healthy environment and diet to maintain red grouper and identification of safe, effective, cost-efficient methods for managing potential disease problems. New methods resulted in the maintenance of healthy red grouper for over three years.

METHODS AND MATERIALS
Management of red grouper, 35.0 - 70.0 cm SL, included careful attention to handling, reducing stress, maintaining good water quality, controlling infections and providing good nutrition.

A three-step approach was developed to maintain healthy grouper:

**Step 1 - Development of Healthy Physical Environment**

Modifications made to the holding facility: improved seawater filtration using a sand filter, diatomaceous earth (DE) filter, vertical screen biofilter and ultraviolet (uv) light treatment; water temperature monitored with electronic sensors; dissolved oxygen regulated with a oxygen injection system; circulation created by air blower connected to airlifts; illumination provided by daylight spectrum bulbs; and, tank dividers to separate aggressive fish.

**Step II - Chemical Treatments**

Chemotherapeutic/chemoprophylactic methods for controlling external parasites were used sparingly and with caution.

Health hazards, high costs, limited availability and the possibility of development of resistant strains of parasites and pathogens have precluded the routine use of drugs and chemicals for treatment (Thoney and Hargis, 1991).

Alternative approaches which included using routine freshwater dips were sought for the control of monogenean trematodes, the most common ectoparasite on fish brought into our facility. Also, prophylactic and aseptic techniques in fish maintenance were followed to control transfer and introduction of parasites and pathogens.
Step III - Diet

A commercially prepared diet was initially fed to grouper. Results from total lipid and fatty acid analysis of liver and abdominal fat were used to help develop a diet in an attempt to reduce stress and improve growth.

RESULTS
Presented in Table 1 are chemicals/drugs used for parasitic and pathogenic infections. Freshwater dips were found to be highly effective as a means of controlling ectoparasites and no adverse effects were experienced by red grouper.

A diet composed of fish flesh, shrimp, powdered kelp and a nutritional supplement contributed to regular weight gains, reduced stress, and healthy red grouper.

DISCUSSION
At our lab the use of increased filtration, routine maintenance with a freshwater dip, and improved nutrition, significantly reduced red grouper mortality. These methods produced such healthy fish that females maintained for three years were gravid.

Freshwater dips of grouper were used routinely because of survivability of trematode eggs. This was found to be a safe, effective, economical method of treatment for trematodes and other parasites in our Lab.

Mueller et al., (1994), suggested that until cost-effective, environmentally sound treatments are developed, marine finfish culture may be constrained by ectoparasitic infections due to parasitic virulence and lack of host specificity. At our Lab the three-step approach has enabled us to maintain healthy red grouper for several years.

REFERENCES


### Table 1. Treatments For External Pathogens/Parasites:

**Bacterial Infections:**

- Dylox \(^a\)
- Organicure \(^b\)
- Fresh Water
- Nitrofurazone \(^c\)
- Neomycin \(^d\)

\(^a\) Dylox = (2, 2, 2-trichloro-1-hydroxy ethyl) Phosphoric Acid Dimethyl Ester
\(^b\) Organicure = A Commercial Mixture of Copper & Formalin
\(^c\) Nitrofurazone = 5-Nitro-2 Furaldehyde Semicarbazone
\(^d\) Neomycin = Neomycin Sulfate as a Powder
A comparison of prey selection of red drum, *Sciaenops ocellatus* (Linnaeus), from three Florida estuaries based on gut content analysis

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INTRODUCTION
Red Drum (*Sciaenops ocellatus*), is an important recreational estuarine-dependent fish found in the Gulf of Mexico and along the Southeastern Coast of the United States. In Florida, red drum stocks have declined during the past decade (Goodyear, 1991). The Florida Department of Environmental Protection (FDEP) supported several studies using red drum as a test species to evaluate the efficacy of marine stock enhancement. Hatchery-reared fingerling and juvenile red drum were marked with coded wire tags or with internal anchor tags and released off the West and East Coasts of Florida. Monitoring studies were performed to provide information on released red drum survival, growth, habitat preference, movement, recruitment and diet.

Mote Marine Laboratory personnel were responsible for identifying and quantifying prey of collected hatchery-reared tagged and wild red drum, based on gut content analysis. Fish were sampled from study sites in Bishop Harbor, Tampa Bay and Bowles Creek, Sarasota Bay on the West Coast and Murray Creek, Volusia County, on the East Coast of Florida. The gut content study provided information on feeding habits of fingerling and juvenile red drum related to size at capture and to collection site. Results demonstrate important transitions in diet that occur in different size groups of red drum.

METHODS AND MATERIALS
To determine food selection of tagged hatchery-reared and nontagged wild red drum fingerlings and juveniles, food items in the stomach and intestines were identified to species using a dissecting microscope, enumerated, and weighed. Fish were measured, total length (TL) and standard length (SL) and weighed (g). The gut was eviscerated by making an incision anterior to the esophagus and another at the anus. The stomach and intestines were removed and weighed (g). Stomach fullness was defined on a scale of 0 (empty) to 5 (distended).

RESULTS
Gut contents from 1,921 (1,008 tagged & 913 wild) fingerling and juvenile red drum from the three study areas were examined. Red drum were divided into three size groups; 50-89mm (TL), 90-129mm (TL), and 130-189mm (TL). The most common prey items were: crustaceans (amphipods, decapods, and mysids), fish (*Anchoa sp.*), and polychaetes. *Corophium sp.* was the most abundant amphipod and was the
predominant food item for the 50-89mm (TL) and 90-129mm (TL) red drum from all sites mysids predominated in red drum (50-89mm [TL] and 90-129mm [TL]) from the West Coast with few found in East Coast red drum. East Coast red drum (all sizes sampled) consumed significantly more fish than West Coast red drum. polychaetes were eaten by all size ranges of red drum, but were more prevalent in the guts of the 90-129mm (TL) red drum from the East Coast. Decapods were the predominant prey item in 130-189mm (TL) red drum from both coasts. Tanaids played a significant role in the diet of 50-89mm (TL) fish from the East Coast.

DISCUSSION
There were no statistical differences observed between the diet of hatchery reared (tagged) and wild fingerling and juvenile red drum from the West or East Coasts of Florida. Peters and McMichael, (1987) found that small juveniles (10-75mm TL) collected in Tampa Bay, Florida, fed predominantly on mysids, amphipods and polychaetes. In West Coast red drum, 50-89mm (TL) amphipods, mysids and polychaetes were predominate, whereas in East Coast red drum, amphipods, tanaids and fish predominanted. The differences in occurrence of fish found in the red drum from each coast was probably related to availability. Additional gut items (plant material, sand and mud) were inadvertently consumed. The red drum appeared to be indiscriminate feeders since most fish contained a combination of two or more distinct prey items. This agreed with observations made by Boothbay and Avault, (1971) from red drum captured in a coastal marsh area off Southeastern Louisiana.

REFERENCES


