

**SCIENCE CONSORTIUM
FOR OCEAN REPLENISHMENT AND ENHANCEMENT
(SCORE)
2003 Proposal**

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Executive Summary

The Science Consortium for Ocean Replenishment and Enhancement (SCORE) addresses critical uncertainties about enhancement of important commercial and recreational species that range the different coastal environments in the U.S. First funded in the 2001 Commerce-Justice-State Appropriations, SCORE is a national partnership established to develop and test the full potential of marine stock enhancement as a solution to the current “maxed out” status of U.S. coastal fisheries. This research consortium is designing and coordinating integrated research programs and field trials needed to develop and test enhancement potential in a variety of coastal habitats. SCORE provides a critical, science-based forum for conducting and evaluating individual fisheries enhancement practices. SCORE scientists are documenting in peer reviewed scientific journals progress on sustainable fisheries production using experimental fish releases, and by testing the validity of the stock enhancement concept.

As scientific gains are made in understanding the potential, SCORE scientists will partner 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 will provide the cadre of citizens needed to support and expand enhancement as a fishery management strategy. SCORE scientists envision that much of 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, SCORE scientists will conduct key experiments to resolve critical uncertainties about how to conduct and monitor the biological, ecological, and economic effectiveness of stock enhancement.

Eventual partners in SCORE include private non-profit institutions, universities, and federal and state agencies. The founding partner organizations are Mote Marine Laboratory (MML) of Florida, the University of New Hampshire (UNH), the Manchester Marine Research Station of the National Marine Fisheries Service (NMFS-MMRS), and the Washington State Department of Fish and Wildlife (WDFW). More partners will be included as the technology is developed and transferred to other coastal States. A close alliance with the Gulf of Mexico Marine Stock Enhancement Consortium (GMSEP) has already been developed that will enable synergistic advances in the science of marine stock enhancement in the U.S.

SCORE brings together a powerful and synergistic combination of scientific expertise and practical experience. Collectively, its members have scientific expertise in fisheries science, genetics, nutrition, pathology, ecology, biometrics, behavior, and reproductive physiology. They also have access to resources in hatchery technology, grow-out, life-support engineering, fish tagging, fisheries economics, recreational and commercial fishing. Each group has done

pioneering work in the relatively new science of marine stock enhancement. Each has the infrastructure and skills to culture at least one economically important species that will serve to develop scientific protocols for responsible stock enhancement. The geographic distribution of the members funded in FY 2003 enables parallel research to be carried out in the cold, temperate, and semi-tropical ocean environments of the Atlantic Northeast, Southeast and Eastern Gulf of Mexico. In this proposal, SCORE researchers will conduct pilot release experiments on two coasts to advance our understanding of critical uncertainties about stocking strategies and the effectiveness of hatchery-releases as a fishery-management tool. Ecological and aquaculture research will also be conducted to provide critical support and information for tests of enhancement potential.

Introduction

The State of World Fisheries and Aquaculture (FAO, 2000) has confirmed that the prospects for growth in annual fishery landings are nearly zero. At least 47% of the 441 major fish stocks in the world are fully exploited and are producing catches at or near their maximum limit. About 28% are over-exploited, and have no room for expansion; 21% are moderately exploited, and only 4% are under-exploited (FAO, 2000). A similar situation exists in the United States, where many of our traditionally harvested species are at record low levels. Fisheries in the Pacific and Atlantic waters reached their maximum production levels almost two decades ago, and are now showing a declining trend in total catches (NMFS, 1999). Annual catches in the Western Central Atlantic, Eastern Central Pacific, and Northeast Pacific appear to have stabilized, having reached a maximum potential a few years ago.

Catch trends indicate the nation's coastal waters have the highest incidence of fully exploited fish stocks and of stocks that are either over exploited, depleted, or recovering after having been depleted. These statistics indicate a general failure in fisheries management and suggest that we will have to harvest less, rather than more from the world's oceans in the future. This failure, combined with growing demand due to exploding human population growth, will lead to a growing gap between supply and demand for fisheries products.

In spite of its diminishing fisheries stocks, the United States remains a leading consumer of seafood. Last year the country posted record imports of \$9.2 billion, widening the annual seafood trade deficit to almost \$6.4 billion. This deficit will never decline. Consumer demand for seafood continues to grow with the high level of disposable income in the country, and relevant changes in population demographics, such as increasing longevity, better health, early retirement, and the steady migration from the interior to the coastal states. However, its speed of growth can be arrested by a national goal of greater self-sufficiency in seafood production.

There are several strategies to attain the objective of increased fisheries production, such as:

- § *Sustainable fisheries management* -- effective management produces results, and about 3% of the major fish stocks are reported to be recovering slowly.
- § *Maximum utilization of the existing fisheries harvest* -- serious attention is now being turned to the waste of discards, which can be as high as 25% of the marine fisheries harvest.
- § *Aquaculture* -- national farmed production now contributes about 15% of the harvest of edible fish and shellfish.
- § *Stock enhancement* -- recreational harvest of the culture-based marine fisheries makes up about 4% of the national landings of edible fish and shellfish

For the most part, all of these strategies are being applied by the U.S. Government in its national plans and policies for fisheries development (NOAA Fisheries Strategic Plan 1998). Some were prioritized for special funding in Ocean 2000 -- the Year of the Ocean Initiative. However, the strategy of stock enhancement is receiving little detailed attention other than activities to improve fish habitats and the possible benefits from new aquaculture technologies for marine fish. This is inadequate. Stock enhancement of both inland and coastal fisheries is a very complex undertaking. It requires significant scientific knowledge in a diverse array of fields, high levels of several technological skills, and finally sensitive application to put into practice. If successful, it is the most cost-effective countermeasure to diminishing stocks. More importantly, it provides interim results well before the long-term benefits of good fisheries management.

Stock Enhancement Background

Stock enhancement lies at the nexus between sustainable fisheries management and fish and shellfish culture. It may be defined as the rearing of fish and shellfish, both juveniles or adults, for their subsequent release in open waters to enhance natural populations. Although the goals of any given stock enhancement program vary, typically they seek to:

- § provide additional catch for commercial and recreational fishermen
- § rebuild spawning stock biomass for the promotion or acceleration of recovery
- § ensure the survival of stocks threatened by extinction
- § mitigate losses due to anthropogenic effects.

Stock enhancement is not a new strategy for replenishing depleted fish stocks. The first marine programs started in the United States in the late nineteenth century, and coastal hatcheries were built and operated successfully in Gloucester and Woods Hole, in Massachusetts, and Boothbay Harbor in Maine. For over sixty years many billions of young cod, haddock, pollock, winter flounder, and even lobster were released in an effort to augment wild populations. Apart from the latter, the programs for marine fish were subsequently discontinued and the hatcheries closed. Primarily, this was because there was no way to correlate the releases of juveniles with measurable increases in the landings of adults. But this was hardly surprising. At that time there were no effective methods for marking hatchery fish and so there was no way to monitor their survival and growth, or to determine whether they actually contributed to rebuilding the population. Furthermore, there was little scientific knowledge of the early life histories of these fish in nature and so their condition and that of the receiving environment at the time of release were largely unknown. Aquaculture techniques for marine fish that spawn in seawater were undeveloped; eggs and yolk-sac fry could be released, but the technology for mass production of fingerlings didn't begin to appear in Europe and North America until the 1970's.

Greater scientific recognition of the challenges, together with significant advances in marine fish breeding and propagation technologies, have given a renewed interest in enhancement as a strategy to replenish declining fish and shellfish stocks. But will today's fisheries scientists succeed where the early pioneers failed? Unquestionably the answer is, yes. First, advances in aquaculture technologies now enable large numbers of high-quality disease-free juveniles to be produced in hatcheries, and there is a far greater understanding of the ecological requirements of the receiving waters. Secondly, there is a wide array of high-tech marking techniques available and more sophisticated methods to measure growth and survival and monitor contribution to the fishery. Finally, many stock enhancement programs are already underway overseas, and early results have been promising, particularly in Japan (Masuda and Tsukamoto, 1998; Hilborn, 1998).

In the 1990's marine stock enhancement began to move beyond the early fact finding stage that Kuhn (1970) observes characterizes new fields of science. After a century of preoccupation with fish culture, researchers in the field of marine enhancement (i.e. focused on marine spawners) began to publish tests of the hypothesis that cultured marine fish could survive in the wild and contribute to fishery landings. A rapid expansion of scientific studies and philosophical debate on marine hatchery releases has begun, following initial publications by researchers in Japan and Norway (Tsukamoto *et al.* 1989; Kristiansen and Svasand, 1990; Svasand and Kristiansen, 1990a,b; Svasand *et al.*, 1990), and earlier work with salmonids in the USA and Canada (e.g., Hager & Nobel, 1976; Bilton *et al.*, 1982)(e.g. see studies and additional citations in Munro and Bell, 1997, and in symposia proceedings edited by Lockwood, 1991; Danielssen *et al.*, 1994; Schramm & Piper, 1995; Coleman & Travis, 1998; and Howell, Moksness and Svasand, 1999).

Marine stock enhancement has begun to be treated scientifically (discussed in Leber, 1999, 2002).

Now, we must deal with the lack of theoretical development in marine stock enhancement and the clear need to reduce uncertainty about the effects of hatchery releases in coastal environments. Wider use of the scientific method and “strong inference” (Platt, 1964) would advance knowledge in this branch of fisheries science considerably faster than its current pace (Leber, 1999). We can characterize much of the experimental work now emerging in this field as a period of trial-and-error evaluation of the hatchery-release hypothesis (releasing cultured fish can increase fishery production). We have entered a *passive-adaptive assessment phase* of marine stock enhancement, which is best described by Walters and Hilborn’s (1978) (and see Hilborn and Walters, 1992) *passive-adaptive management* approach. Platt (1964) argues that, for exploring the unknown, there is no faster method than “strong inference” — the *systematic application* of the age-old scientific method of inductive inference that dates back to Francis Bacon. What makes “strong inference” so effective is systematically “...recycling the procedure, making sub-hypotheses or sequential hypotheses to refine the possibilities that remain; and so on” (Platt, 1964). A key component of “strong inference” is acknowledging the competing alternative hypotheses (major uncertainties) that could explain an observation, and then rigorously weeding out the false alternatives through experimentation. Platt reminds us that for a hypothesis to be testable we must be able to state what conditions would show the hypothesis is false (Popper, 1959, 1965).

Walters and Hilborn (1978) reiterate Platt’s argument about exploring the unknown, but add a caveat for fishery management, “We learn most rapidly by introducing large disturbances and much monitoring, but we incur high risks and costs by doing so” — the “dual control problem.” The paradox is that to advance this field we must experiment; yet funding for marine stock enhancement research lies largely within the management agencies that are implementing hatchery releases. By mandate, the agencies must manage resources (i.e. implement enhancement, not study it). The solution to this paradox is to gear up long overdue research programs to evaluate the range of critical uncertainties that cloud our understanding of stock-enhancement potential, and to engage resource management in *active-adaptive management* (Hilborn and Walters, 1992). With this approach, risks of failure are restricted to substocks of the stocks being managed, allowing systematic experimental evaluation of critical uncertainties to become an integral part of fisheries management strategy. Active-adaptive management is essentially “strong inference” adapted to fishery science. A quick scan of Platt’s (1964) paper reminds us also that it is the *systematic application* of a logical tree of hypothesis tests, and exclusions, that produces much more rapid progress than in fields of science that use other approaches. Coupling “strong inference” and active-adaptive management principles to marine stock enhancement research is the logical next phase of this field.

The new pioneers in marine enhancement are fisheries scientists and fishermen, working together on a shared but carefully allocated resource, as exemplified in Japan. The Japanese program involves about 80 species of marine fish, mollusks, and crustaceans. The principal enhanced marine fisheries are yesso scallop, Kuruma prawn, red sea bream, and flounders. The practice benefits from the country’s extensive continental shelf but it is estimated that stocking accounts for 90% of the chum salmon fishery, 50% of the Kuruma prawn catch, up to 75% of red sea bream, almost all the scallop harvest, and up to 40% of the flounders (Kitada 1996). Techniques used by the Japanese to support hatchery releases include habitat restoration, predator removal, and behavioral conditioning. There is also a strong commitment to the program by coastal fishing communities.

Another pioneering country is Iran, which, since the break up of the U.S.S.R., now shares the fisheries resources of the Caspian Sea with four other states. Within its zone, fisheries scientists in Iran raise and release about 12 million juveniles of the indigenous sturgeon species, which support almost the entire nationally-controlled fishery and therefore the valuable caviar industry. In addition, state hatcheries release juvenile bream, kutum, pike-perch, and Caspian trout, all of which support fisheries harvested by licensed coastal cooperatives.

Other countries active in marine fish enhancement include Norway, Denmark, Iceland, U.K., France, Spain, Australia, Thailand, and China. Many Island nations of Oceania have active programs for restocking their indigenous populations of mollusks, such as giant clams, pearl oysters, trochus, and green snails.

There are few marine stock enhancement programs underway in the U.S., but several research and development projects are underway. In addition to the well-established program for lobsters in Massachusetts, there are pioneering projects for red drum (Florida, South Carolina and Texas), Pacific threadfin, mullet, and snapper (Hawaii), red snapper (Mississippi, Alabama and Florida), white seabass (California), summer flounder (North Carolina), cod (Maine), lingcod (Washington), snook (Florida), and winter flounder (New Hampshire). Among the largest of the marine enhancement programs in the U.S. has been the red drum program in the Gulf of Mexico, where hatchery reared juveniles are reported to comprise some 20% of the population (Anon. 1992).

SCORE

SCORE addresses critical uncertainties about enhancement of important commercial and recreational species, which range the different coastal environments in the country. This research consortium will design and coordinate integrated research programs and field trials needed to develop and test enhancement potential in a variety of coastal habitats. SCORE provides a critical, science-based forum for conducting and evaluating individual fisheries enhancement practices. SCORE scientists will monitor and document progress on sustainable fisheries production using experimental fish releases, and by testing the validity of the stock enhancement concept.

Partners in SCORE include private non-profit institutions, universities, and federal and state agencies. Founding partner organizations are Mote Marine Laboratory (MML) of Florida, the University of New Hampshire (UNH), the Manchester Marine Research Station of the National Marine Fisheries Service (NMFS-MMRS), and the Washington State Department of Fish and Wildlife (WDFW). FY 2003 congressional funding for SCORE was specified for the University of New Hampshire and Mote Marine Laboratory. More partners will be included as the technology is developed and transferred to other coastal States.

The intent of SCORE is to bring together a powerful and synergistic combination of scientific expertise and practical experience. Collectively, its members have scientific expertise in fisheries science, genetics, nutrition, pathology, ecology, biometrics, behavior, and reproductive physiology. They also have access to resources in hatchery technology, grow-out, life-support engineering, fish tagging, fisheries economics, recreational and commercial fishing. Each group has done pioneering work in the relatively new science of marine stock enhancement. Each has the infrastructure and skills to culture at least one economically important species that will serve to develop scientific protocols for responsible stock enhancement. The geographic distribution of the members enables parallel research to be carried out in the cold, temperate, and semi-tropical ocean environments of the Atlantic Northeast, Southeast and Eastern Gulf of Mexico.

Alternate Views and Critical Uncertainties about Stock Enhancement

Alternate views to the concept of marine stock enhancement can be expected from some conservation biologists, resource managers, and environmentalists. Opposition to marine stock enhancement is fueled by the current controversy over salmonid hatcheries in the Pacific Northwest, where opposition has focused on potential damage to ESA-listed wild stocks from a hatchery system that presently produces fish that are captured in mixed stock fisheries containing both wild and hatchery fish. Hatchery fish are harvested at high rates, which cannot be sustained by wild populations. Additionally, it is believed that inbred or introduced strains of hatchery fish, when spawning with wild fish, will result in loss of wild-stock fitness. It can be expected that these same legitimate concerns will be expressed as new proposals for marine stock enhancement appear. More specifically, conservation biologists will be concerned that stock enhancement will disrupt natural evolutionary processes and result in loss of local adaptability in naturally spawning stocks. Fishery resource managers may favor a science-based regulatory fishery management approach over the stocking of marine fish as a means to recovery. The environmental community, on the other hand, may oppose the philosophy of marine stock enhancement on the basis that technological fixes for the depletion of fish stocks simply substitute one problem for another and divert attention from the real problem, the need for better management of wild stocks.

These concerns are generally classified as genetic, ecological, management, and philosophical in nature. The main issues and some of the critical uncertainties are as follows:

(i) Effects of stock enhancement on ecosystem dynamics

- § Is there sufficient environmental carrying capacity to support additional production at release sites?
- § Do hatchery fish add overall population size or simply replace wild stocks in their habitat?
- § Can habitat be expanded to incorporate larger numbers of stocked fish without depleting wild stocks?
- § What are the health effects on wild fish through transmitted disease?
- § What are optimal release strategies that maximize survival without impacting wild populations?
- § What are key measures of suitable habitat for releases?

(ii) Effects of stock enhancement on wild fish genetics

- § What is the genetic structure of the population to be enhanced?
- § Is the genetic structure of released fish representative of the natural population that is to be enhanced?
- § Is inbreeding /outbreeding depression or domestication selection a problem during the culture phase?

(iii) Fishery enhancement vs. Fishery management

- § When should hatchery releases be used, and what issues need to be resolved before beginning a hatchery-release program?
- § What are the goals of stock enhancement?
- § What are the explicit indicators of success, and how will 'success' be measured?
- § What determines when to stop?
- § What other fishery management strategies need to be coupled with stock enhancement?
- § Do the costs of stock enhancement outweigh the gains?
- § Can the same level of enhancement gained from hatchery releases be achieved through stronger fishing regulations and enforcement?

(iv) Philosophical issues

- § Will fishery enhancement be viewed as a panacea that can replace other management strategies?
- § If stock enhancement proves to be successful will it replace harvest reductions and habitat protection/restoration as viable alternatives? Will it be hard to turn off?
- § Is marine fisheries enhancement an environmentally viable fishery management tool or simply another case of 'techno-arrogance'?
- § If successful, will stock enhancement result in loss of funds for other management techniques?

Clearly, a critical, science-based evaluation is needed of the potential for stock enhancement to replenish depleted marine fisheries and increase fisheries production. Historical emphasis on release magnitude has overshadowed key questions, like those stated above, about stocking effectiveness and effects on fisheries landings and ecological/genetic impacts to fish populations. Furthermore, the inattention of the science community in applying rigorous scientific inquiry in evaluating release impact has fueled opposition to marine stock enhancement. Advances in 'enhancement effect' have not kept pace with advances in development of new hatchery culture technology, and this has resulted in uncontrolled and unevaluated releases.

If the current trend continues, new marine aquaculture capabilities will likely spawn many new stocking programs, and the debate over stock enhancement will intensify. Until there is a concerted scientific effort to make major progress in developing and testing stock-enhancement theory, we believe that these new programs will fail to meet their expectations.

To develop a sound marine stock-enhancement technology, we must integrate and coordinate research and expertise in several essential sub-disciplines to solve the pressing issues in this field. As a new science, stock-enhancement of marine fishes (that spawn in seawater) is handicapped by 100 years of questionable stocking practices before quantitative evaluations of effectiveness began in the 1990s. Lack of consensus on key research issues and failure to treat marine stock enhancement as a science have constrained advances in this branch of fisheries science. Given this history, and because of rapidly expanding interest worldwide in starting new stock enhancement programs, we must apply a substantial amount of science towards solving several key constraints to responsible application of stock enhancement technology (Blankenship and Leber, 1995; Leber, 1999, 2002).

Clearly, research collaborations are needed to integrate the principal sub-disciplines of enhancement research to resolve such a wide range of issues. Rapid advances can be made in understanding marine stock enhancement potential by focusing such collaborations on resolving major uncertainties, using a scientific and responsible approach.

2003-2004 SCORE Work Plan

The SCORE Approach

Research by SCORE focuses on all scientific questions that have arisen as a result of concerns expressed within the scientific community, and on the economic effectiveness of enhancement strategies. The Consortium embraces a responsible approach to the development of sustainable and effective stock enhancement schemes, incorporating principles outlined in the publication by Blankenship and Leber (1995).

The goal of SCORE is to advance the science and effectiveness of marine stock enhancement in a responsible fashion. SCORE scientists will conduct a large number of concurrent multi-disciplinary research activities in three coastal areas of the U.S.A. These activities are summarized below:

- § selecting appropriate species for testing marine enhancement potential
- § incorporating regional stock rebuilding goals
- § developing techniques for raising sufficient numbers of fish
- § assessing reason(s) for the decline of the wild population
- § developing and adapting tagging and tracking techniques
- § implementing a health management program to prevent spread of disease
- § achieving an understanding of all genetic issues and addressing them
- § developing and testing optimal release strategies
- § determining ecological requirements of released and wild fish
- § assessing the performance and fishery contribution of released fish
- § studying the ecological impact of released fish
- § developing quantitative measures to monitor and evaluate success
- § evaluating economic feasibility
- § involving and educating the beneficiaries
- § integrating an adaptive management process into enhancement management strategies

For any given species, simultaneous consideration of all these issues requires a well-staffed multi-disciplinary research team, which can only be assembled by pooling existing institutional resources. Collectively, SCORE scientists span the range of disciplines and expertise needed to advance responsibly the science of stock enhancement and its development as an additional fishery management tool. SCORE will achieve this by focusing research and development on the activities listed above. The knowledge gained by SCORE research will also be used to provide tests of the marine stock enhancement concept in fundamentally different habitats, and to demonstrate its potential as an additional fishery-management strategy that could complement current approaches.

Coordination and Planning

The Science Consortium for Ocean Replenishment and Enhancement (SCORE) is coordinated and directed by a 'Management Committee' composed of the project's 'Research Coordinators' from each of the principal institutions (the partner organizations in the current 2003-2004 phase: Mote Marine Laboratory and the Zoology Department at the University of New Hampshire, plus advisors from the founding partner organizations). The Research Coordinators will meet regularly to evaluate research priorities, direction, and progress (at least twice per year, but more frequently if needed). Mote Marine Laboratory will serve as principal contractor and administrative liaison with NOAA/NMFS.

Partners in SCORE operate through a Memorandum of Agreement. Individual regional projects are being carefully planned and executed by the Consortium, with feedback on key research results integrated into the planning process. Each project will develop milestones and indicators of success, and mechanisms for accountability. The Consortium has already developed open lines of communication with other research projects in stock enhancement. The research coordinators all have strong associations with stock enhancement programs and scientists at local, national and international levels and serve on many enhancement planning teams. These associations include several research collaborations on stock enhancement projects at all three geographical levels. Thus, the planners for SCORE activities have detailed awareness and knowledge of the approaches, status, successes and challenges of stock-enhancement programs worldwide.

Work Plan Regional Background

Florida Background

The State of Florida's Fish and Wildlife Conservation Commission (FWC) is evaluating the potential of marine stock enhancement to be an effective resource management tool in the 21st century. The FWC enhancement program was initiated in the mid 1980's, and from the onset, Mote Marine Laboratory (MML) has been a partner in this effort to enhance inshore marine fish stocks. In 1996, the FWC-MML partnership was reorganized and the new Director (K. Leber) of MML's Center for Fisheries Enhancement (CFE) was invited to be a co-leader of the FWC marine stock enhancement team. The team includes managers and biologists at FWC's Florida Marine Research Institute (FMRI), the FWC Stock Enhancement Research Facility (SERF) at Port Manatee, Florida, MML's Center for Fisheries Enhancement, and, since its formation by FWC in 1998, Florida's Marine Stock Enhancement Advisory Board (MSEAB, composed of stakeholders and scientists). The FWC has identified red drum *Sciaenops ocellata*, common snook *Centropomus undecimalis* (classified a decade ago as a "species of special concern"), and spotted seatrout *Cynoscion nebulosus* as the three priority species for stock enhancement research and development in Florida. All three species support important recreational fisheries throughout their range, and abundances of all three are depressed.

The FWC has reoriented its red drum enhancement program to follow the recommendations of Blankenship and Leber (1995) for a responsible approach to stock enhancement. This follows a decade of enhancement activities in Florida that were not nearly as focused as they are now on using a scientific approach to develop, evaluate and implement a sound marine stock enhancement technology.

The FWC has the resources needed to implement and evaluate red-drum stock enhancement effectiveness, and maintain a small stock-enhancement oriented research program needed to determine adaptive management strategies for this species. But, the FWC does not currently have the resources needed to fully evaluate all of the scientific issues underlying red drum enhancement, or to develop and test all stock enhancement technology needed with other species. In response, MML's Center for Fisheries Enhancement has been assisting FWC in developing and testing new enhancement technology.

Since 1997, and in partnership with FWC, MML's Center for Fisheries Enhancement has been conducting pilot hatchery-release experiments with common snook, reared at MML, to evaluate the potential of hatchery releases to significantly increase snook population size in Sarasota Bay, Florida. The pilot releases have shown substantial enhancement potential. Though very small-scale releases (only ~ 42,000 fingerlings, 70-150 mm in length, have been released to date), the

hatchery-released snook comprise around 15% of the young-of-the-year at release sites and 4% of the adult spawning stock in Sarasota Bay (Brennan and Leber, 2000, 2001). MML's pilot experiments have identified release 'microhabitats' where snook growth and survival are apparently much greater than at other release sites in the Bay. Although much more research is needed to develop optimal release strategies and understand causal mechanisms, the pilot-release experiments indicate that hatchery snook have high site fidelity and that this species is an ideal test animal for evaluating enhancement potential in marine and estuarine environments. Strategy and protocols for conserving health and genetics of wild snook stocks affected by enhancement activities have also been developed through the partnership with FWC (Tringali and Leber, 1999).

This work was originally funded through a cooperative research partnership involving the State of Florida's FMRI, a private foundation -- Mote Scientific Foundation (MSF), and by the Federally funded Gulf of Mexico Marine Stock Enhancement Consortium (GMSEP). However, the GMSEP research consortium is now concentrating all of its research efforts on developing enhancement techniques for red snapper. The partnership involving FMRI and Mote Marine Laboratory continues to provide some state of Florida fishing-license funds to help support part of the snook stock enhancement research conducted by MML's Center for Fisheries Enhancement.

SCORE provides the additional resources and expertise needed to significantly advance enhancement research in Florida. We now need to scale up release magnitude to examine key questions about stocking density, carrying capacity, and the potential to actually increase snook population size. A better understanding of predator-prey interactions and food availability is needed, and a whole suite of issues related to Blankenship and Leber's (1995) responsible approach to stock enhancement must be evaluated — e.g., a greater understanding of hatchery fish interactions with wild stocks, conserving genetics, maintaining high health, developing risk-averse enhancement strategies, acclimation of hatchery fish, optimizing release strategies and cost-yield ratios, cost-benefit, controlling enhancement effect, modeling enhancement impact, and dealing with uncertainty in a variable environment in order to maintain high predictability of enhancement effect.

Advances in snook aquaculture technology are also needed to provide the capability to scale up release magnitude to address the issues above. Since 1996, Mote Marine Laboratory has been rearing snook in its finfish hatchery facilities to provide the fingerlings needed for the pilot-release experiments in Sarasota Bay. Currently, the technology exists to produce common snook on a very small scale (10,000 100-mm long fingerlings per year in two 100' long x 40' wide greenhouses). Gains in aquaculture technology have been made, but emphasis on fingerling production to support the pilot releases must now be coupled with basic aquaculture research to provide the advances needed to develop an economically viable, mass-production aquaculture technology for snook.

Development of common snook mass culture technology will allow us to proceed to the next research priority in evaluating stock enhancement potential -- to learn how to control stocking magnitude, we must increase release magnitude in pilot experiments to examine the processes that mediate recruitment success and abundance in nursery habitats. We must also develop cost effective production technology, which is needed for snook enhancement to be an economically viable fisheries management option. The major impediments now to mass culture of snook are poor egg quality and poor survival during the larval rearing phase.

New Hampshire Background

The winter flounder, *Pseudopleuronectes americanus*, is widely distributed along the east coast of North America, ranging from Labrador to Georgia, and may attain sizes up to 64 cm (25 in.) total length. It supports important commercial and recreational fisheries throughout its range, but is most abundant in the Gulf of Maine. The species is typically exploited in coastal locations, although offshore areas, particularly Georges Bank and Nantucket Shoals, support important winter flounder fisheries as well. The principal commercial fishing gear used is the otter trawl. Winter flounder are managed by the New England Fishery Management Council (within the EEZ) and the Atlantic States Marine Fisheries Commission (within state waters). Regulations include time/area closures, gear restrictions, minimum size limits, a moratorium on commercial fishing permits, and days-at-sea restrictions.

As with most groundfish species, catches have declined precipitously in recent years. For example, Gulf of Maine commercial and recreational catch in 1981 were about 5,000 metric tons (mt). Since then, it has declined dramatically, such that the total Gulf of Maine catch in 1998 (most recent year for which data are published) was only about 600 mt (Nitschke et al. 2001). The cause(s) of the declines in winter flounder catch are not well understood, but they include, in probable order of importance, overfishing, unfavorable environmental conditions for recruitment, and habitat degradation. The goal of the current management program is to reduce fishing mortality to levels that will allow stocks to rebuild above minimum biomass thresholds, and then remain at or near target biomass levels. While it is hoped that these more stringent fisheries regulations will allow winter flounder populations to rebuild to historic levels, recovery could take a decade or more. The proposed research builds upon past Sea Grant and SCORE projects designed to assess the feasibility of winter flounder stock enhancement. A secondary goal of this project is to develop and demonstrate commercial scale aquaculture production techniques for this species. The majority of our research has related to the development of optimal release strategies that are critical to the success of any stock enhancement program. In particular, we have conducted a number of experiments to determine how lab-reared juvenile winter flounder differ from wild caught juveniles. We have also been using field-deployed microcosms, stocked with both cultured and wild-caught juveniles, to examine differences in growth, survival, and diets, and vulnerability to predation. We have conducted pilot scale releases of fish, and collected growth and survival data through field sampling programs. Finally, we have done intensive research on the habitats of juvenile winter flounder, and used mathematical modeling to identify appropriate release locations.

Score Project Objectives:

To develop and test the potential of marine stock enhancement, SCORE scientists will conduct research to resolve critical uncertainties about the effectiveness of using hatchery releases as a fishery management tool (discussed above under ‘alternate views and critical uncertainties about stock enhancement’). The project objectives were established to cover the range of issues involved in developing viable stock enhancement technologies.

The 10 objectives below comprise the framework for SCORE research. They provide a common thread that will focus planning and research among the organizations involved in the project. The principal investigators in SCORE have been conducting stock enhancement research and some of these objectives below have already been accomplished – a testament to the rapid progress we expect to achieve in this research consortium, because research is already underway. The SCORE project brings expertise together that is needed to resolve key uncertainties now constraining the use of stock enhancement as a marine fisheries management tool.

Objective 1: Select Appropriate Species

Justification: In the absence of a candid and straightforward method, targeting species for stock enhancement work can become a biased process. Unless attention is focused on a set of criteria that can be used to prioritize species, consideration of an immediate need by an advocacy group or simply the availability of aquaculture technology can become the driving factors in species selection. Commercial and recreational demand is obviously important criteria, but should they take precedence over other factors? Our approach is to select species for SCORE research based on several criteria. This objective has already been satisfied in Florida, Washington and New Hampshire, but we include here the rationale for species selection in each state to justify the species selected for SCORE research.

Approach and Procedures:

Florida

A two-day meeting was held in Biloxi, Mississippi February 5-6, 1998, to bring together stakeholders in a stock enhancement program for the northern Gulf of Mexico (GMSEP) to deal with issues related to marine stock enhancement. The focus here was to develop and discuss and select criteria for species selection for enhancement research in the Gulf, and to prioritize finfish species for research in the Northern Gulf. Stakeholders included representatives from the National Marine Fisheries Service, state natural resource agencies from Florida, Alabama, Mississippi, Louisiana and Texas, and selected environmental groups. Invited speakers were Lee Blankenship, Washington Department of Fish and Wildlife, and Don Kent, Hubbs Research Institute. Discussions focused on determining criteria upon which species selection should be based and on selection of species for enhancement research to take place in Mississippi.

Using the criteria developed in the Biloxi workshop, Mote Marine Laboratory's Center for Fisheries Enhancement assisted the Florida Department of Environmental Protection's Marine Research Institute (FMRI, now within a new agency, the Florida Fish and Wildlife Conservation Commission [FWC]) in the planning and conduct of a species selection workshop at FMRI in October 1998 to prioritize species for stock enhancement in Florida. The goal was to complete the species selection process using the approaches developed in Biloxi and a similar workshop conducted earlier in Hawaii (Leber, 1994). The process in Florida involved soliciting a species pool from stakeholders, followed by scoring the species based on the suite of criteria (identified below). The species list was developed through a mail-out questionnaire to a group of 500 licensed anglers, scientists, and others involved in stock enhancement or resource utilization in Florida.

The criteria developed in Biloxi were: species not responding to traditional management, user group value, culture history, species role or impact on ecosystems, ability to monitor impact, political support, understanding the sub-population, suitable life history parameters, and likelihood of an effect. Based on these, the October 1998 workshop at FMRI identified red drum *Sciaenops ocellata*, common snook *Centropomus undecimalis*, and spotted seatrout *Cynoscion*

nebulosus as the three priority species for stock enhancement research and development in Florida. MML will concentrate its research efforts initially on common snook to assist the State of Florida in developing a multi-species enhancement technology.

New Hampshire

The concomitant decline in US flounder catch, and documented successes with marine fish stock enhancement, led a panel of fisheries and aquaculture experts to conclude that both aquaculture and stock enhancement should be explored as means of increasing the availability of flounder in the US, and they recommended further research on flounder aquaculture and stock enhancement (Waters 1996). Winter flounder were among the species strongly suggested for further study. The species has a number of attributes that make it an excellent candidate for stock enhancement and/or commercial aquaculture production. First, it supports significant commercial and recreational fisheries throughout its range, and thus represents an important natural resource to New England. A second reason for selecting winter flounder is that culturing techniques have been developed (see review by Howell and Litvak 2000), so it is possible to produce the large numbers of fish needed for stock enhancement. A third reason for selecting winter flounder is that their life-history characteristics make them an ideal candidate for assessing stock enhancement in northern New England. Most spawn in estuarine locations, and the young fish spend their first two years in or near their natal waters (Perlmutter 1947; Topp 1967), living in shallow sand and silt areas (Buckley 1989), and making short tidal excursions (Tyler 1971). Further, Saucerman & Deegan (1991), working in a Massachusetts estuary, found that there was very little movement of the young-of-the-year fish, with 98% of released fish recaptured within 100 m of the release site. These characteristics of early estuarine dependence and non-migratory nature make winter flounder an ideal candidate for studying marine stock enhancement. Their estuarine location guarantees that field study sites are numerous and easily accessible, and that sampling of young fish can be done using inexpensive techniques. Further, because New England estuaries are extremely well studied, there is a wealth of biological, physical, and chemical information that is available to support enhancement research. Another reason for the selection of winter flounder is that their biology and ecology are very well known relative to most fish species. Detailed reviews of these topics are provided in Bigelow & Schroeder (1953), Klein-MacPhee (1978), and Buckley (1989). This wealth of background information is essential when considering stock enhancement, and winter flounder are nearly unique in this regard. Lastly, there are well-established winter flounder sampling programs in all of the New England states, so there are very good records of abundance and distribution in time and space. This historical data will be invaluable in assessing the success of any winter flounder enhancement program, and the sampling done by these agencies will supplement the sampling done in conjunction with the research program. This will be further supplemented by data collected from the extensive commercial and recreational fisheries for this species.

Objective 2: Define Goals and Objectives of Enhancement, Incorporating Regional Stock Rebuilding Goals

Justification: The goals and objectives of stock enhancement programs should be clearly defined and understood prior to implementation. The assumptions, risks and expectations about the performance and operation of an enhancement program necessary to make it successful should be determined (post-release survival, interactions with wild stocks, long-term fitness, disease, etc.).

A regional management plan should identify the context into which enhancement fits, as one tool, into the total strategy for managing stocks. The genetic structure of wild stocks targeted for

enhancement should be identified and managed according to objectives of the enhancement program. The plan should

A clear rationale is needed for when to use enhancement and when to stop. Thus, the benefits and risks of marine stock enhancement that are common to all enhancement activities should be understood and modeled. This includes hatchery technologies and effects of hatchery releases on population size, catch, genetic structure and ecosystem function.

Approach and Procedures:

Florida

The project at Mote Marine Laboratory to evaluate stock-enhancement potential is well integrated into the strategic planning process for marine enhancement in Florida. MML will continue to help lead planning meetings with administrators and stakeholders involved in Florida's FWC marine stock enhancement program to identify short- and long-term objectives to aim for in developing snook stock enhancement capabilities. We will also consider how enhancement would be used in context with existing management efforts to rebuild stocks. Target indicators of success will be developed for the range of release magnitudes and issues involved in evaluating stock enhancement potential (e.g. stocking effectiveness, economic viability, conservation) and the other principles embraced in a "Responsible Approach" concept (Blankenship and Leber, 1995).

Through conference calls and planning meetings involving the principal SCORE scientists, we will also continue to develop a conceptual model to evaluate enhancement benefits and risks that can be adapted to the priority species (red drum and snook) targeted by the FWC for research to evaluate stock enhancement potential.

As marine stock enhancement is developed and evaluated in Florida, implementation will be guided by the goals and measures of success developed. Attention to critical uncertainties about stocking effectiveness is the key to success.

Testing the capability of urban snook fisheries

Preliminary work will address the potential to create urban fisheries in hard-water inland ponds in Florida, using the novel approach of stocking catadromous common snook into freshwater ponds. This work may afford a possible extension of the state of Florida's stock enhancement program. In 2003-2004, we will begin investigating the capability of using the common snook in urban fishery environments. Juvenile snook will be tagged and stocked (on a small scale) into selected freshwater ponds in southwestern Florida. We will investigate appropriate predator-prey ratios, and potential impacts on existing largemouth bass populations in the ponds. Different sizes of snook will be stocked. We will also begin looking into the socio-economic potential of these systems.

New Hampshire

The overall goal of a winter flounder stock enhancement program is to accelerate recovery of the fishery by increasing spawning stock biomass. Before this goal can be achieved, we need to further develop and evaluate a winter flounder stock enhancement program. To start on this, a

long-range research plan will be developed in the coming year, in consultation with the New Hampshire Dept. of Fish and Game.

Objective 3: Develop a Genetic Management Plan

Justification: The need for genetic resource management in stock enhancement programs is currently the subject of intense public debate, and its importance cannot be over-rated. Responsible guidelines are now becoming available to aid resource managers in revitalizing stocks without loss of genetic fitness that could follow from inbreeding in the hatchery and subsequent outbreeding depression in the wild (e.g., Kapuscinski and Jacobson 1987; Shaklee et al. 1993a, 1993b). Once the genetic status of the target stock and the genetic goals of the enhancement program are identified, the approach for managing genetic resources is similar to the approach for managing other enhancement objectives (e.g., controlling the level of impact of stocked fish on abundances of the target population). This includes: (1) identifying the genetic risks and consequences of enhancement; (2) defining an enhancement strategy; (3) implementing genetic controls in the hatchery and a monitoring and evaluation program for wild stocks; (4) outlining research needs and objectives; and (5) developing a feedback mechanism. These points are discussed in detail in the three published guidelines referenced above.

Approach and Procedures:

Florida

Genetic guidelines and safeguards for snook stock enhancement have been developed in Florida (Tringali and Leber, 1999). In Florida, snook populations are biologically and genetically divergent between the Atlantic Ocean and the Gulf of Mexico. Thus, no transfers will occur between the Atlantic and Gulf snook populations. In the Gulf, snook may be further subdivided into interconnected demes having limited genetic exchange. Based on Tringali and Leber's (1999) recommendations, broodstock sources for hatchery releases will be limited to the targeted system or an adjacent estuary. Compared to other marine and estuarine fishes, allozyme and mitochondrial DNA polymorphism is low in common snook. Most allozyme polymorphism is maintained in the form of rare alleles occurring at frequencies of <0.05 . During the expanded phases of stocking, it is recommended that at least 100 wild-caught adults per generation interval (GI), ~3 years, be used to found hatchery populations and that the genetic effective sizes of those populations be > 50 . This should preserve $> 99\%$ of the original heterozygosity and incorporate rare alleles into hatchery populations. We modeled the potential reductive effects of stocking on the effective sizes of enhanced snook populations. Assuming 50 effective hatchery breeders are used, hatchery contributions to Atlantic or Gulf populations should not exceed 31% per GI. Conservatively estimating hatchling survivorship and wild spawning stock abundance, we've proposed stocking guidelines that satisfy this requirement.

New Hampshire

Tagging and meristic studies indicate three separate stocks of winter flounder, including one north of Cape Cod (Gulf of Maine stock), one east and south of Cape Cod (Southern New England - Middle Atlantic stock), and one on Georges Bank (Georges Bank stock) (Brown and Gabriel 1998). Because all of our broodstock come from the Gulf of Maine stock, and our release location is north of Cape Cod, the fish we release should have a similar genetic make up as the wild fish in the area. Nevertheless, we plan to verify this by comparing the gene frequencies of our reared juveniles to those of wild juveniles. We

anticipate that this will be done in association with Dr. Joe Crivello at the University of Connecticut, with whom we are collaborating.

Objective 4: Develop Culture Technology

Justification: Aquaculture technology is relatively new for common snook and queen conch, but fairly well established for winter flounder. In order to conduct the pilot release experiments needed to evaluate critical uncertainties and cost effectiveness of enhancement, a reliable, efficient mass-culture technology is needed for all three species. Significant work is needed in this area for snook and additional research is needed for queen conch.

Approach and Procedures:

Florida

The goal in Florida of the aquaculture technology component is to develop the culture technology to produce common snook and queen conch for stock enhancement. In Year 1, research efforts focused on determining the optimal diet for first feeding larvae (i.e., enrichment of live feeds), environmental factors that increase egg quality and larval survival in wild strip-spawning of snook, and establishing captive broodstock and experimental larval rearing systems for snook culture. In 2003-2004, we will continue the work to develop controlled maturation and spawning techniques and improve larval survival with common snook. We will also initiate a new effort to develop the culture technology for queen conch. Recirculating aquaculture systems will be used for all culture efforts and the results of this research will be transferred to the aquaculture industry in reports, presentations at local, regional and national aquaculture meetings and in scientific publications.

A major obstacle to expansion of marine fish culture is in rearing early life stages. In contrast to salmonids, catfish and other species, the larvae of most marine fish do not possess large yolk sacs and they depend mainly on exogenous sources of food during early development. Presently, early life stages are cultured on live food, including microalgae, rotifers and *Artemia*; however, live feeds are costly, not always readily available and often of variable quality. Inadequate nutrition is likely to be major contributing cause to high mortality rates of cultured marine fish larvae used in enhancement and aquaculture programs.

We are proposing to develop formulated diets for marine fish larvae to reduce dependency on live feeds and to improve success in larval culture. Formulated diets for early life stages need be acceptable and digestible by larvae as well as providing all required nutrients in optimal proportions. The ability of early larvae to utilize dietary protein and other large complex nutrients appears to be limited. Therefore, inclusion particle types that can deliver vitamins, amino acids, peptides and other low-molecular weight, water-soluble (LMWS) nutrients to larvae will be developed and included in carrier particles to provide larvae with complete, complex microdiets.

To achieve all of these goals, we will use the following approach:

A. To develop a year round captive spawning protocol for common snook.

Temporary broodstock holding systems (17,386 liters/tank) were constructed at Mote Marine Laboratory's main campus and stocked with snook in year 1 of this project. In 2003-2004, we will construct three new larger broodstock holding systems at Mote Aquaculture Park (54,315 liters/tank) and initiate studies to induce spawning using temperature and photoperiod manipulation (Bromage 1995; Bjornsson et. al., 1998). The three tank systems will be exposed to a shortened winter and spring temperature and photoperiod cycle and then maintained at summer spawning conditions for 2-3 months. Larval success is dependent on the production and ovulation of mature, viable oocytes. The percent fertility and survival to day 14 will be quantified in each cohort.

B. To determine an optimal conditioning diet for common snook broodstock.

Broodstock nutrition has long been recognized as a critical determinant of hatchery success. Fish will be fed a fresh broodstock diet (i.e., squid, shrimp and kapelin) in combination with a commercial marine broodstock pelleted diet and an experimental broodstock diet supplemented with vitamins and capelin oil. We will monitor the spawning success (# viable eggs per spawn) under photoperiod and temperature controlled conditions.

C. To develop cost-effective larval rearing culture technology for common snook. Current larval-rearing practices include addition of live feeds at 3 day and 1 night time periods. In addition, live feeds (i.e., rotifers) have been provided at high densities (30 rotifers/ml). These practices result in high production costs and limit the total production capacity of the hatchery. The goal of the larval rearing studies is to determine the optimal feeding strategy to produce large numbers of snook for stock enhancement studies.

Although the technique of feeding snook larvae at night has been a standard practice at MML for the past 6 years, this technique may not be required to ensure the success of snook larval culture. Two experimental larval rearing systems (each system includes 4 130-l tanks) will be stocked with larvae at a stocking rate of 25 larvae/l. Half of the tanks in each system will be fed at 15 rotifers/ml at 8 a.m., Noon, 4 p.m., and 10 p.m. (night feeding) and the other half will be fed at 8 a.m., Noon, and 4 p.m. (no night feeding). Both experimental systems will be harvested at 14DAH and survival will be compared.

In order to determine the live feeds requirements for larval snook, we will conduct an experiment to compare the survival and growth at two different rotifer densities. Two experimental larval rearing systems (each system includes 4 130-l tanks) will be stocked with larvae at a stocking rate of 25 larvae/l. Half of the tanks in each system will be fed 15 rotifers/ml and the other half will be fed 30 rotifers/ml. The number of feedings per day will be determined by the results of the night-feeding experiment described above. Both experimental systems will be harvested at 14DAH and growth and survival will be compared between the two treatments.

D. To reduce cannibalism during larval rearing of common snook.

Cannibalism becomes a serious problem in larval snook culture from day 12 to day 30-32 when fry can first be handled and size graded. Successful strategies to reduce predation may include increased turbidity, water currents, habitats, diets, etc. This experiment will compare survival in systems where turbidity is increased using two different greenwater culture strategies (addition of algae paste versus live algae). Three experimental larval rearing systems (each system

includes 4 130-l tanks) will be stocked with larvae at a stocking rate of 25 larvae/l. Turbidity will be increased in one of the experimental systems by the addition of algal paste and the second system will include the addition of live algae. Survival and growth in the greenwater culture systems to a clear-water control system. The number of feedings per day will be determined by the results of the night-feeding experiment described above. Both experimental systems will be harvested at 30DAH and growth and survival will be compared between the two treatments.

E. To develop nursery culture methods for juvenile queen conch.

The techniques to farm queen conch have been advancing rapidly since the 1970s (Davis 2000). Florida Fish and Wildlife Conservation Commission (FWC) conducted restoration research using queen conch in the Florida Keys (Glazer and Berg, 1994) and restocking of hatchery-reared and wild conch was conducted in the Bahamas by Stoner and Davis (1994). Stoner and Glazer (1998) found variations in morphology between hatchery and wild stocks, which may explain differences in survival between the two groups. Juvenile production efforts this year will target 3000-5000 queen conch for stock enhancement trials. The conch will be cultured in recirculating raceway production systems on a aragonite substrate.

F. To develop complex microparticles (particles made up of separate inclusion and carrier particles) capable of supporting growth and development in marine fish larvae

(Subcontracted to University of Idaho; Investigators:

Dr. Ronald Hardy, 3059F National Fish Hatchery Rd, University of Idaho, Hagerman, ID 83332

Dr. Mike Rust, Northwest Fisheries Science Center, 2725 Montlake Blvd. E., Seattle, WA, 98112)

In the 2003-2004 phase of this project, we will focus on the following two specific tasks.

A) To evaluate protein micro-sponges as inclusion and carrier particles

(Subcontracted to University of Idaho; Investigators:

Dr. Ronald Hardy, 3059F National Fish Hatchery Rd, University of Idaho, Hagerman, ID 83332

Dr. Mike Rust, Northwest Fisheries Science Center, 2725 Montlake Blvd. E., Seattle, WA, 98112)

Protein microsponges will be evaluated as means of delivering LMWS nutrients to larvae. The digestibility of particle types that have satisfactory retention efficiencies for amino acids will be qualitatively determined in experiments with larvae using markers. Promising particle types will be evaluated in terms of leaching of soluble protein, larval feed acceptability and if resources allow, growth experiments.

B) To develop a method to determine apparent protein digestion and absorption efficiency for fish larvae and use it to evaluate promising microparticulate diets.

(Subcontracted to University of Idaho; Investigators:

Dr. Ronald Hardy, 3059F National Fish Hatchery Rd, University of Idaho, Hagerman, ID 83332

Dr. Mike Rust, Northwest Fisheries Science Center, 2725 Montlake Blvd. E., Seattle, WA, 98112)

A sensitive quantitative method will be developed to determine dietary protein digestibility by fish larvae by comparing the ratio of protein to inert marker in the food and feces of larvae. The development of this method will provide us with a powerful tool to determine diet utilization and will greatly facilitate microparticulate diet development in the future.

Initially, feeding and growth experiments will be conducted with larvae of clown fish (*Amphiprion percula*) as model species - both of which are relatively easy to spawn on a regular basis and are presently cultured in the laboratories of Dr. Rust. When available, experiments will also be conducted with larval rockfish (*Sebastes sp.*), lingcod (*Opeladon elongatus*) or other species that are cultured in the laboratory of Dr. Rust, as well as other commercially important species, depending on availability.

Testing of microparticulate feeds for marine fish will require larvae from target species to determine digestibility and whether or not nutritional needs are being met. Juveniles will be needed to test volitional entry for the automated tagging and vaccination equipment.

New Hampshire

The basic techniques for culturing winter flounder have been developed, and are reviewed in Howell and Litvak (2000). Nevertheless, there are two issues that relate to culture technology that need to be addressed. The first is the recent difficulty of obtaining good weaning diets. The diet we have used in the past (Biokyowa) is manufactured in Japan, and has become nearly impossible to obtain because of importation issues. Because of the importance of good diets, we intend to assist personnel at the Washington Department of Fish and Wildlife who will collect sexually mature adults of species identified as potential candidates for enhancement. These will be delivered to the National Marine Fisheries Manchester Washington laboratory for spawning and subsequent larval rearing and feed trials.

The second issue we intend to examine is sexual differentiation, and the sex ratio of cultured fish as described later in the proposal.

To provide researchers with adults capable of producing larvae and juveniles for the experimentation and testing in A) and B) above

(Subcontracted to Washington Department of Fish and Wildlife; Investigator: Gregory G. Bargmann, Program Manager. Washington Department of Fish and Wildlife

Target species identified as potential candidates for enhancement will be used. The following tasks will be conducted in support of the work being done at the Northwest Fisheries Science Center, Seattle Washington and University of Idaho, Hagerman, Idaho to develop microparticulate feeds.

Collect sexually mature adults of species requested by the primary investigators. Deliver live adults to the National Marine Fisheries Manchester WA. Laboratory for spawning and subsequent larval rearing and feed trials. Collect, rear, or import appropriately sized juveniles of target species for volitional entry studies.

Objective 5: Manage Disease and Health

Justification: Disease and health guidelines are important to both the survival of the fish being released and the wild populations of the same species or other species with which they interact. Standard health management protocols are needed as part of a certification process to prevent spread of parasites and disease pathogens. Protocols underdevelopment will be utilized in 2003-2004 in the production-tag-release process.

Additional work is needed to develop a rapid vaccination process for known pathogens. This objective will seek to produce a working protocol that can be broadly applied to all the SCORE species. The use of coded wire tags to mark fish prior to release has become a standard method for determining stocking impact for all SCORE projects. In addition, the desire to vaccinate fingerlings prior to release is also desirable to minimize the potential for loss of stocked fish to common known pathogens. We propose to test a combined machine that simultaneously tags and vaccinates fish at one time. This approach should greatly reduce the stress on the fish and the labor required for these two procedures. This machine will be tested in marine fish for the first time to generate operational protocols that can be applied to all SCORE species.

Approach and Procedures:

Florida

Develop and utilize health-management protocols tailored to stocking marine organisms

FWC's Florida Marine Research Institute has developed an aggressive and responsible approach in this area in association with their red drum enhancement project (Landsberg et al. 1991). Their policy requires that all groups of fish pass a certified inspection for bacterial and viral infections and parasites prior to release. Maximum acceptable levels of parasites, etc., in the hatchery populations are established based on the results of screening healthy wild populations.

In year 1 of SCORE, we adopted the FWC protocols for acquiring certified inspection by a USDA-certified veterinarian prior to release of any hatchery-reared snook into the wild. Currently, little is known about natural levels of diseases and parasites in snook. Research on diseases and parasites in natural snook populations will be addressed in the future.

Test the use of a combination vaccination/coded wire-tagging machine on a marine species

(Subcontracted to University of Idaho; Investigators:

Dr. Ronald Hardy, 3059F National Fish Hatchery Rd, University of Idaho, Hagerman, ID 83332

Dr. Mike Rust, Northwest Fisheries Science Center, 2725 Montlake Blvd. E., Seattle, WA, 98112)

An automated tagging/vaccination machine developed by Northwest Marine Technology for salmonids will be tested to determine if the equipment can be adapted for marine fish. The device relies on a fish's natural behavioral traits like swimming into currents and choosing between different environments (lighted versus darkened areas, deep versus shallow water, etc.)

to volitionally enter the system without anesthetic to be tagged and/or vaccinated. A variety of marine species will be tested to see if volitional entry is similar to that experienced with salmonids. If volitional entry is not as good with marine species, experimentation and testing with other environmental factors will be conducted. Results will be used to re-design, if necessary, a volitional entry system for marine fish so entry into the automated tagging and vaccinating is feasible.

New Hampshire

There are standard health management protocols already in place at the UNH finfish hatchery. In this, a diagnostic laboratory checks a random sample of fish, on a predetermined schedule, for bacteria, viruses and parasites. Any pathogens found are reported, along with prescribed treatments. Because it is essential that all released fish be free of any communicable disease, we will continue to have each batch of fish tested, and certified as healthy, before release to the wild.

Objective 6: Describe Life History Patterns and Ecological Interactions

Justification: The design phase of enhancement programs should include consideration of ecological factors that can contribute to the success or failure of hatchery releases. Predators, feeding habits and food availability, accessibility of critical habitat, competition over food and space, environmental carrying capacity and abiotic factors, such as temperature and salinity, are all key variables that can affect survival, growth, dispersal, and reproduction of cultured fish in the wild. Predatory losses and food availability have long been thought to be among the principal variables that mediate recruitment success in wild populations (Lasker, 1987, Houde, 1987).

Habitat degradation in marine environments can also affect recruitment success. In vegetated aquatic environments, habitat availability and habitat quality (e.g. structural complexity) have been shown to mediate survival from predators (Crowder and Cooper, 1982, Stoner, 1982, Leber, 1985, Main, 1987). In some cases, habitat degradation in marine environments may be so complete that certain habitats are unsuitable for stock enhancement (Stoner, 1994). To enhance fisheries in some locales, restoration of coastal habitat may be the first priority.

A solid understanding of the ecological mechanisms mediating target species abundances can require exhaustive field studies for each species considered for enhancement. Whole careers have been dedicated to understanding mechanisms behind animal distributions and abundance; it does not seem practical to hold off on stock enhancement research until the ecological mechanisms are completely understood. However, failure to consider such factors can result in poor performance of released fish at best and negative impacts on natural stocks (Murphy and Kelso, 1986).

Our viewpoint is that preliminary, pilot-scale experimental releases with subsequent monitoring of cultured fish afford a direct method for evaluating assumptions about the impacts of uncontrolled ecological interactions and environmental factors. For example, assumptions about carrying capacity in particular release habitats should be evaluated through pilot releases conducted prior to full-scale enhancement at those sites (Leber et al., 1995). That approach is elaborated on under Objective 8 below. Ecological studies that are needed to design some of the key pilot release experiments in future phases of SCORE are presented here.

Approach and Procedures:

Florida

An evaluation of cannibalism risk in juvenile snook

Cannibalism in snook has been well documented in artificial rearing situations (unpublished data, Mote Aquaculture) and has even been documented to occur in the wild (Aaron Adams, unpublished data). However, as a top predator in the near shore estuaries, the degree that inter-cohort and intra-cohort cannibalism influence abundance levels of juveniles has never been quantified and remains unclear. In artificial rearing situations, cannibalistic behavior has been observed to be especially intense during early stages and this early life cannibalistic tendency (if it exists) may serve as strong evidence for density-dependent mechanisms operating in juvenile snook populations.

We propose to quantify allometric cannibalistic tendencies of age-0 and age-1 snook in a natural environment. Four 20' diameter enclosures will serve as the experimental arenas. These enclosures will be placed along shoreline habitat in estuaries where juvenile snook are common. Two enclosures will include forms of typical refugia found in these habitats such as submerged branches, logs, sea grasses, and mangrove prop roots. Two other enclosures will not include refugia however. Prior to stocking all observed fish and potential predators will be removed from the enclosures. Different size structures of juvenile snook will be tested for cannibalistic tendencies. For each enclosure cannibalism trial, two different size classes will be tagged according to size and will be introduced together into the enclosure. Two weeks later, the enclosures will be harvested and counts will be made of each surviving size class. Different combinations of sizes, stock density, and wild and hatchery fish will be tested.

New Hampshire

Winter flounder are certainly among the most thoroughly studied of all marine fish species. Reasons for this include their commercial and recreational importance, their distribution in east coast estuaries near large numbers of government and university laboratories, their frequent use as a model in fish physiology research, and their vulnerability to impact by power generating stations, which has resulted in detailed monitoring of early life-history stages. Their entire life history is very well known, and there has been an enormous amount of research on their ecology. This wealth of information will facilitate the development of a winter flounder stock enhancement program. Our most recent contribution to this, funded through SCORE, has been to use Habitat Suitability Index (HSI) modeling to predict appropriate release locations for winter flounder in the Great Bay Estuary of New Hampshire (Wanat 2002). Habitat variables used in the modeling included temperature, salinity, depth, substrate type, prey availability, and predator abundance.

Objective 7: Identify Released Hatchery Fish

Justification: One of the most critical components of any enhancement effort is the ability to quantify success or failure. Without some form of assessment, one has no idea to what degree the enhancement was effective or, more critically, which approaches were totally successful, partially successful, or a downright failure. Natural fluctuations in marine stock abundance can mask successes and failures. Maximization of benefits cannot be realized without the proper monitoring and evaluation system.

Approach and Procedures:

Florida

To identify experimental treatment conditions in pilot release experiments, all released fish will be tagged with coded wire tags (CWTs) and at least some portion with visible implant elastomers (VIE tags, Northwest Marine Technology (NMT), Inc.). Coded-wire tag codes will identify release site, release microhabitat, size-at-release, release lot (date) and number of fish per treatment condition. In snook, tags will be implanted in the cheek area with an automatic injector. Previous studies have shown a coded-wire tag retention rate of 95% and greater in snook (Brennan et al., 2000). The elastomer tag is used to provide a visible indicator of hatchery fish. Elastomer material will be injected into the caudal fins using a hydraulic injector (NMT, Inc.). Previous studies have shown an elastomer retention rate of 90% and greater in snook (Brennan et al., 2000).

Refine tag technology for stock enhancement and ecological studies

Additional experimental work is needed to refine tagging capabilities for SCORE research in Florida. 2003-2004, we will:

Refine VIE tag capability for stock enhancement with juvenile snook

Previous studies at Mote Marine Laboratory with VIE in juvenile snook (Brennan *et al*, in review) reported promising VIE retention in the caudal fin rays while poor retention was reported in the head and jaw areas of the juvenile snook. In 2003-2004, we will investigate the potential for use of this tag in a new site--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. We will test this in the wild to determine potential harmful effects on fish survival and growth.

Develop capability for the use of PIT tags in ecological studies with juveniles

Many studies show PIT (passive integrated transponder) tags to be extremely useful in ecological studies requiring multiple recaptures, individual information, and long-term data collection needs. We will adapt PIT tags to juvenile snook for use in future studies testing density dependence. In these experiments, we will tag wild juveniles throughout a nursery system according to size, microhabitat capture location, and date. Follow-up large-scale releases will be performed in these systems and recapture efforts will determine the impact of large-scale releases on existing wild snook populations. We will look into the potential of developing and employing benign monitoring systems with these tags.

Develop capability of PIT tags used in adult snook for spawning behavioral studies

A protracted spawning season has many biological implications such as multiple spawning efforts of individuals, energy allocation over a spawning season, changes in egg quality, 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 these is important for responsibly producing progeny for year class supplementation.

Wild caught female snook will be tagged with Passive Integrated Transponder (PIT) tags throughout the spawning season of summer 2003. Each female will be scanned for previous tags, measured, aged (from scale annuli, aging only first time tagged fish), and given an index of maturation. Individual female maturation will be categorized by maturation stages as follows:

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

Snook will be collected during spawning cycles on the new moon and full moon phases from May through September. Collections will be conducted at spawning sites from Venice Inlet to Rattlesnake Key. Snook will be captured with seines and trammel nets. All tagged fish will be released after processing.

Develop sonic tracking capability for juvenile snook

As an additional means for evaluating released hatchery fish in the wild, sonic tags will be tested and applied toward hatchery juvenile snook. Data obtained from these tags will allow researchers to evaluate short-term diurnal movement patterns within microhabitat to further elucidate the effects of microhabitat on hatchery snook in the wild. Additionally, snook tagged with sonic tags will be tracked seasonally to determine seasonal habitat preferences and the extent of juvenile emigration from the creeks during the fall when CPE have been determine to significantly decline in the creeks (Brennan, unpublished data). These studies will help determine important ontogenetic habitat shifts in the snook life history. Pseudo tags will be applied towards juvenile snook using various surgical methods to determine optimal tagging methods prior to tagging snook with sonic tags. These fish will be held for 1 month at the snook culture facility and retention and healing will be monitored. Optimal procedures will then be employed with sonic tags in the juvenile snook to develop the capability for future sonic tracking studies.

Preliminary sonic-tagging studies investigating seasonal migration patterns of juvenile snook

We will begin to evaluate seasonal migration patterns of age-1 snook and age-2 snook to understand important ontogenetic habitat preferences in the snook life history. Currently, very little information exists on ontogenetic habitat shifts in juvenile snook. Understanding these processes will aid us in understanding density dependent mechanisms operating in juvenile snook habitats. We will begin working with the sonic tracking equipment (Vemco) and track sonic tagged age-1 snook in the nursery habitats during summer 2003 through March 2004. These preliminary tracking investigations will aid us in planning a large-scale evaluation of seasonal migrational patterns of juvenile snook.

New Hampshire

Two tagging methods have been used successfully with winter flounder juveniles in our laboratory. These include Visible Implant Florescent Elastomer tags, and Microwire tags, both developed by Northwest Marine Technologies. Fish as small as 25mm TL are routinely tagged, and we have done long-term tag retention studies indicating the tags remain in place, and detectable, for up to a year. Because we are very interested in studying the movement of small, released fish, we are eager to work with ultrasonic tags as well, and plan to work with ultrasonic tag manufacturers in the development of tags small enough to place on juvenile winter flounder.

Objective 8: Optimize Release Strategies

Justification: Just as preliminary releases can be used to evaluate ecological assumptions, pilot release experiments afford a means of quantifying and learning how to control the effects of release variables and their influence on the performance of cultured fish in coastal environments (e.g. Tsukamoto et al. 1989; Svasand et al. 1990; Willis et al. 1995; Leber, et al., 1995, 1996, 1997, 1998;).

Experiments to evaluate release strategies (e.g. size-at-release, release season, release habitat (and microhabitat), release magnitude, and various ways to acclimate, etc.) should always be conducted prior to launching full-scale enhancement programs. These experiments are a critical step in identifying enhancement capabilities and limitations and in determining release strategy. They also provide the empirical data needed to plan enhancement objectives, test assumptions about survival and cost-effectiveness, and model enhancement potential. The lack of monitoring to assess survival of the fish released by marine enhancement programs early in this century (through the 1950's) was the single greatest reason for the failure of those programs to increase stock abundances and fishery yields (Richards and Edwards 1986).

Approach and Procedures:

Florida

Researchers in MML's Center for Fisheries Enhancement have been conducting very small-scale pilot-release experiments with common snook (*Centropomus undecimalis*) to

develop optimal release strategies (Brennan and Leber, 2000, 2001). This research has shown a significant effect of release-site location on subsequent growth and survival of hatchery snook released at snook nursery sites in Sarasota Bay, Florida. We hypothesize that the five most important (and interactive) factors mediating habitat effect on growth and survival of released snook are (1) predation and refugia, (2) environmental variables, particularly dissolved oxygen, temperature and salinity, (3) acclimation to various directive factors in the natural environment, (4) prey availability, and (5) competitive displacement over food and space. Clearly, a host of other logistical, ecological and biological factors may also impact performance of released fish, such as fish size-at-release, handling stress, behavior, timing of releases, tide stage, weather patterns, etc., but the five factors above top our list of priority issues at this time. We will prioritize and evaluate all of the factors in subsequent phases of the project.

In year 1 of the SCORE project, we began to evaluate factor (1) and continued ongoing research to evaluate factors (2) and (3). Factor (4) will not be considered until after we gain an understanding of snook feeding habits at our study sites. To fully consider factor (5), we must first make more progress in advancing snook aquaculture technology to provide the greater number of fingerlings needed to evaluate carrying capacity at our release sites and then develop release strategies to accommodate carrying capacity considerations. That (aquaculture) work is considered above under Objective 4. Meanwhile, preliminary field studies were started in year 1 of SCORE to investigate actual densities of wild snook in their nursery habitats in Sarasota Bay. These studies included a preliminary examination of density-dependent interactions of hatchery and wild snook, discussed below.

To produce cultured snook for the Florida pilot release studies in 2003-2004 described below, eggs and milt will be collected during the natural spawning season from wild snook using a 200 m surround net placed around spawning aggregations that occur biweekly throughout the spawning season (June through August) in the inlets of Sarasota Bay. Fertilized eggs will be transported to the laboratory and hatched in the MML fish hatchery. The young snook will be cultured for approximately 9 mos to 100 to 150 mm fork length (FL), harvested, and tagged with coded-wire tags and elastomer tags to identify treatment variables. Prior to release, the tagged snook will be given a couple of days to recover from tagging stress.

The tagged snook will be released at established field sites where snook pilot releases have been conducted previously by MML. The release sites will be located at documented snook nursery habitats from results of our previous studies. In the pilot releases below, all experimental treatment and control conditions will be replicated on at least 2 successive days and in at least two nursery habitats (streams) within the bay, using a randomized-block experimental design (treatments blocked over time and space, as in Leber et al., 1998).

Factor (1) – *Predation and refugia:*

Microhabitat suitability and survival patterns of released snook

Escape from predators during the first couple of days following releases is perhaps the single most important factor in survival patterns of hatchery-released fishes. Survival of organisms in their environment is closely associated with habitat quality, as 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.

Streams and tributaries are the primary nursery habitats of common snook. Previous MML studies in Sarasota Bay show much higher recapture rates for those juvenile snook released upstream -- and subsequently recaptured in seine samples made at release sites upstream, downstream and across the bay. 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. These studies suggest that upstream release sites afford much greater survival (where low salinity provides refuge from most predatory marine fishes) than releases near the mouths of streams. However, emigration patterns cloud this picture; lower recapture rates of snook released downstream could be explained by chance alone, owing to greater dispersal out of the study area of the fish released near the mouths of streams. To resolve this issue, recapture data are needed for released snook captured on spawning grounds and other adult snook habitats. As follow-up to our earlier studies, we will collect information from the adult fishery, which will provide data on ultimate survival and growth rates of our released fish. Fish recovered from the various release microhabitats will provide data unbiased by dispersal patterns of early juveniles, because the spawning habitats are miles from the juvenile habitats and fish recruited to these habitats originate from a collective assortment of all of the release microhabitats.

To examine relative survival of snook released in upstream and down stream habitats by MML researchers in previous studies, researchers will attempt to recover hatchery fish from snook spawning grounds and adult snook habitats along Gulf of Mexico beaches of barrier islands in the vicinity of Sarasota Bay. Adult snook populations will be sampled in their spawning habitats during summer 2003. We will use a 450' seine, a 150' seine, heavy cast nets, and hook and line equipment to capture adult snook. Sampling will occur in conjunction with trips aimed at collecting fertilized eggs for rearing in the hatchery. Beach seines and surround nets will be employed during the seasons when snook aggregate to spawn in those habitats. Snook caught in these collections will be examined with a field-sampling detector (NMT, Inc) to detect coded-wire tags. All tagged fish will be placed on ice and returned to the laboratory, where coded wire tags will be extracted and examined to retrieve tag codes and evaluate the influence of release habitat on survival patterns.

Relative Predation Intensity on Juvenile Snook Populations in Various Nursery Habitats

This study aims to quantify predation intensity on juvenile snook populations among varying habitats. As stock enhancement research with snook populations in Florida progresses and continued resources are invested, it is critical to understand the role that predation plays in determining the ultimate abundance of a year class. This study will have broad ramifications for stock enhancement research with cannibalistic species, and for quantifying the importance of habitat refuges for survival of juvenile fishes. It may also help for predicting the impact of man-made structural habitat alterations (via dredging) on an important recreational species such as the common snook. We hypothesize that predation intensity on age-0 snook is minimized in natural

creek nursery habitats where shoreline refuge is abundant, and that older year classes of snook are the primary predators of the age-0 year class.

Predation has a direct effect on reducing abundance of a year class, and quantifying this by age class and habitat type is an integral component in understanding survival patterns. Many methods are used to measure predation levels and tethering is one widely used approach (Aronson and Heck 1995). Although tethering experiments are not suitable representations of natural predation rates, they are accepted as a measure of relative predation risk in comparisons measuring alternative predators and habitat circumstances.

In 1998 and 1999, we experimented with the effects of release microhabitat on survival and growth rates of hatchery snook (Brennan and Leber, unpublished). We released juvenile hatchery-reared snook into four basic “microhabitats” of a stream system including: (1) upstream habitats, (2) midstream habitats, (3) stream mouths, and (4) inter-coastal island habitats. Subsequent recaptures of snook in nursery habitats and even years later in adult spawning habitat showed us that the highest survival rates were from snook released in the upstream and midstream habitats and lowest rates were from snook released along island shoreline habitats. We found that fish assemblages at the island habitats were dominated by larger snook, while in the upstream creek habitats, juvenile (age-0 and 1) snook were more common; we hypothesized that predation by larger snook was the primary factor that influenced the post-release survival rates.

The objectives of this study are (1) to compare relative predation rates on age-0 snook (60-250 mm fork length [FL]) in different nursery microhabitats, (2) to identify primary predators of age-0 snook, (3) to compare predation rates on age-0 snook between fall and spring seasons, and (4) to quantify the influence of various physical habitat characteristics on predation rates in age-0 snook.

Age-0 snook will be tethered in different habitats and seasons to evaluate predation risk. A modified tethering system will be used to identify snook predators using a treble hook and a strike indicator buoy system. Hatchery-reared age-0 snook will be tethered in four general microhabitats along an estuarine creek system: upstream, midstream, stream mouth, and island shoreline habitat. Tethering will also be performed in habitat of various microhabitat categories defined in terms of depth, bank slope, and distance from shore. Tethering will also be performed in fall and spring seasons. A minimum of three replicates will be tested for each microhabitat-season combination. Tethering duration will span no longer than 4 hours. Optimal time of day for eliciting a strike will be initially tested and subsequently applied for future tethering trials. Water quality parameters (temperature, salinity, dissolved oxygen, and pH) will also be measured during each trial.

Factor (2) – *environmental variables*:

No work is planned in this area during 2003-2004

Factor (3) – acclimation to various directive factors in the natural environment:

To evaluate the effects of acclimation on survival of released hatchery fish, hatchery snook were subjected in year 1 to various treatment conditions prior to release. The effect of acclimation to the release site was evaluated using cages to corral and protect snook overnight from predators. To control for this, non-acclimated snook were also released directly into the wild. This work showed the cage acclimation increased subsequent recapture rates, and presumably survival, by 100%. Based on these results, most release experiments conducted during 2003-2004 will include an initial acclimation period in cages at release sites

Factor (4) *prey availability*

No work is planned in this area during 2003-2004

Factor (5) *competitive displacement over food and space*

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

A primary emphasis of SCORE year 1 snook experimental releases has been to identify potential density-dependent effects exhibited in juvenile snook populations. The basic theory behind density-dependence is that population size is controlled by intra-specific competition or predation for a limited resource. Therefore, interspecific competition or predation limits further expansion of the population. In many species, density-dependent mechanisms only operate within a specific age category, or life stages, such as among juveniles. The common snook exhibit signs of density-dependency such as being piscivorous and cannibalistic. As part of a responsible approach toward the pilot studies in stock enhancement with juvenile snook, the potential for density-dependence with juvenile snook must be investigated. Since 1997, stocking activities have focused on effects of release strategies on survival and growth of hatchery-released snook. We are now focusing our attention on the effects of hatchery-released fish on wild snook populations.

During year 1 of SCORE, we began to investigate density dependence and the influence of large-scale releases on the existing juvenile snook populations. These studies are a series of long-term studies that manipulate recruitment through release of hatchery fish into several nursery habitats of varying abundance levels. In 2002 we released juveniles into four creek systems of different densities. In two creeks we attempted to double existing abundances, while in two others we attempted to increase abundance only minimally (~10% increase).

Before releases occurred, estimates of age-0 and age-1 abundances of snook in the nursery habitats were necessary. Four Sarasota Bay creeks harboring nursery habitats were selected for this experiment: South Creek, North Creek, Bowlees Creek, and Whitaker Bayou. Sampling occurred in all creeks to determine snook abundances. Because juvenile snook abundance in these areas is generally associated with shoreline habitat, sampling effort was related to total shoreline distance. Aerial photographs were used to obtain total shoreline habitat within the creeks and every third 100' section of the shoreline was sampled. A 220 foot seine was used as

the standard sampling gear, and on open shorelines (i.e. shorelines with no opposite banks within 70' of the shore) the net was deployed 70' offshore. Immediately 70' of the net was pulled to the shore while the other end was arched toward the shore approximately 100' away. Thus, 100' of shoreline was sampled. In stream and canal habitats, both shores were sampled, and again, 70' of the net was used to block off the lower half of the sample section while the remaining net was pulled down one of the shorelines approximately 100' and arched across to the opposite shore. With this method, roughly every third 100' section of shoreline habitat within the creek was sampled. In many cases shoreline habitat was not sampled because of logistical difficulties, however.

Pre-Release April 2002 Sampling

The total net sets per creek, estimated shoreline distance, and snook abundance from the April 2002 samples were as follows:

Creek	# net sets	# shore sets sampled	Total shoreline (feet)	% shore sampled	Expanded Estimate Mean age-0/100 feet
Whitaker Bayou	18		25100	7.2%	2.30
Bowlees Creek	28		46100	6.1%	1.11
North Creek	45	66	79200	8.3%	3.00
South Creek	26		79570	3.3%	1.15
Total Sets	117 Sets				

All captured snook were tagged with VIE tags in the caudal fins and with sequential CWTs before they were released. Different VIE colors were used to identify the creek where the snook were captured, and fin location to identify snook recaptured multiple times.

Age 0 and age-1 snook abundance were estimated in the following way:

$$\text{total age-0 snook} = x * \text{ts}/100$$

where, x = mean catch of age-0 snook within a creek
 ts = total shoreline habitat (in feet)

May 2002 Tagging and Release

In May 2002, 2477 juvenile hatchery-reared snook were tagged and released into creeks and estuaries around the Sarasota area. Tagging activities occurred on May 14-15, 2002 at the Mote Marine Laboratory Aquaculture Facility. All released snook were tagged with a coded-wire tag (CWT) and a visible implant elastomer (VIE) tag. CWTs were injected into the left cheek muscle with automatic CWT injectors. CWTs identified the experimental treatments outlined above, as well as general size class (small: 70-125mm FL; medium: 125-160mm FL; and large: 165-270mm FL). Fluorescent red VIE tags were injected into the caudal fins of each snook for release. This tag provided a visible identification of the released fish as hatchery reared snook.

Release numbers were then calculated for each creek using the available hatchery snook (~ 2800 snook). Because Whitaker Bayou and Bowlees Creek were estimated to have lower age-0 snook populations, they were chosen for “high density” releases and North Creek and South Creek were chosen for “low density” releases. Pre-release estimates of age-0 snook in the creeks were as follows:

Creek	Wild population	Releases			
		Proposed % increase	*Est. Release #	Actual % inc.	Actual Release #
Bowlees Creek	281 age-0 snook	100	562	158.2	889
Whitaker Bayou	523 age-0 snook	100	1046	97.8	1024
North Creek	2509 age-0 snook	10	502	8.7	436
South Creek	765 age-0 snook	10	153	8.4	128

The estimated release number is twice the actual number needed because previous work has shown that at least 50% mortality occurs when fish are not acclimated in pens at the release site for 3 days prior to release (Brennan et al. Unpublished data). An unknown additional post-release mortality occurs with the hatchery snook - - even with acclimation, and therefore, as a rule of thumb, 2x the actual number needed was released.

Post-release sampling occurred in June 2002, August, October, December, and January 2003, and results from the follow up sampling indicated potential for an additive effect of the hatchery releases, although these effects are confounded with seasonal dispersal patterns and uncertainty in potential immigration of non-release system juveniles. We feel that these experiments need further investigation in several areas:

(1) Release magnitude needs to be increased by several fold so detection of effect size is more apparent. Releases may even need to be scaled up by an order of magnitude. (2) Intrinsic differences among release sites call for a need of crossover experiments with recruitment manipulation and creek density. To perform this, several release years are needed, and careful within-year estimations of abundance are needed. (3) We need to evaluate the effects of releases of age-1 snook on the wild age-0 snook, in addition to the effects within year classes.

In achieving these goals, in 2003-2004 we will continue to perform seasonal standardized sampling in the nursery habitats as baseline data for future releases when more hatchery-reared juveniles are available for release. Sampling will occur in June 2003, October/November 2003, and January-March 2004. If the proposed snook aquaculture research and development work identified in Objective 4 provides sufficient numbers of juveniles (we estimate about 10,000 are needed), we will repeat the above experiment, attempting to triple or quadruple snook densities at the primary treatment sites, while maintaining control abundances at ~ a 10% increase.

New Hampshire

Two of the most important issues in any stock enhancement program are the timing, and location(s) of the releases. Timing is critical because of the temporal variability in most environmental attributes (e.g. seasonally changing temperature, salinity, prey availability, predator abundance). Similarly, there is a great deal of spatial variability (e.g. differences in substrate type, amount of vegetation, current velocities, predator abundance, prey availability, etc.). Thus the set of all biotic and abiotic variables, which collectively form the organism's habitat, vary in both space and time. This complexity presents real challenges to those interested in marine stock enhancement, because maximal success can only be achieved if the organisms are released into the "optimal habitat", i.e. the set of conditions that will maximize their growth and probability of survival.

One of the most promising approaches to defining, and then identifying an organism's optimal habitat is through mathematical modeling. Examples include the use of linear discriminate function analyses, Categorical Analysis Regression Tree (CART) and Habitat Suitability Index (HSI) models (Norcross et al. 1995, 1997; Rubec et al. 1998; Jury 1999; Gallaway et al. 1999). In such approaches, the spatial and temporal distributions of important environmental attributes are determined through field sampling programs. These are then digitized to create detailed, grid-like environmental maps. Because each grid cell contains the geometric mean value of each of the attributes, each cell can be assigned a "suitability index", which measures, based on what is known about the biology of the organism in question, how suitable any specific area (box within the grid) is. While modeling studies such as these have been used extensively in fisheries research to identify nursery areas and to study essential fish habitat, they have yet to be used in stock enhancement studies.

The first year's goal of the UNH research was to apply these very powerful modeling techniques in our stock enhancement research. Results were very promising, and the interested reader can find details in our interim reports. As part of the proposed research we intend to verify the modeling results using *in-situ* growth and survival studies (see below).

UNH Research Plan-2003.

The proposed research will expand a winter flounder (*Pseudopleuronectes americanus*) stock enhancement research program underway in New Hampshire. In this, we have been examining the fitness of hatchery-reared fish, including their growth and survival and their vulnerability to predators (Fairchild 1998, Fairchild and Howell 2000). We have also completed an intensive study of winter flounder habitats in the Great Bay Estuary, and used this information to predict optimal release locations (Wanat 2002). The proposed research uses these studies as a foundation upon which to build.

All six parts of the proposed research will rely on the production of juveniles, so we begin with a brief outline of the techniques that will be used. Following that, we will present each of the six parts.

Juvenile Production

Production of juveniles will follow the methods outlined in Fairchild (1998) and Howell and Litvak (2000). Briefly, adult winter flounder will be obtained in March from local commercial fishermen and transported to the UNH Coastal Marine Laboratory (CML). Several groups, each consisting of 2 males and 1 female, will be held in 1m³ circular tanks supplied with flowing seawater. Experience has shown that spawning occurs volitionally in these systems, and that fertilization rates are very high. Embryos will be moved to 6m³ tanks supplied (1 liter/min) with filtered (5 micron), ultraviolet treated, ambient temperature (5-6°C) seawater. Three to four days after hatching, microalgae are added to the tanks each day, and the larvae are fed microalgae-enriched rotifers (*Brachionus sp.*) twice daily at a rate of 4000 per liter. This diet is replaced by DHA Selco™ enriched *Artemia* nauplii as the larvae increase in size. After the fish have metamorphosed, which occurs 35-40 days after hatching, weaning begins by co-feeding

enriched *Artemia* and the weaning diet (Biokyowa™). Over the course of 10 days, the ratio of live food to dry diet decreases until the fish are only offered formulated food. As the fish increase in size, the weaning diet will be replaced by a formulated diet produced by Nutreco (Gemma). Particle size will increase as the fish grow. Because winter flounder >20mm TL are considerably less vulnerable to predation by green crabs (*Carcinus maenas*) and seven-spine bay shrimp (*Crangon septemspinosa*) (Witting and Able 1993,1995; Fairchild and Howell 2000), juveniles will be held in the laboratory until they are >20mm TL, which should occur in July.

Part 1. Test the predictive capabilities of HSI models through *in-situ* experiments.

Wanat (2002) developed Habitat Suitability Index (HSI) models designed to identify the optimal habitat of juvenile winter in the Great Bay Estuary of New Hampshire. The approach was to intensively study the temporal and spatial distribution of wild juvenile flounder, and to characterize their habitat in terms of temperature, salinity, depth, substrate type, prey availability, and predator abundance. A number of mathematical modeling techniques were used to calculate the overall HSI for each site and month, which were then compared to the observed abundances of winter flounder to see if the distribution of fish fit the habitat model. The abundance of winter flounder juveniles also was compared with each variable within the model to determine which component(s) are driving habitat selection. Finally, the HSI model predictions were tested with actual temporal and spatial distribution patterns of the fish to determine the utility of the model.

The intensive field work and the habitat suitability modeling approach proved useful in many ways. We now understand what the small fish are eating in nature, we know the temporal and spatial distribution of these prey taxa, as well as their seasonal abundances. We also have the same data for their potential predators. Further, we have identified 2 sites that, at least on the basis of 4 variables, seem to be quite suitable for winter flounder juveniles. We intend to complete the study in the summer of 2003 by testing the predictive capabilities of the HSI models through *in-situ* experiments. In this work, we will test growth and survival at 5 geographically separate locations. Two of these will be the locations that the HSI modeling predicted as “best” in the Great Bay Estuary, and 2 will be locations the modeling predicted as “worst” in the Great Bay Estuary. The fifth location will be in the Hampton-Seabrook estuary at the proposed pilot-scale release location.

Experiments will be conducted in 1m³ enclosures that we, and others (Sogard 1992, Phelan et al. 2000) have used successfully in the past. Each enclosure has 6mm plastic mesh sides and top panel, but the base is open to allow fish to contact the natural substrate. Metal extensions of the side walls extend into the sediments to anchor the enclosures in place, and also prevent the escape of fish or the entrance of predators. Three replicates of these will be placed in each of our 5 study sites. Laboratory reared juvenile winter flounder, approximately 25mm TL, will be CMWT tagged, stocked (n=10 per enclosure), and held in the enclosures from July through November, and their growth and survival will be measured at weekly intervals. Prey abundance inside and outside of the enclosures will be tracked from monthly core samples, and temperature will be recorded using data loggers attached to one enclosure at each site. Our prediction is that the fish held in areas with high HSI values will display good growth and survival

relative to those in areas with low HSI values, and thereby confirm the habitat modeling approach.

Part 2. Pilot Scale Releases

We intend to release 10,000 juvenile winter flounder in the coming year. This experimental release will allow us to: 1) estimate the mortality rate of released fish, and compare it to wild fish; 2) estimate the growth rate of released fish, and compare it to wild fish; 3) describe the diet of released fish, and compare it to wild fish; 4) study the movements of released fish, and compare them to wild fish; and 5) gain insights about the carrying capacity of the release location.

Tagging and releasing

All released juveniles will be tagged to differentiate them from wild fish. Two tagging methods have been used successfully with winter flounder juveniles in our laboratory. These include Visible Implant Florescent Elastomer tags, and Coded Micro Wire Tags (CMWT), both developed by Northwest Marine Technologies, Inc. For this study we intend to use CMWT tags because they are quickly applied, easily detected, and fish can be identified as individuals. Our previous tagging studies have shown that there is a size-specific mortality associated with coded wire tags. Fish ≤ 21 (+/- 4.0) mm and 0.2 (+/-0.1) g are mortally wounded by the tagging process and /or the tags themselves (Fairchild 2002). Therefore, only fish greater than 25 mm will be tagged. Immediately prior to release, all fish will be inspected to ensure that tags are still in place. We expect a 9% tag loss based on our prior tag-retention studies (Fairchild 2002). Prior to stocking, all juveniles will be anesthetized (MS222), tagged with a CMWT, and allowed to recover for 48 hours. A sample of 100 fish will be held in the laboratory to confirm tag retention. In addition to the laboratory-reared juveniles, a sample of 1000 wild juveniles will be caught at the release site and marked (CMWT) in an identical fashion. This will be done 2 days before the release, and the fish will be held in the laboratory in tanks containing natural substrates and supplied with flowing seawater.

We also hope to use ultrasonic tags to monitor the small-scale (10's of meters) movements of the released fish. Because ultrasonic tags small enough to place on fish as small as 25 mm do not currently exist, we intend to develop the technology in conjunction with a commercial ultrasonic tag manufacturer, Sonotronics. We are currently working with them to adapt their SMT-02 tags to suit our purposes. These tags are 13 x 7 mm cylinders, weighing 0.5 g in water and have a battery life of 8 days. We can extend the battery life by manipulating pinger rates and intervals, and we hope to reduce the tag weight by 50%. The tagging protocol for the SMT-02 tags for juvenile winter flounder will be developed in the laboratory. Fish will be anesthetized with MS-222, and the tags will be applied externally (dorsal surface) using a combination of SuperGlue™ and small sutures. We have already begun preliminary experiments with this, and the combination seems to be effective. Once we are confident that the tags can be attached effectively, we will observe the swimming and feeding ability of the fish in the laboratory to ensure that tag does not affect the fish's behavior or probability of survival.

Releases will occur in July because: a) the fish will >25mm, and thus less vulnerable to predators (Witting and Able 1993, 1995; Fairchild 2000); b) it is the time when juveniles are most abundant in our field sampling programs, and when preferred prey are relatively abundant (Wanat 2002); and c) releasing fish in July ensures that we will have several months for field work before winter weather limits our sampling activities. We have selected the release location based on the known environmental requirements of juvenile winter flounder. Although the species is eurythermal (Casterlin and Reynolds 1982), euryhaline (Bigelow and Schroeder 1953), and found over a wide range of depths and substrate types (Able and Fahay 1998), highest abundances of wild fish have been found in small coves just inside of estuarine inlets (Witting 1995, Able and Fahay 1998). Such areas may serve as critical habitats because of their low current speeds, the deposition of fine-grain sediments that often occur in such locations, and their close proximity to adults as they enter the estuary for spawning. Our chosen release location will be in the Hampton-Seabrook estuary. The specific location (approximately 1km²) has a sandy-mud substrate, is relatively shallow (<2m) at low tide, is bounded by a deep channel, and has relatively high salinities (28-30ppt) and cool temperatures in the autumn (6-14°C). There is also good access for sampling vessels and very few features that would obstruct towed nets. Finally, it is a site that has been extensively sampled (>20 years) for winter flounder juveniles by a local utility company (Normandeau Associates 2001) and the state of New Hampshire. These long, and continuous databases indicate that winter flounder juveniles are more abundant in this location than any others in either the Great Bay or Hampton-Seabrook estuaries. As importantly, the long-term data base will allow us to evaluate, at least in a general sense, the effectiveness of the stocking program.

To begin the release, tagged fish, both cultured and wild, will be collected from laboratory tanks, placed 100 each in insulated containers (0.5m² bottom surface area) filled with seawater, and moved to the transport vessel. Containers will be supplied with seawater and oxygen during the 1.5 hour transit by boat. At the release site, fish will be placed (SCUBA divers) into 2 pre-deployed, vinyl-coated wire acclimation chambers (10 x 10 x 0.5m). Fish will be held in these acclimation chambers for 24 hours, and then released.

Field sampling

Fish sampling will begin the day after release, and will occur daily for the first 2 weeks, and at weekly intervals for the following 10 weeks. Three types of collecting gear will be used on each sampling occasion. In shallow water (<1.5m) we will employ a 33m x 2m beach seine. Three replicate samples, each with an approximate swept area of 1500m², will be taken near high tide. In mid-depth areas (1.5-3m) we will employ a 1m beam trawl. For the first 4 weeks of sampling this will have a 3mm mesh net. As the fish grow, the mesh size will be increased to 6mm. Three replicate 50m long tows will be taken within the 1.5-3m depth interval, each parallel to the shore. In the deeper areas (>3m) we will use 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 will be taken within the depth interval, each parallel to the shore, and each approximately 100m length. The catch from all fish sampling will be identified and enumerated. All winter flounder caught will be checked for CMWT. Abundance will be estimated as catch-per-unit-effort (CPUE), given as

number caught per m² sampled. On each sampling occasion we will also measure and record bottom temperature, salinity, dissolved oxygen, and depth.

To characterize prey availability, we will take a weekly series of 6 replicate benthic cores (0.0079m² to a depth of 10cm), beginning at release, and continuing for 3 months. Cores will be stored in Zip-Lock™ bags, placed in ice, and returned to the laboratory where they will be sieved through a 1mm mesh sieve. All prey taxa will be fixed in 10% formalin and stored in 40% ethanol until they are identified, counted and weighed.

Estimating mortality

Quantitative estimates of mortality assume no emigration from the release site, however we should be able to know if this assumption is violated from our emigration/dispersal study (described below). Instantaneous natural mortality rates (M) will be calculated from survival estimates (S) as:

$$S = N_{t+1}/N_t$$

and

$$M = -(\log_e N_{t+1} - \log_e N_t)$$

where N = number caught, and t = time interval. For the first two weeks after release, the time interval will be one day, yielding daily mortality estimates. From weeks 3-12 after release, sampling will be done weekly, yielding weekly mortality estimates. A longer term, monthly instantaneous natural mortality rate estimate (M_{mo}), will be calculated using catch curve analyses based on weekly abundance data (Ricker 1975). The estimates described above will be made for both wild-caught and laboratory-reared fish. Rates for wild-caught and laboratory-reared fish will be statistically compared to determine if they suffer differential mortality.

Estimating growth

Growth rates of both cultured and wild fish will be calculated from increases in sizes (lengths and weights) over time of recaptured fish. Daily, weekly, and monthly rates will be calculated in each year of the project, and the rates of wild-caught and laboratory-reared fish will be statistically compared.

Describing diets

To compare the feeding ecology of laboratory-reared and wild-caught fish, we will dissect out and preserve stomachs from a representative sample of 10 recaptured fish, and 10 similarly sized wild fish, at weekly intervals. Prey taxa will be identified, to the lowest possible taxonomic level, using a dissecting microscope. The number and total weight of each taxa will be recorded. An Index of Relative Importance (IRI) will be calculated for each prey taxa, for laboratory-reared and wild-caught fish. These data will allow us to determine if cultured and wild fish have similar or different diets, and also to examine ontogenetic changes in diets of the two groups.

Estimating movement/emigration

Movement away from the release location, here considered emigration, will be quantified using recapture data. The magnitude of movement will be estimated from the mean of all straight-line distances between recapture locations and release location. The rate of movement will be estimated from distance traveled per unit time. Data will also be grouped by size class and month to determine how these two variables affect distances moved and rates of movement.

Although we will have very accurate positional data (differential GPS) at the start and end of all beam trawl and otter trawl samples, small scale (<50m) movements will be difficult to quantify because it will be impossible to know exactly where the fish were caught during the sampling tow. For this reason, we intend to develop and use the small ultrasonic tags described above. Ten tags, each with a discrete acoustic signature that allows each fish to be identified as an individual, will be used. These ultrasonically tagged fish will be released, along with the other fish, in each release event. By using a hydrophone and receiver onboard a research boat, the fish will be tracked multiple times per day to monitor their movements. If we are unable to discern whether the fish is still alive, or fear that the tag has been shed (e.g. no movement or activity noticeable by hydrophone), divers will be used to locate the fish and/or tag. Added benefits of using ultrasonic tags to monitor small-scale movements include the acquisition of data on fine-scale habitat preference within the release site, and movements of the fish in association with diel and tidal cycles.

Part 3. Studying carrying capacity

One of the central questions in marine stock enhancement is whether the release of fish increases abundance in the wild, or simply displaces the wild fish targeted for enhancement. To a large extent, the answer to this question depends on the carrying capacity of the environment. Unfortunately, carrying capacity is extremely difficult to quantify because of the complexity of the aquatic ecosystems, and characteristic temporal and spatial variability. Because of this, the question of increases to, or displacement of, wild populations, and the issue of carrying capacity, have been studied indirectly in most stock enhancement research. Ecological theory predicts that if a population is at carrying capacity, the addition of more individuals (via stock enhancement) would trigger density dependent mechanisms. These could include a reduction in individual growth rate because of limited food resources, dispersal of portions of the population into unfavorable habitats, changes in diet, decreases in survival, and/or increases in agonistic behavior.

Although we do not know what the carrying capacity of the Great Bay or Hampton-Seabrook estuaries is for winter flounder, we do know that recent estimates of abundance, all of which are 2 fish per 100m² (Armstrong 1995, Normandeau Associates 2001, Wanat 2002, Fairchild 2002), are much lower than estimates for some other estuaries, where abundances as high as 35 to 40 fish per 100m² have been reported (Sogard 1992, Goldberg et al. 2002). Although there is no long-term data series that can be used to track abundance in the Great Bay estuary over time, a 24 year data set is available for the Hampton-Seabrook estuary which is located <20 miles away.

This shows a significant decrease in winter flounder abundance over the last decade (Normandeau Associates 2001), which is suggestive that similar decreases have occurred in the Great Bay estuary. If this is true, the Great Bay flounder population may be well below carrying capacity, perhaps due to decreases in recruitment caused by the over fishing of adults. Alternatively, it is possible that the habitat has been altered in some way, and that the resources needed to support larger numbers of fish no longer exist. Limited catch due to restricted carrying capacity appears improbable, because the species has demonstrated an exceptional ability to successfully inhabit a wide range of conditions and habitat types. Understanding the reason(s) for the observed regional decline is the subject of intense study by others, but our research should contribute to this understanding.

Although it seems unlikely that winter flounder are at their carrying capacity, we plan to examine the question of whether the release of winter flounder would result in an increase in the wild population, or simply displace the wild fish. To accomplish this, we will take advantage of the species' susceptibility to density-dependent processes. DeLong et al. (2001) found, using an 11 year time series from Narragansett Bay, that the population had declined dramatically, and that both mortality and growth rates of juvenile winter flounders were negatively correlated with fish density. Thus if we observe a decline in growth and survival after stocking, it would be suggestive that density dependent mechanisms were operating, and that the carrying capacity of the localized habitat had been exceeded. Similarly, studying the feeding ecology of released and wild fish, and how natural prey density changes, will provide clues about carrying capacity. If, for example, we observe changes in the diets, in the amounts of food eaten, or in the prey community, it would be suggestive that preferred food resources had become limited, and that stocking had caused the population to exceed carrying capacity. Finally, following small-scale changes in the temporal and spatial distribution of each fish type will provide clues about the carrying capacity. For example, if wild fish emigrate rapidly from the release site, compared to the cultured fish, it would suggest that carrying capacity had been exceeded. Conversely, if there are no changes in growth and survival rates, no changes in feeding ecology, and no differential movements, we would interpret this as evidence that carrying capacity had not been exceeded, and that the experimental stocking was enhancing the wild population rather than displacing it.

Part 4. Study stress physiology associated with fish transport

Aquaculture and stock enhancement practices unavoidably subject fish to a variety of husbandry related stressors (Waring et al. 1992; Pickering 1993). One particular stressor is the movement of fish (Carmichael et al. 1984; Davis and Parker 1986; Robertson et al. 1988). The adverse stimuli produced by initial capture, loading into transport containers, the actual transport, unloading, and finally stocking have been shown to cause hypersecretion of both catecholamines and corticosteroids in teleosts (Robertson et al. 1988; Barton and Iwama 1991; Barnett and Pankhurst 1998). This primary stress response, which may occur immediately following extended handling and/or transport, can induce a cascade of secondary effects, including osmoregulatory, metabolic, and immune disturbances resulting in detrimental affects to fish health, growth, and survival (Carmichael et al. 1984; Robertson et al. 1988; Barton and Iwama 1991).

Examining the aforementioned disturbances and their related physiological changes have proven useful in modifying capture and transport techniques (Robertson et al. 1988; Pickering 1993).

Unfortunately, much of the published literature on teleost stress physiology has focused on adults (Barton and Peter 1982; Bergheim et al. 1990; Robertson et al. 1988; Barton and Iwama 1991; Waring et al. 1992; Wedemeyer 1996) while information describing the transport and/or handling stress response of juveniles is lacking (De Carvalho et al. 2002). The few juvenile species that have been studied suggest that handling and confinement associated with fish transport are likely to impact the performance and scope of juvenile survival (Serafy et al., 1999; Shrimpton et al., 2001; De Carvalho et al., 2002). We intend to evaluate the physiological stress responses associated with movement of hatchery reared juvenile winter flounder from the laboratory to the release site. These results will contribute to knowledge on the stress response in juvenile fish, and indicate if, and how, our transport and acclimation practices should be modified. Two stress physiology experiments will be undertaken. The first seeks to quantify physiological stress associated with tagging, and to determine whether physiological differences exist in winter flounder juveniles when tagged with elastomer tags and coded wire tags. To accomplish this, 60 cultured juvenile flounder will be hand netted, and anesthetized. Half will be tagged using elastomer tags and half using coded wire tags. All will be immediately snap frozen on dry ice (-70 °C), and stored at -20 °C until further analyses of cortisol and glucose levels. A control treatment will consist of 30 flounders hand netted and anesthetized but not tagged, immediately snap frozen on dry ice (-70 °C), and stored (-20 °C) until further analyses. An additional 360 fish also will be tagged (120 elastomer, 120 coded wire, 120 no tag control) and stocked into holding tanks at the CML. Thirty each from the two treatments and control will be sampled at 12 h intervals, for 48 h, to determine the time period needed for cortisol and glucose to return to baseline values after tagging.

A second experiment will measure physiological differences in cultured winter flounder during and after transportation from the hatchery to the release site, and also determine if stocking density during transport affects stress physiology. To accomplish this, juvenile flounder will be hand netted, loaded into insulated, 0.5m² containers on board a UNH research vessel and transported to the release site. Transit time will be about 1.5 hours. Fresh seawater will be continuously pumped through each container during the transport and stocking procedure. To ensure water quality is maintained during transport, dissolved oxygen, temperature, and pH levels will be monitored continuously. Three replicates each of three different transfer densities (100, 200, and 400% bottom area coverage) will be examined in order to quantify which, if any, density is most optimal for juvenile winter flounder transport and release. Before the transfer process starts, 10 juvenile flounder from the hatchery tank will be hand netted, immediately snap frozen on dry ice (-70°C), and stored at -20°C until further analyses. These fish will constitute the control portion of this study. At the beginning and end of transfer, and at half hour intervals during the boat trip, 10 fish from each replicate in each density treatment will be hand netted, anesthetized, immediately snap frozen on dry ice (-70°C), and stored at -20°C until further analyses. All remaining juvenile flounder will be released into the stock enhancement site.

Physiological measurements for both Experiments 1 and 2

Preparation of juvenile flounder will follow a modified technique of Hiroi et al (1997). The frozen samples weighing approx. 100-400 mg will be homogenized in a five fold volume of ice cold PBS (0.06M Na₂HPO₄*7H₂O, 0.04M NaH₂PO₄*H₂O, 0.15M NaCl, 0.1% sodium azide) using a Polytron homogenizer and 36mm generator (Glenn mills, USA). The homogenate

(approximately 500 μ l) will be extracted twice with 5 ml of diethyl ether by vortexing for 2 minutes. The aqueous layer will be snap frozen in an acetone/dry ice bath before decanting the organic layer into 16 x 100cm borosilicate glass tubes. The ether will then be evaporated to dryness in a 37° C water bath under a stream of nitrogen. The aqueous phase will be re-extracted as described above and the extracts combined. To reconstitute the dry residue, 500 μ l of tetrachloromethane will be added and vortexed for 4 minutes. PBS containing 0.1% gelatin (500 μ l) will then be added and vortexed for 2 minutes. The mixture will then be centrifuged (3000 rpm, 10 min, 4 °C), and the aqueous layer removed and stored at – 20 °C until further use. Triplicate samples of plasma glucose (mg/dl) and cortisol (ng/ml) will be analyzed by the Atlantic Veterinary College (Prince Edward Island, Canada) using enzymatic colorimetric assay (Diagnostic Products Corporation Los Angeles, CA., USA; Boehinger Mannheim/Hitachi 917 analyzer) and competitive immunoassay (Diagnostic Products Corporation Los Angeles, CA., USA), respectively. Results will be presented as mean \pm 1 SEM (N) and analyzed using ANOVA, followed by a Tukey's test.

Statistical data analysis

Results will be presented as mean \pm 1 SEM (N). Statistical analyses will be conducted by ANOVA, followed by a Tukey's test using SYSTAT version 10 software (SPSS, Inc., Chicago, Illinois, USA). A probability (P) value of < 0.05 will be considered significant.

Part 5. Sampling gear efficiency

In order to assess the impacts of released cultured fish, both cultured and wild winter flounder populations need to be sampled effectively, i.e. the sampling gear must be able to catch a sample of each population that accurately reflects their abundance. While there are many ways to sample flatfish populations, beam trawls have become the international standard for surveying juvenile flounder (Kuipers et al. 1992). Unlike an otter trawl, the mouth of the beam trawl is fixed, thereby providing a standardized way to measure the area swept by the net. The goal of this study is to determine how effective our beam trawl is and, if necessary, to modify it to increase its likelihood of catching small juvenile flounder.

Previous studies have compared research beam trawls to 1 m² drop nets which are nearly 100% efficient (Wennhage et al. 1997, Nicolajsen et al. 2002). In order to conduct this type of efficiency study, however, the target species density must be >0.4 per m², otherwise the number of drop trap replicates needed is too great (Wennhage et al. 1997). In Great Bay, winter flounder abundance is < 2 fish per 100 m² (Armstrong 1995, Fairchild 2002, Wanat 2002), so a comparison of beam trawls and drop traps is not practical.

An alternative calibration technique has been suggested by Kuipers et al. (1992), in which glass beads, similar in size and density to the fish, are deposited by divers in 1 m² quadrats on both sandy and muddy substrates. The beam trawl is then towed through these quadrats, and the percentage of beads captured by the net gives an estimate of gear efficiency. Divers also observe how the gear fishes (e.g. whether it's riding smoothly or skips over the bottom) so that

appropriate alterations can be made to increase its efficiency. Our intention is to perform similar tests in our field-sampling site, and to adjust the net to maximize its efficiency.

Once the beam trawl gear is adjusted for sediment type, its efficiency at capturing juvenile winter flounder will be tested. A rectangular *in situ* enclosure, measuring 3m by 50m, will be constructed near our release site. The enclosure will consist of a 1m high, 5mm mesh wall, with float lines at the surface and lead lines at the base. The enclosure will be stocked with a total of 400 measured (TL) and tagged juvenile winter flounder. Half will be wild fish and half will be cultured fish. Immediately after stocking, the area will be sampled with one pass of the beam trawl. The number of captured tagged flounder and their lengths will be recorded. The known abundance (2.67 fish per m²) will be compared to the abundance from the beam trawl. This experiment will be repeated 5 times, using different fish on different days, and an average gear efficiency will be calculated. Because we expect efficiency to change with fish size and water temperature, we will conduct this exercise once each month during the field-sampling season. These final efficiency values will be used to adjust our catch-per-unit-effort data during the study.

Part 6. Determination of Sex Ratio

Studies have shown that sexual differentiation, and therefore male:female sex ratio, in some flatfish species can be influenced by juvenile incubation temperature (Yamamoto 1999, Luckenbach et al. 2003). This also may be true for winter flounder, whose juveniles are quite eurythermal (Klein-MacPhee 1978), but sexual differentiation and the sex ratio of cultured fish have never been investigated. This is significant because the sex ratio of fish used in stock enhancement programs can affect the wild population. For example, Kanaiwa and Harada (2002) used modeling techniques to study the impacts of stocking sex reversed males, which were genetically XX females but phenotypic males. They found that this could result in the extinction of the gene on the Y chromosome that determines sex, resulting in profound changes in sex ratio and population dynamics of the wild population.

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 intend to study sexual differentiation and cultured fish sex ratio as part of this study. To accomplish this, we will sample fish from the general culture population at approximately 10mm total length (TL) intervals, starting at metamorphosis and continuing through the first year. On each sampling occasion, we will randomly collect 30 fish. Tissues will be fixed in Bouin's solution for 48 hours, washed in 50% ethanol, and stored in 70% ethanol until processed. Sex of the fish will be determined histologically. Histological processing will involve embedding the tissues, sectioning (2-6 microns), and staining with hematoxylin and eosin. Slides will be examined to view structures and cells associated with gonadal tissue. By examining the size series of fish collected, we will be able to determine the size and age when sexual differentiation occurs, as well as the sex ratio of the cultured population. Results of these studies will form the foundation of future work designed to determine which variables affect sex ratio.

Objective 9: Conduct Economic Analysis

Justification: The success of an enhancement program depends upon the desired objectives; a fish biologist may simply be concerned with whether the program can raise viable juveniles and release them, a fisherman may care only whether it provides more fish to catch than would otherwise be available, and a politician or manager may care only whether the program relieves political pressure to do something. We should want to ask how much the enhancement program costs and what benefits are obtained; how else can we decide whether marine enhancement is a good use of public funds (Hilborn, 1998)?

Costs and benefits should be estimated and economic models developed to predict the value of enhancement. This information can be used to generate funding support through legislation or user fees. The information can contribute to an explicit understanding with policy makers and the general public on the time frame that is needed before full-scale releases can begin for such components as adaptation of culture technology and pilot release experiments to initiate development of effective release strategies. Education of the public and policy makers on the need and benefits of a responsible approach is also important, because it will cost more than merely raising fish and releasing them.

Approach and Procedures:

An economic analysis of any stock enhancement programs will be complex, and will require quantities of financial data. In the first year of the project we began to build the financial data base by keeping accurate records of all costs associated with fish production (labor, supplies, etc.). Although these data will change given economies of scale, they will allow us to estimate the cost per released fish.

No activities are planned in 2003-2004.

Objective 10: Develop Adaptive Management Strategies

Justification: Adaptive management is a critical aspect of any stock enhancement program (Blankenship and Leber, 1995; Leber, 1999, 2002, in press; Walters and Martel, 2003). To be able to respond to changes in fishing mortality and other causes of reduction in stock abundance, the effects and effectiveness of management actions must be understood. Imposing sound design on the application of management treatments and tests is especially needed in the field of stock enhancement, where there has been little theoretical consideration of how to ensure it works. A paradigm shift is starting in the field of fisheries management, where focus in the field needs to shift towards insuring all fisheries-management activities are treated as part of large-scale management experiments (Walters and Martel, 2003).

When critical uncertainties about the basic assumptions made about the outcome of enhancement are identified and prioritized, choice of production and management strategies can be tailored to new information about these basic assumptions. A process is needed to evaluate these uncertainties as an integral part of the species management plan. The plan should include a feedback loop to evaluate and change production and management objectives based upon new information gained from assessment of enhancement effect. Such 'active adaptive management' is discussed by Hilborn and Walters (1992).

To use active adaptive management, three important protocols need to be established – (1) experimental objectives must be woven into the stocking process to provide

information on unresolved critical uncertainties about stock enhancement effectiveness, (2) an assessment strategy must be provided that will recover unbiased release-impact / release-effectiveness data, and (3) a feedback loop must be established that enables and guides modification to existing enhancement strategy, and agency management of that strategy, on the basis of new information about critical uncertainties.

Approach and Procedures:

Florida

Work has already begun in Florida to incorporate active adaptive management into the State of Florida's marine stock enhancement strategy. In 2001, a strategic plan entitled, "Florida Stock Enhancement: Long Range Plan for Success" (Leber et al., 2001) was submitted by FMRI to its parent agency, the Florida Fish and Wildlife Conservation Commission for consideration by the Commissioners. Integral to the success of this strategic plan is the incorporation of active adaptive management as part of a small research program, maintained by FMRI, to track the success (and failures) of stock enhancement activities in Florida. The plan will enable constant refinements to the enhancement process as critical uncertainties are evaluated in conjunction with each hatchery release. No further SCORE work is planned for 2003-2004 in Florida on this topic.

New Hampshire

Because the winter flounder program is still in the very early stages, no activities are planned in 2003-2004.

RELEVANT LITERATURE

- Able, K.W. and M.P. Fahay. 1998. The first year in the life of estuarine fishes in the mid-Atlantic Bight. Rutgers Univ. Press. New Brunswick, N.J., 342p.
- Andoh, T., K. Watanabe and T. Matsubara. 1999. Problems and perspectives in stock enhancement of barfin flounder. Bull. Hokkaido Natl. Fish. Res. Inst. 63: 19-33.
- Armstrong, M.P. 1995. A comparative study of the ecology of smooth flounder, *Liopsetta putnami*, and winter flounder, *Pleuronectes americanus*, from Great Bay Estuary, New Hampshire. PhD Dissertation, Univ. of New Hampshire, Durham, N.H.
- Bargmann, G.G. 1982. The biology and fisheries for lingcod (*Ophiodon elongatus*) in Puget Sound. Wash. Dept. of Fisheries, Tech. Rept. 66: 69p
- Bargmann, G. 1985. Management studies on lingcod in Puget Sound, Washington 1982 to 1984. Wash. Dept. Fisheries, Prog. Rept. 234: 39p.
- Barnett, C.W. and N.W. Pankhurst. 1998. The effects of common laboratory practices on the stress response of greenback flounder *Rhombosolea tapirina* (Gunther, 1862). Aquaculture 162: 313-329.
- Barton, B.A. G.A. and Iwama. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annual Review of Fish Disease 1: 3-26.
- Barton, B.A. and R.E. Peter. 1982. Plasma cortisol stress response in fingerling rainbow trout, *Salmo gairdneri* (Richardson) to various transport conditions, anesthesia, and cold stocking. Journal of Fish Biology 20: 39-51.
- Bergheim, A., F. Kroglund, D.F. Vatne, and B.O. Rosseland. 1990. Blood plasma parameters in farmed Atlantic Salmon (*Salmo salar* L.) transferred to sea cages at age eight to ten months. Aquaculture 84: 159-165.
- Bigelow, H.B. and W.C. Schroeder. 1953. Fishes of the Gulf of Maine. U.S. Fish Wildlife. Serv., Fish. Bull. U.S., Vol. 53, 577 p.
- Bilton, H.T., D.F. Alderdice, and J.T. Schnute. 1982. Influence of Time and Size at Release of Juvenile Coho Salmon (*Oncorhynchus kisutch*) on Returns at Maturity. Canadian Journal of Fisheries and Aquatic Sciences 39:426-447.
- Bjornsson, B.T., Halldorsson, O., Haux, C., Norberg, B., and Brown, C.L. 1998. Photoperiod manipulation of Atlantic halibut (*Hippoglossus hippoglossus*) sexual maturation: thyroid hormones and total plasma calcium levels. Aquaculture 166:117-140.
- Blankenship, H.L. and K.M. Leber. 1995. A responsible approach to marine stock enhancement. In H.L. Schramm Jr. and R.G. Piper (eds.), Uses and effects of cultured fish in aquatic ecosystems. Amer. Fish. Soc. Symp. 15: 167-175.

- Brennan, N.P., and K.M. Leber. 2001. Survival, growth, and recruitment of stocked juvenile snook to an adult fishery in Sarasota Bay, Florida. Abstract. January 21-25, 2001 World Aquaculture Society annual convention. Orlando, Florida, USA.
- Brennan, N.P., and K.M. Leber. 2000. Relative effects of release strategies on the survival of hatchery-reared juvenile snook in the wild. Abstract. May 2-6, 2000 World Aquaculture Society annual convention. Nice, France.
- Brown, R. and W. Gabriel. 1998. Status of fishery resources off the northeastern United States for 1998. NOAA Tech. Memo. NMFS-NE-115.
- Bromage, N. 1995. Broodstock management and seed quality – General considerations. IN: Broodstock Management and Egg and Larval Quality, Institute of Aquaculture, Blackwell Science. pp. 1-24.
- Brown, R. and W. Gabriel. 1998. Status of fishery resources off the northeastern United States for 1998. NOAA Tech. Memo. NMFS-NE-115.
- Buckley, J. 1989. Species profiles: life histories and environmental requirements of coastal fish and invertebrates (North Atlantic) - winter flounder. U.S. Fish Wildl. Biol. Rept. 82(11.87). 12 p.
- Buckley, R., G. Hueckel, B. Benson, S. Quinnell, M. Canfield. 1984. Enhancement research on lingcod (*Ophiodon elongatus*) in Puget Sound. Wash. Dept. Fisheries, Prog. Rept. 216: 93p.
- Carr, W.E.S., and C.A. Adams. 1972. Food habits of juvenile marine fishes: evidence of the cleaning habit in the leatherjacket, *Oligopolites saurus*, and the spottail pinfish, *Diplodus holbrooki*. U.S. Fishery Bulletin 70:1111-1120.
- Carmichael, G.J. 1984. Long distance truck transport of intensively reared largemouth bass. Prog. Fish Cult. 46: 111-115.
- Casterlin, M.E. and W.W. Reynolds. 1982. Thermoregulatory behavior and diel activity of yearling winter flounder, *Pseudopleuronectes americanus* (Walbaum). Environ. Biol. Fish. 7: 177-180.
- Coleman, F., J. Travis, and A.B. Thistle (eds). 1996. Marine stock enhancement: a new perspective. Proceedings of the First William R. and Lenore Mote International Symposium in Fisheries Ecology, November 20-23, Sarasota, Florida. Bulletin of Marine Science 62(2) 714 pp.
- Costanza, R., D. Duplisea and U. Kautsky. 1998. Ecological modeling and economic systems with STELLA. Ecological Modeling 110: 1-4.
- Cowx, I.G. 1994. Stocking strategies. Fish. Manage. and Ecol. 1: 15-30.
- Crowder, L. B. and W. E. Cooper. 1982. Habitat structural complexity and interaction between bluegills and their prey. Ecology. 63:1802-1813

- Danielssen, D.S., B.R. Howell and E. Moksness. (eds.) 1994. An international symposium on sea ranching of cod and other marine species, Arendal, Norway, 15-18 June 1993). *Aquaculture and Fish. Manage.* 25(1): 1-264.
- Davis, M. 2000. Queen conch (*Strombus gigas*) culture techniques for research, stock enhancement and growout markets. IN: Fingerman, M. and Nagabhushanam, R. (eds.) *Recent Advances in Marine Biotechnology*, Vol. 4, Aquaculture, Science Publishers, Inc. New Hampshire, USA, pp. 127-159.
- DeLong, A.K., J. S. Collie, C. J. Meise and J.C. Powell. 2001. Estimating growth and mortality of juvenile winter flounder, *Pseudopleuronectes americanus*, with a length-based model. *Can. J. Fish. Aquat. Sci.* 58: 2233-2246.
- De Carvalho, G.L., R. Roubach, and CARM. Araujo-Lima. 2002. Transportation of Tambaqui juveniles (*Colossoma macropomum*) in Amazon: main problems. *World Aquacult.* 33 (1): 51-53.
- Drawbridge, M.A., D.B. Kent, M.A. Shane and R.F. Ford. 1995. The assessment of marine stock enhancement in southern California: a case study involving the white seabass. In H.L. Schramm Jr. and R.G. Piper (eds.), *Uses and effects of cultured fish in aquatic ecosystems*. *Am. Fish. Soc. Symp.* 15: 568-569.
- FAO. 2000. *The State of World Fisheries and Aquaculture 2000*. Food and Agriculture Organization of the United Nations. , Viale delle Terme di Caracalla, 00100 Rome, Italy. ISBN 92-5-104492-9.
<http://www.fao.org/DOCREP/003/X8002E/X8002E00.htm>.
- Fairchild, E.A. 1998. Winter flounder, *Pseudopleuronectes americanus*, stock enhancement: a preliminary investigation into the performance of cultured, juvenile fish. MS Thesis, Univ. of New Hampshire, Durham, NH.
- Fairchild, E.A. 2002. Winter flounder, *Pseudopleuronectes americanus*, stock enhancement in New Hampshire: developing optimal release strategies. PhD Dissertation, University of New Hampshire, Durham, N.H. 142 p
- Fairchild, E.A. and W.H. Howell. 2000. Predator-prey size relationship between *Pseudopleuronectes americanus* and *Carcinus maenas*. *J. Sea Research* 44:81-90.
- Folkvord, A., G. Blom, O. Dragesund, A. Johannessen, O. Nakken and G. Naevdal. 1994. A conceptual framework for enhancing and studying recruitment of marine fish stocks. *Aquaculture and Fish. Manage.* 25: Supplement 1, 245-258.
- Goldberg, R., B. Phelan, J. Pereira, S. Hagan, P. Clark, A. Bejda, A. Calabrese, A. Studholme and K.W. Able. 2002. Variability in habitat use by young-of-the-year winter flounder, *Pseudopleuronectes americanus*, in three northeastern U.S. estuaries. *Estuaries* 25: 215-226.

- Galloway, B.J., J.G. Cole, R. Meyer and P. Roscigno. 1999. Delineation of essential habitat for juvenile red snapper in the northwestern Gulf of Mexico. *Trans. Amer. Fish. Soc.* 128: 713-726.
- Glazer, R.A. and C.J. Berg, Jr. 1994. Queen conch research in Florida: an overview. IN: R.S. Appeldoorn (ed.) *Proc. 1st Latin American Malacological Conference. Special Workshop on the Management and Culture of Queen Conch*, pp. 79-95.
- Hager, R.C., and R.E. Noble. 1976. Relation of size at release of hatchery-reared coho salmon to age, size, and sex composition of returning adults. *Progressive Fish-Culturist* 38:144-147.
- Hilborn R. & Walters C.J. 1992. *Quantitative Fisheries Stock Assessment*, Chapman and Hall, New York and London. 570 pp.
- Hilborn, R. 1998. The economic performance of marine stock enhancement projects. *Bulletin of Marine Science* 62(2):661-674.
- Hiro, J., Y. Sakakura, M. Tagawa, T. Seikai, and M. Tanaka. 1997. Developmental changes in low-salinity tolerance and responses of prolactin, cortisol and thyroid hormones to low salinity environment in larvae and juveniles of Japanese flounder, *Paralichthys olivaceus*. *Zool. Sci.* 14:987-992.
- Howell, W.H. and M. K. Litvak. 2000. Winter flounder culture. *In: Encyclopedia of Aquaculture*, R.R. Stickney (ed.), John Wiley and Sons, NY, pp. 998-1005.
- Howell, B. R., E. Moksness, and T. Svasand. 1999. *Stock enhancement and sea ranching*. Fishing News Books, Blackwell Science Ltd., Oxford. 606 pp.
- Houde, E.D. 1987. Fish early life dynamics and recruitment variability. *American Fisheries Society Symposium* 2:17-29.
- Iglesias, J. and G. Rodriguez-Ojea. 1994. Fitness of hatchery reared turbot, *Scophthalmus maximus* L., for survival in the sea: first year results on feeding, growth and distribution. *Aquaculture and Fisheries Manage.* 25(1): 179-188.
- Jury, S.H. 1999. Modeling the effect of temperature and salinity on lobster distribution and abundance in the Great Bay Estuary. Doctoral Dissertation, Univ. of New Hampshire, Durham, NH.
- Kanaiwa, M. and Y. Hirada. 2002. Genetic risk involved in stock enhancement of fish having environmental sex determination. *Population Ecology* 44: 7-15.
- Kapuscinski, A. R. and L. D. Jacobson. 1987. Genetic guidelines for fisheries management. Sea-Grant Research Report 17. Minnesota Sea-Grant. University of Minnesota, St. Paul. 66pp.

- Kent, D.B. and M. A. Drawbridge. 1995. Accomplishments and roadblocks of a marine stock enhancement program for white seabass in California. *American Fisheries Society Symposium* 15: 492-498.
- Klein-MacPhee, G. 1978. Synopsis of biological data for the winter flounder, *Pseudopleuronectes americanus* (Walbaum). NOAA Tech. Rept. NMFS Circular 414, 43 p.
- Knauss, J.A. 1994. The state of the world's marine resources. In: C.W. Voigtlander (ed.), *The State of the World's Fisheries Resources*. Proc. of the World Fisheries Congress Plenary Sessions. Inter. Science Publisher, USA. p.19_24.
- Kristiansen T.S. & Svåsand, T. (1990) Enhancement studies of coastal cod in western Norway. Part III. Interrelationships between reared and indigenous cod in a nearly land-locked fjord. *Journal du Conseil International pour l'Exploration de la mer*, 47, 23-29.
- Kuhn T.S. (1970). *The Structure of Scientific Revolutions*, 2nd edition. University of Chicago Press, Ltd., London.
- Kuipers, B.R., B. Maccurrin, J.M. Miller, H.W. van der Veer, and J.I.J. White. 1992. Small trawls in juvenile flatfish research: their development and efficiency. *Neth. J. Sea Res.* 29(1-3): 109-117.
- Landsberg, J.H., G. K. Vermeer, S. A. Richards, and N. Perry. 1991. Control of the parasitic copepod *Caligus elongatus* on pond-reared red drum. *Journal of Aquatic Animal Health* 3:206-209.
- Leber, K.M. 1985. Influence of decapod foraging and microhabitat complexity on seagrass communities: a field test of the refuge hypothesis. *Ecology* 66(6): 1951-1964.
- Leber, K.M. 1994. Prioritization of marine fishes for stock enhancement in Hawaii. The Oceanic Institute. Honolulu, Hawaii. 34pp.
- Leber, K.M. 1995. Significance of fish size-at-release on enhancement of striped mullet fisheries in Hawaii. *Journal of the World Aquaculture Society* 26:143-153.
- Leber, K.M., N.P. Brennan, and S.M. Arce. 1995. Marine Enhancement with striped mullet: Are hatchery releases replenishing or displacing wild stocks? *American Fisheries Society Symposium* 15:376-387.
- Leber, K.M., Brennan N.P., & Arce S.M. 1998. Recruitment patterns of cultured juvenile Pacific threadfin, *Polydactylus sexfilis* (Polynemidae), released along sandy marine shores in Hawaii. *Bulletin of Marine Science*. 62(2):389-408.

- Leber, K.M. 1999. Rationale for an experimental approach to stock enhancement. Pp 63-65 *In: Stock Enhancement and Sea Ranching* (eds. B.R. Howell, E. Moksness and T. Svasand). Chapter 5. Fishing News Books, Blackwell Science Ltd., Oxford. 606 pp.
- Leber, K.M., B. Halstead, and K. Haddad. 2001. Florida Stock Enhancement: Long Range Plan for Success. Unpublished document presented to Florida Fish and Wildlife Conservation Commission. 7 pp.
- Leber, K. M. 2002. Advances in marine stock enhancement: shifting emphasis to theory and accountability. Pp 79-90 *In* Stickney, R. R. and J. P. McVey (eds) *Responsible Marine Aquaculture* CABI Publishing, New York.
- Leber, K. M. In Press. Marine Stock Enhancement in the USA: Status, trends and needs. *In* Leber, K.M., S. Kitada, H.L. Blankenship and T. Svåsand (eds) *Stock Enhancement and Sea Ranching II*. Blackwell Scientific Publications, Oxford.
- Lockwood, S.J. (ed.). 1991. The Ecology and Management Aspects of Extensive Mariculture. ICES Marine Science Symposium 192:248 pp.
- Luckenbach, J.A., J. Godwin, H.V. Daniels and R.J. Borski. 2003. Gonadal differentiation and effects of temperature on sex determination in southern flounder (*Paralichthys lethostigma*). *Aquaculture* 216: 315-327.
- Main, K. L. 1987. Predator avoidance in seagrass meadows: prey behavior, microhabitat selection and cryptic coloration. *Ecology* 68(1):170-180.
- Martell, S. J. D, 1999a. Estimating lingcod biomass in Hecate Strait using stock reduction analysis. *In: N. Haggan and A. Beattie* (eds) "Back to the future: Reconstructing the Hecate Strait ecosystem.", Fisheries Centre Research Reports, Vancouver BC, 7:3, pp 49-55.
- Martell, S. J. D, 1999b. Ph. D. dissertation, University of British Columbia, Vancouver, BC.
- Martell, S. J. D., C. J. Walters and S. S. Wallace. 2000. The use of Marine Protected Areas for conservation of lingcod (*Ophiodon elongatus*). *Bull of Mar. Sco.* 66(3):729-743.
- Masuda, R. and K. Tsukamoto. 1998. Stock enhancement in Japan: Review and perspective. *Bulletin of Marine Science* 62(2):337-358.
- McEachron, L.W. and K. Daniels. 1995. Red drum in Texas: a success story in partnership and commitment. *Fisheries* 20(3): 6-8.
- Mills, M. L., 1994. Marine Fish Stocks in Washington: Status and Enhancement Considerations. *In: T. Noshio and K. Freeman*, "Marine Fish Culture and

- Enhancement Conference Proceedings”, Washington Sea Grant Pub # WSG-WO-94-1, pp 10-16.
- Moulton, L. L., 1977. An ecological analysis of fishes inhabiting the rocky nearshore regions of northern Puget Sound, Washington. Ph. D. dissertation, University of Washington, Seattle, WA. 181 pp.
- Munro J.L. & Bell J.D. (1997) Enhancement of Marine Fisheries Resources. *Reviews in Fisheries Science* 5(2):185-222.
- Murai, T. and Y. Koshiishi. 1998. Prospects in stock enhancement of Japanese flounder. *In: Howell, W.H. et al. (eds). Nutrition and Technical Development of Aquaculture. UJNR Tech. Rept. 26: 115-123.*
- Murphy, B. R. and W. E. Kelso. 1986. Strategies for evaluating freshwater stocking programs: past practices and future needs. *In Fish Culture in Fisheries Management. R. H. Stroud, editor. American Fisheries Society. pp. 303-316.*
- NMFS. 1999. Our Living Oceans. Report on the status of U.S. living marine resources, 1999. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-F/SPO-41, on-line version, <http://spo.nwr.noaa.gov/olo99.htm>.
- NOAA 1992. Our Living Oceans. NOAA Tech. Memo. NMFS-F/SPO-2, 148 pages.
- NOAA 1995. Our Living Oceans. NOAA Tech. Memo. NMFS-F/SPO-19, 160 pages.
- Neidig, C.L., D.P. Skapura, H.J. Grier, and C.W. Dennis. 2000. Techniques For Spawning Common Snook: Broodstock Handling, Oocyte Staging, And Egg Quality. *North American Journal of Aquaculture. 62:103-113*
- Nicolajsen, H., J. Carl, C.R. Sparrevohn, and O.R. Eigaard. 2002. On the efficiency of a hand-towed two-metre beam trawl and two different designs of push nets for catching juvenile flounder (*Plathichthys flesus*). *In Proceedings from the fifth international symposium on flatfish ecology, Isle of Man, 3-7 November, 2002.*
- Nitschke, P., R. Brown, and L. Hendrickson. 2001. Status of Fisheries Resources off Northeastern United States – Winter Flounder. <http://www.nefsc.noaa.gov/sos/spsyn/fldrs/winter/>
- Norcross, B.L., B.A. Halladay, and F.J. Muter. 1995. Nursery area characteristics of pleuronectids in coastal Alaska, USA. *Netherlands J. of Sea Res. 34(1-3): 161-175.*
- Norcross, B.L., F.J. Muter and B.A. Halladay. 1997. Habitat models for juvenile pleuronectids around Kodiak Island, Alaska. *Fish. Bull. 95(3): 504-520.*
- Normandeau Associates Inc. 2001. Seabrook Station 2000 Environmental Monitoring in the Hampton-Seabrook Area: A Characterization of Environmental Conditions.

- Palsson, W.A., J.C. Hoeman, G.G. Bargmann, and D.E. Day. 1996. 1995 status of Puget Sound bottomfish stocks. Wash. Dept. Fish and Wildlife, Manuscript Rept, 600 Capitol Way N., Olympia, WA 98501-1091, 98p.
- Perlmutter, A. 1947. The blackback flounder and its fishery in New England and New York. Bull. Bingham Oceanographic Collect., Yale Univ. 11(2), 92p.
- Phelan, B.A., J.P. Manderson, A.W. Stoner and A.J. Bejda. 2000. Estuarine and habitat related differences in growth rates of young-of-the-year winter flounder (*Pseudopleuronectes americanus*) and tautog (*Tautoga onitis*) in three northeastern U.S. estuaries. J. Exp. Mar. Biol. Ecol. 247: 1-28.
- Pickering, A.D. 1993. Husbandry and stress. Pages 155-169. *In:* J.F. Muir and R.J. Roberts (eds.). Recent Advances in Aquaculture IV. Oxford, Blackwell.
- Platt J. R. (1964) Strong Inference. *Science* 146:347-353.
- Polovina, J.J. 1991. Evaluation of hatchery releases of juveniles to enhance rockfish stocks, with application to Pacific ocean perch *Sebastes alutus*. Fish. Bull. U.S. 89: 129-136.
- Popper K.R. (1959) *The Logic of Scientific Discovery*. Basic Books, New York.
- Popper K.R. (1965) *Conjectures and refutations, the growth of scientific knowledge*. Harper and Row, New York.
- Richards, W.J., and R.E. Edwards. 1986. Stocking to restore or enhance marine fisheries. *In:* Fish Culture in Fisheries Management. (ed. by R. H. Stroud). pp. 75-80. American Fisheries Society, Bethesda, Maryland.
- Ricker, W.E. 1975. Computation and Interpretation of Biological Statistics of Fish Populations. Bull. 191, Fisheries Research Board of Canada. 382p.
- Roberts, C.M., N. Quinn, J.W. Tucker and P.N. Woodward. 1995. Introduction of hatchery reared Nassua grouper to a coral reef environment. N. Amer. J. Fish. Manage. 15(1): 159-164.
- Robertson, L., P. Thomas, and C.R. Arnold. 1988. Plasma cortisol and secondary stress responses of cultured red drum (*Sciaenops ocellatus*) to several transport procedures. Aquaculture 68: 115-130.
- Rubec, P.J., M.S. Coyne, R.H. McMichael and M.E. Monaco. 1998. Methods being developed in Florida to determine essential fish habitat. Fisheries 23: 21-25.
- Saucerman, S.E. and L.A. Deegan. 1991. Lateral and cross-channel movement of young-of-the-year winter flounder (*Pseudopleuronectes americanus*) in Waquoit Bay, Massachusetts. Estuaries 14(4): 440-446.
- Schramm, H.L. Jr., and R.G. Piper. (eds.). 1995. Uses and effects of cultured fishes in aquatic ecosystems. American Fisheries Society Symposium 15, 608 pp.
- Searafy, J.E., J.S. Ault, T.R. Capo and D.R. Schultz. 1999. Red drum, *Sciaenops*

- ocellatus*, stock enhancement in Biscayne Bay, FL, USA: assessment of releasing unmarked early juveniles. *Aquaculture Research* 30(10): 737-750.
- Shaklee, J. B., C. A. Busack, and C. W. Hopley, Jr. 1993a. Conservation Genetics Programs for Pacific Salmon at the Washington Department of Fisheries: Living with and learning from the past, looking to the future. In *Selective Breeding of Fisheries in Asia and the United States*. K. L. Main and E. Reynolds, editors. The Oceanic Institute. Honolulu, Hawaii. pp 110-141.
- Shaklee, J. B., J. Salini, and R. N. Garrett. 1993b. Electrophoretic characterization of multiple genetic stocks of barramundi perch in Queensland, Australia. *Trans. Amer. Fish. Soc.* 122:685-701.
- Shaw, W. N and T. J. Hassler 1989. *Species Profiles: Life histories and environmental requirements of coastal fishes and invertebrates (Pacific Northwest)*. Lingcod. Biol. Rep. U. S. Fish. Wildl. Serv., 19pp
- Sogard, S.M. 1992. Variability in growth rates of juvenile fishes in different estuarine habitats. *Mar. Ecol. Prog. Ser.* 85: 35-53.
- Stoner, A. W. 1982. The influence of benthic macrophytes on foraging behavior of pinfish, *Lagodon rhomboides*. *Journal of Experimental Marine Biology and Ecology* 104:249-274.
- Stoner, A.W. 1994. Significance of habitat and stock pre-testing for enhancement of natural fisheries: experimental analyses with queen conch, *Strombus gigas*. *Journal of the World Aquaculture Society* 25:155-165.
- Stoner, A.W. and M. Davis 1994. Experimental outplanting of juvenile queen conch, *Strombus gigas*: comparison of wild and hatchery-reared stocks. *Fish. Bull., U.S.* 92: 390-411.
- Stoner, A.W. and R.A. Glazer 1998. Variation in natural mortality: Implications for queen conch stock enhancement. *Bull. Mar. Sci.* 62(2) 427-442.
- Svåsand, T., K.E. Jørstad and T.S. Kristiansen. 1990. Enhancement studies of coastal cod in western Norway. I. Recruitment of wild and reared cod to a local spawning stock. *J. Cons. int. Explor. Mer* 47: 5-12.
- Svåsand, T. & Kristiansen T.S. (1990a) Enhancement studies of coastal cod in western Norway. Part II. Migration of reared coastal cod. *Journal du Conseil International pour l'Exploration de la Mer*, 47, 13-22.
- Svåsand, T. & Kristiansen T.S. (1990b) Enhancement studies of coastal cod in western Norway. Part IV. Mortality of reared cod after release. *Journal du Conseil International pour l'Exploration de la Mer*, 47, 30-39.
- Svåsand, T., T.S. Kristiansen, T. Pedersen, A.G.V. Salvanes, R. Engelsen, G. Naevdal and M. Nodtvedt. 2000. The enhancement of cod stocks. *Fish and Fisheries*. 1: 173-205.
- Tanaka, M., T. Seikai, E. Yamamoto and S. Furuata. 1998. Significance of larval and

- juvenile ecophysiology for stock enhancement of the Japanese flounder, *Paralichthys olivaceous*. Bull. Mar. Sci. 62(2): 551-571.
- Topp, R.W. 1967. An estimate of fecundity of the winter flounder, *Pseudopleuronectes americanus*. J. Fish. Res. Bd. Can. 25: 1299-1302.
- Travis, J., F.C. Coleman, C.B. Grimes, D. Conover, T.M. Bert and M. Tringali. 1998. Critically assessing stock enhancement: an introduction to the Mote Symposium. Bull. Mar. Sci. 62(2): 305-311.
- Tringali, M. D. and K. M. Leber. 1999. Genetic considerations during the experimental and expanded phases of snook stock enhancement. Bulletin of the National Research Institute of Aquaculture, Suppl. 1:109-119 (Japan, in English).
- Tucker, J.W. 1998. Marine Fish Culture. Kluwer Academic Publishers, Massachusetts. 750 pp.
- Tyler, A.V. 1971. Surges of winter flounder *Pseudopleuronectes americanus* into the intertidal zone. J. Fish. Res. Board Can. 28(11): 1727-1732.
- Walters, C. J., & Martell, S. (2003) *Harvest management for aquatic ecosystems*. Princeton University Press, Princeton.
- Walters C.J. & Hilborn R. (1978). Ecological optimization and adaptive management. *Annual Reviews of Ecology and Systematics*, 9, 157-88.
- Wanat, J.M. 2002. Using habitat suitability models to identify essential fish habitat for the winter flounder, *Pseudopleuronectes americanus*, in Great Bay Estuary, N.H. Master's thesis, University of New Hampshire.
- Waring, C.P., R.M. Stagg, and M.G. Poxton. 1992. The effects of handling on flounder (*Platichthys flesus* L.) and Atlantic salmon (*Salmo salar* L.). Journal of Fish biology 41: 131-144.
- Waters, E.B. 1996. Sustainable flounder culture and fisheries. Univ. of North Carolina Sea Grant College Program, Raleigh, N.C., Pub. No. UNC-SG-96-14, 12pp.
- Wedemeyer, G. A. 1996. Transportation and handling. Pages 727-758 in W. Pennell, and B.A. Barton, editors. Principles of Salmonid Culture. Elsevier, Amsterdam.
- Wennhage, H., R.N. Gibson, and L. Robb. 1997. The use of drop traps to estimate the efficiency of two beam trawls commonly used for sampling juvenile flatfishes. J. Fish Bio. 51: 441-445.
- Wespestad, V.G., Bargmann, G.G. and Hays, D.E. 1994. Opportunities for the enhancement of the lingcod (*Ophiodon elongatus*) in Puget Sound and Georgia Straits. Aquaculture and Fisheries Management 25:189-197.

- West, J.E. 1997. Protection and restoration of marine life in the inland waters of Washington state. Puget Sound/Georgia Basin Environmental Report Series, 6: 144p.
- Willis, S. A., W. W. Falls, C. W. Dennis, D. E. Roberts, and P. G. Whitchurch. 1995. Assessment of season-of-release and size-at-release on recapture rates of hatchery-reared red drum (*Sciaenops ocellatus*) in a marine stock enhancement program in Florida. American Fisheries Society Symposium 15:354-365.
- Witting, D.A. 1995. Influence of invertebrate predators on survival and growth of juvenile winter flounder. PhD Dissertation. Rutgers Univ., New Brunswick, N.J.
- Witting, D.A. and K.W. Able. 1993. Effects of body size on probability of predation for juvenile summer and winter flounder based on laboratory experiments. Fish. Bull. 91: 577-581.
- Witting, D.A. and K.W. Able. 1995. Predation by seven spine bay shrimp *Crangon septemspinosa* on winter flounder *Pleuronectes americanus* during settlement: laboratory observations. Mar. Ecol. Prog. Ser. 123: 23-31.
- Yamamoto, E. 1999. Studies on sex manipulation and production of cloned populations of hirame, *Paralichthys olivaceous* (Temminck et Schlegel). Aquaculture 173: 235-246.
- Ziemann, D.A. and K.M. Leber. 1997. Stock enhancement of Pacific threadfin (*Polydactylus sexfilis*) in Hawaii. World Aquaculture '97, Seattle, WA. (abstract).

**2003-2004 SCORE BUDGET
Mote Marine Laboratory (MML)**

DIRECT EXPENSE	TOTAL
Salaries	\$ 138,185
Benefits ¹	45,601
Subtotal	\$ 183,786
Travel	\$ 9,500
Equipment	48,326
Materials & Supplies	18,932
Outside Services	529,400
Other Direct Costs	11,703
Subtotal	\$ 617,861
TOTAL DIRECT	\$ 801,647
INDIRECT COSTS ²	\$ 143,353
TOTAL BUDGET	\$ 945,000

¹ Fringe benefits for full-time employees of Mote Marine Laboratory are calculated at 33.0% of direct salary costs.

² MML overhead on this project is equivalent to 52.66 % of total direct costs less subcontracts (MML overhead computation is described below)

BUDGET JUSTIFICATION (MML)

DIRECT EXPENSES

Direct Salaries - Covers the salaries for the principal investigator and professional, technical and secretarial staff involved in the program.

POSITION	PERSONNEL	HOURS
(1) Senior Scientist	Kenneth M. Leber, Ph.D.	312 ³
(1) Senior Scientist	Kevan L. Main, Ph.D.	104
(1) Senior Biologist	Nathan P. Brennan, M.S.	1352
(1) Senior Biologist	New Hire, M.S.	2038
(1) Staff Biologist	Vicki Mooney, B.S.	957
(1) Staff Biologist	Roger Debruler, B.S.	624
(1) Staff Biologist	Harry Ruiz, A.A.	1040
(1) Admin. Assist.	Terri Deppe	520
(3) Technicians	Staff, B.S.	1040 x 2_

³ Matching funds for an additional 450 hours on this particular project are provided for the principal investigator (Kenneth M. Leber) by MML through the endowed Charles M. Breder Chair in Fisheries Ecology. Thus total time for the PI on this project is 762 hours. Matching funds are also provided by The Florida Fish and Wildlife Conservation Commission (\$100,000) and Mote Scientific Foundation (\$75,000).

Fringe Benefits - For budget estimation purposes, benefits are computed at 33.0 % of direct salary costs. This is the current average for full-time MML employees. Actual benefits rates vary by individual employee. Only salaries and benefits specifically related to this project will be charged to the grant.

Travel - Supports airfare and per diem costs connected with round-trip travel between MML in Florida and UNH to complete work associated with the project, and for attendance and scientific presentations at scientific symposia (World Aquaculture Society, March, 2004 in Honolulu) highlighting marine stock enhancement. (\$9,500 domestic travel, \$0 International travel).

Equipment -

IBM compatible computer and printer for data analysis associated with the project (\$2,250)

PIT tag technology (\$10,076)

Coded-wire tag detector (\$7,000)

Acoustic transmitters and receivers (50 VR8 transmitters, 9 VR2 receivers; \$24,000)

Filtration systems (\$5,000)

Materials and Supplies -

Laboratory supplies - coded wire tags, elastomer tags, tagging and release accessories, air pumps and stones, sampling nets, buckets, foul-weather gear, lanterns, chemicals and gases, environmental monitoring meters, office supplies, etc. (\$11,876).

Animal Maintenance Supplies (\$5,556)

Computer supplies including software to support data analysis, graphics, manuscript and report preparation, printer cartridges, and computer backup hardware (\$1,500)

Outside Services – Subcontracts:

University of New Hampshire	\$435,000
University of Idaho	\$ 80,000

Other Direct -

Telecommunications for contact with collaborators and within the project (\$1,200)

Postage and freight charges associated with supplies and communications (\$324).

Automobile expenses for use of MML vehicles for field collections, local meetings, purchase of supplies, etc. (\$1000).

Boat Expenses (22' Trembley -- \$2,000)

Photocopy costs for duplicating project documents and printing informational materials to assist with field sampling. (\$800).

Conference registration for stock-enhancement presentations (\$500)

Photography expenses associated with the preparation of publications and seminars (\$379).

Safety Supplies (\$500)

Miscellaneous expenses associated with the project including miscellaneous supplies and costs to process the subcontracts (\$5,000)

Indirect Costs - the MML indirect cost rate (overhead rate) on this grant is equivalent to 52.66 % of total direct costs less subcontracts. It is important to note that, for computational purposes, the Laboratory's overhead rate is determined on a base of direct labor only (only the salaries and benefits portion of direct costs). This direct labor computational rate was established by our federal cognizant agency to be 78.0 % of direct salaries and benefits of MML employees.

SCORE SUB-BUDGET
University of New Hampshire

DIRECT EXPENSE	TOTAL
Salaries	\$ 184,925
Benefits ¹	36,242
Subtotal	\$ 221,167
Travel	9,000
Equipment	25,200
Materials & Supplies	29,117
Outside Services	10,000
Other Direct Costs ²	\$ 15,310
Subtotal	\$ 88,627
TOTAL DIRECT	\$ 309,794
INDIRECT COSTS ³	\$ 125,206
TOTAL BUDGET	\$ 435,000

Budget Justification (UNH)

A. Rates:

The UNH fringe benefit rate is 8.4% for graduate student summer salary, and non-status (hourly) employees. Fringe rate for faculty salary and status employees is 40.5%.

The UNH indirect cost rate is 46%, excluding equipment and graduate student tuition.

The UNH indirect cost rate on subcontracts is 26%.

B. Direct Expenses:

Faculty Salary - Covers 4.5 months for W.H. Howell, the principal investigator, who will be on sabbatical leave for one semester. He will devote full time to this project during this time.

Stipend and summer salary are requested for 1 graduate student who will use the project for thesis research.

Hourly funds are requested to support 2 scientists full time, and one research technician part time. They will assist in all aspects of this labor-intensive project.

Supplies include miscellaneous items needed to conduct the research, including glassware and reagents, materials to build in-situ cages, CMWT tags and tagging machine rental, a bench-top homogenizer, histological slide preparation, cortisol and glucose assays, SCUBA fills, fish food (both live and formulated), etc.

Travel funds will be used to present findings at the World Aquaculture Society meetings in HI (n=3), and the Flatfish Biology Conference in CT (n=3). Funds will also be used to meet with the other PI's on the project, as well as colleagues in Europe who are doing similar studies.

Equipment includes 2 CMWT detection wands, a new outboard engine for the boat we will use in the fieldwork, and a personal computer for data analyses and manuscript preparation.

A subcontract is included for personnel at the Washington Department of Fish and Wildlife who will develop microparticulate diets.

Other direct costs include graduate student tuition for 1 year (\$8,060 for 1 student), small boat user fees (\$5,250), phone, photocopy and postage (\$2,000).

REFERENCES
OF
KEY PROJECT PERSONELL

C U R R I C U L U M V I T A E

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Academic Training:

B.A. Biology, 1969	Otterbein College, Westerville, OH
M.S. Zoology, 1975	University Rhode Island, Kingston, RI
Ph.D. Zoology, 1980	University Rhode Island, Kingston, RI

Employment:

5/96 to present -- Professor of Zoology, UNH, Durham, NH
6/86 to 4/96-- Associate Professor of Zoology, UNH, Durham, NH
9/80 to 5/86 -- Assistant Professor of Zoology, UNH, Durham, NH

Recent Publications (1996-2000):

Saila, S.B., E. Lorda, D. Miller, R. Sher, and W. Howell. (1997). Equivalent adult estimates from egg, larval and juvenile fish losses at Seabrook Station. *N. Amer. J. Fish. Manag.* 17: 811-825.

Savage, G.C., W.H. Howell, R. Barnaby and B. Celikkol. 1998. Demonstration of open-ocean aquaculture for groundfish. In: *Open-Ocean Aquaculture '97: Charting the Future for Ocean Farming*. C.E. Helsley (ed.). U. Hawaii Sea Grant Rept. #CP-98-08. pp.175-200.

Crossin, G.T., S.Abdulazziz-Al-Ayoub, S.H. Jury, W.H. Howell and W.H. Watson. (1998). Behavioral thermoregulation in the American lobster, *Homarus americanus*. *J. Exp. Biol.* 201: 365-374.

Bucklin, A. and W.H. Howell. 1999. Progress and prospects for the UNH Open Ocean Aquaculture Demonstration Project. In: R.R. Stickney (ed.), *Joining Forces with Industry, Proceedings of the Third International Conference on Open Ocean Aquaculture, Corpus Christi, Texas, May 10-15, 1998*. Pp. 7-30.

Howell, W.H., B.J. Keller, P.K. Park, J.P. McVey, K. Takayanagi, and Y. Uekita (eds.). 1999. Nutrition and Technical Development of Aquaculture. *Proc. of Twenty-sixth U.S-Japan Aquaculture Symposium, Durham, NH, USA*. UJNR Tech. Rept. No. 26, 243 p.

Howell, W.H. and W.H. Watson. (1999). Skewed sex ratio in an estuarine lobster (*Homarus americanus*) population. *J. Shellfish Res.* 18(1): 193-201.

King, N.J., W.H. Howell and E.A. Fairchild. (1999). The effect of stocking density on the growth of juvenile summer flounder, *Paralichthys dentatus*. In: Howell, W.H. et al. (eds.) *Nutrition and Technical Development of Aquaculture. Proc. of Twenty-sixth U.S-Japan Aquaculture Symposium, Durham, NH, USA*. UJNR Tech. Rept. No. 26, 243 p.

- King, N.J. and W.H. Howell. (1999). Effects of microalgae and live diet type on the growth of first feeding winter flounders, *Pleuronectes americanus*. In: Howell, W.H. et al. (eds.) Nutrition and Technical Development of Aquaculture. Proc. of Twenty-sixth U.S-Japan Aquaculture Symposium, Durham, NH, USA. UJNR Tech. Rept. No. 26, 243 p.
- Rabe, J.H., J.A. Brown, D.A. Bidwell and W.H. Howell. 1999. Preliminary observations on the larviculture of witch flounder (*Glyptocephalus cynoglossus*). Bull. Aqua. Assoc. Canada 98-2: 19-20.
- Watson, W.H., A. Vetrovs, and W.H. Howell. (1999). Lobster movements in an estuary. Mar. Biol. 134(1): 65-75.
- Fairchild, E.A. and W.H. Howell. 2000. Predator-prey size relationship between *Pseudopleuronectes americanus* and *Carcinus maenas*. Journal of Sea Research 44 (1-2): 81-90.
- Howell, W.H. and M.K. Livak. (2000). Winter flounder (*Pseudopleuronectes americanus*) aquaculture. In: Stickney, R. (ed.) Encyclopedia of Aquaculture, John Wiley and Sons, New York, N.Y., p.998-1005.
- King, N.J., W.H. Howell, M. Huber and D. Bengtson. 2000. Effect of larval stocking density on laboratory-scale and commercial- scale production of summer flounder, *Paralichthys dentatus*. J. World Aquaculture Soc. 31: 436-445.

Manuscripts in Review:

- Bidwell, D.A. and W.H. Howell. The effect of temperature on first feeding, growth and survival of larval witch flounder, *Glyptocephalus cynoglossus*. Journal World Aquaculture Society.
- Bidwell, D.A. and W.H. Howell. The effect of incubation temperature on the embryonic development of the witch flounder. Journal World Aquaculture Society.
- Fairchild, E.A. and W.H. Howell. Optimal stocking density for juvenile winter flounder, *Pseudopleuronectes americanus*. Journal World Aquaculture Society.

Recent Funded Research Activities (1996-2000):

- | | |
|---------|---|
| 1995-98 | NOAA/NMFS Sea Grant – Development of commercially viable aquaculture industries in New England based on cod and haddock (with T. Bradley, L. Buckley, L.Kling)
\$174,807 |
| 1995-98 | NOAA/NMFS Sea Grant – Developing indices necessary for predicting commercial catches of the American lobster (<i>Homarus americanus</i>) (with W. Watson)
\$168,177 |
| 1995-98 | USDA Hatch -- The influence of habitat on the distribution and abundance of lobsters: The role of coastal and estuarine eelgrass beds (with W. Watson)
\$33,000 |

1996-98 USDA, Northeast Regional Aquaculture Center -- Development of the summer flounder aquaculture industry (with D. Bengtson et al.)
\$146,939

1998 NOAA /NMFS Sea Grant - An analysis of trap saturation and the behavioral basis of catchability (with Win Watson)
\$31,818

1997-98 Canadian Center for Fisheries Innovation -- Feasibility of witch flounder aquaculture (with D. Bidwell and J. Brown)
\$17,000

1998-99 NOAA/NMFS Sea Grant - Open Ocean Aquaculture Demonstration Project: Biology (with R. Langan et al.)
\$74,999

1997-98 NOAA/NMFS Sea Grant -- Development of an open ocean aquaculture demonstration project.
\$65,000

1998-0 NOAA/NMFS Sea Grant -- Development of an open ocean aquaculture demonstration projec (with R. Langan, G.Nardi, and R. Hayman).
\$396,665

1998-00 NOAA/NMFS Sea Grant – Developing indices necessary for predicting commercial catches of the American lobster (*Homarus americanus*) (with W. Watson)
\$168,177

1998 USDA Hatch -- The influence of habitat on the distribution and abundance of lobsters: The role of coastal and estuarine eelgrass beds (with W. Watson)
\$33,000

1999-01 NOAA/NMFS Sea Grant - Assessing the feasibility of winter flounder stock enhancement
\$186,764

1999-01 NOAA/NMFS Sea Grant - A field analysis of the behavioral and ecological factors determining lobster catchability and trap saturation (with W. Watson)
\$98,351

1999-0 NOAA/Oceanic & Atmospheric Research - Demonstration of net pen culture of haddock, cod and black seabass (with several others)
\$445,994

2000 Hubbard - Aspects of the life history and ecology of Raja spp.
\$8,000

2000 NOAA/Oceanic & Atmospheric Research – Summer flounder aquaculture
\$79,480

2000-01 NOAA – Northeast Consortium – Determining groundfish species movement patterns in the western Gulf of Maine using mark and recapture techniques.
\$214,640

2001-02 NOAA/Oceanic & Atmospheric Research – The production of juvenile cod.
\$111,000

Pending Grants:

2001-03 NOAA/Oceanic & Atmospheric Research – Net pen culture of cod and halibut.
\$106,000

2001-02 NOAA/NMFS – Improving marine fish stock enhancement through the use of Habitat Suitability Indices.
\$200,000

KENNETH M. LEBER, Ph.D.
MOTE MARINE LABORATORY
June 5, 2001

Academic Background

George Mason University, Fairfax, Virginia. B.S. Biology. 1969.
East Carolina University, Greenville, North Carolina. M.S. Biology. 1977.
Florida State University, Tallahassee, Florida. Ph.D. Biology. 1983.

Positions and Experience

Mote Marine Laboratory, Sarasota, Florida. Charles M. Breder Chair in Fisheries Ecology.
Director of the Center for Fisheries Enhancement. Development of responsible stock-enhancement technology and marine aquaculture technology for replenishing depleted populations of marine organisms. Research focus: ecological and habitat requirements of target species, optimal release strategies, assessment of stocking impact, adaptive management techniques for stock enhancement, conserving wild-stock genetic diversity, economic benefits of enhancement, cost-effective aquaculture production technology. Expertise in fish and crustacean fishery ecology, habitat selection, foraging ecology, predator-prey interactions and aquaculture. 1996 - present.

The Oceanic Institute, Waimanalo, Hawaii. Program Manager, Stock Enhancement Program. Developed stock-enhancement technology to replenish marine fishes. Developed prototype marine enhancement program for Hawaii. Research involved assessment of survival, growth, and dispersal of cultured fish released in coastal habitats; hatchery contribution to wild stocks and commercial or recreational fisheries, aquaculture technology for marine finfish; socioeconomic and ecological impact; adapting fish tagging systems to tropical and subtropical species. 1988 - 1996.

Research Scientist, U.S. Marine Shrimp Farming Program. Nutrition and feeding ecology of penaeid shrimp; Evaluation of foraging patterns and secondary productivity in aquaculture ponds; Design and execution of lab and field experiments aimed at increasing yields and carrying capacity in shrimp aquaculture ponds. 1985 - 1988.

Harbor Branch Oceanographic Institution, Ft. Pierce, Florida. Postdoctoral Fellow with R.W. Virnstein. 1984 - 1985.

Florida A&M University, Tallahassee, Florida. Assistant Professor, Zoology. 1983 - 1984.

Academic and Professional Honors

Dr. Leber holds the Charles M. Breder Chair in Fisheries Ecology at Mote Marine Laboratory. 1996 - present.

Panel member, United States--Japan Cooperative Program in Natural Resources (UJNR) established by Cabinet-Level meeting of Joint U.S.--Japan Committee on Trade and Economic Affairs; and charged with exploring and developing bilateral cooperation by exchanging information related to aquaculture that could benefit both countries. (member since 1995).

Member, International Scientific Committee for Norwegian Programme for the Development and Encouragement of Sea Ranching. Committee planned the 1st International Symposium on Stock Enhancement and Sea Ranching held in Bergen, Norway, in 1997.

Chairman, International Scientific Committee for 2nd International Symposium on Stock Enhancement and Sea Ranching. Sponsored by the Japan Fisheries Agency and Japan Sea-Farming (stock enhancement) Association. 2000-2002.

Chairman, World Aquaculture Society International Working Group on Stock Enhancement. The charter is focused on fostering scientific debate about a responsible approach to stock enhancement. Also plans stock-enhancement symposia for Annual Conference of World Aquaculture Society. 1997-2000.

President, American Fisheries Society, Hawaii Chapter. 1995-1996.

Secretary-Treasurer, American Fisheries Society, Hawaii Chapter. 1992-1993.

Postdoctoral Fellowship: Department of Benthic Ecology, Harbor Branch Oceanographic Institution, Ft. Pierce, Florida. 1984 - 1985.

James R. Fisher Award: Presented by Sigma Xi at Florida State University for manuscript entitled "Influence of decapod foraging and microhabitat complexity on seagrass communities: a field test of the refuge hypothesis." 1983 recipient.

Best Student Paper Award: Presented by Atlantic Estuarine Research Society. 1977.

Outstanding Graduate Student: monetary award. East Carolina University Biology Department's 1977 recipient.

National Science Foundation Undergraduate Research Fellow: Competitive award University of Maryland's Chesapeake Biological Laboratory. Summer 1969.

Peer-Reviewed Publications

Leber, K. M. 1981. Spatial patterns of *Ocypode quadrata*: A re-evaluation. (Decapods, Brachyura). *Crustaceana* 41: 110-112.

- Leber, K. M. 1982. Seasonality of microinvertebrates on a temperate, high wave energy sandy beach. *Bulletin of Marine Science* 32: 86-98.
- Leber, K. M. 1982. Bivalves (Tellinacea: Donacidea) on a North Carolina Beach: Contrasting population size structures and tidal migrations. *Marine Ecology Progress Series* 7: 297-301.
- Leber, K. M. 1985. Influence of decapod foraging and microhabitat complexity on seagrass communities: a field test of the refuge hypothesis. *Ecology* 66(6): 1951-1964.
- Leber, K. M. and H. S. Greening. 1986. Community studies in seagrass meadows: a comparison of two methods for sampling microinvertebrates and fishes. *Fishery Bulletin* 84(2): 443-450.
- Leber, K. M. and G. D. Pruder. 1988. Using experimental microcosms in shrimp research: the growth-enhancing effect of shrimp pond water. *Journal World Aquaculture Society* 19(4): 197-203.
- Wyban, J. A., G. D. Pruder, K. M. Leber and L. Burzell. 1989. Paddlewheel effects on shrimp growth, production and crop value in commercial earthen ponds. *Journal World Aquaculture Society* 20(1): 18-23.
- Moss, S. M., G. D. Pruder, K. M. Leber and J. A. Wyban. 1992. Shrimp microcosm results: the relative enhancement of *Penaeus vannamei* growth by selected fractions of shrimp pond water. *Aquaculture* 101: 229-239
- Freeman, D. W., E. O. Duerr, and K. M. Leber. 1992. Use of bagasse as a feed input to semi-intensive shrimp growout ponds. *Journal World Aquaculture Society* 23: 23-30.

(Continued)

Additional Peer-Reviewed Publications -- re: Marine Stock Enhancement

- Leber, K. M. 1995. Significance of fish size-at-release on enhancement of striped mullet fisheries in Hawaii. *Journal World Aquaculture Society* 26(2):143-153.
- Leber, K. M., N. P. Brennan, and S. M. Arce. 1995. Marine enhancement with striped mullet: are hatchery releases replenishing or displacing wild stocks? *in* Uses and effects of cultured fishes in aquatic ecosystems. *American Fisheries Society Symposium* 15:376-387.
- Blankenship, H. L. and K. M. Leber. 1995. A responsible approach to marine stock enhancement. *In* Uses and effects of cultured fishes in aquatic ecosystems. *American Fisheries Society Symposium* 15:165-175.
- Leber, K. M., S. M. Arce, D. A. Sterritt, and N. P. Brennan. 1996. Marine stock-enhancement potential in nursery habitats of striped mullet, *Mugil cephalus*, in Hawaii. *Fishery Bulletin* 94(3):452-471.
- Leber, K. M. and S. M. Arce. 1996. Stock enhancement effect in a commercial mullet *Mugil cephalus* fishery in Hawaii. *Fisheries Management and Ecology* 3:261-278.
- Leber, K. M., H. L. Blankenship, S. M. Arce, and N. P. Brennan. 1997. Influence of release season on size-dependent survival of cultured striped mullet, *Mugil cephalus*, in a Hawaiian estuary. *Fishery Bulletin*, 95(2):267-279.
- Leber, K. M. and C-S. Lee. 1997. Marine stock-enhancement potential with striped mullet, *Mugil cephalus*, in Hawaii. *Bulletin of Natural Research Institute for Aquaculture*, Suppl.3:117-134.
- Leber, K. M., N. P. Brennan and S. M. Arce. 1998. Recruitment patterns of juvenile, cultured Pacific threadfin, *Polydactylus sexfilis* (Polynemidae), released along sandy marine shores in Hawaii. *Bulletin of Marine Science* 62(2):389-408.
- Leber, K. M. 1999. Rationale for an Experimental Approach to Stock Enhancement. Pages 63-75 *In* *Stock Enhancement and Sea Ranching* (Ed. by B.R. Howell, E. Moksness, and T. Svasand) Blackwell Scientific Publications, Oxford. 606 pages.
- Tringali, M.D. and K.M. Leber. 1999. Genetic considerations during the experimental and expanded phases of snook stock enhancement. *Bull. National Research Institute Aquaculture (Japan) Suppl.* 1:109-119.
- Leber, K. M. 2002. Advances in marine stock enhancement: shifting emphasis to theory and accountability. Pp 79-90 *In* Stickney, R. R. and J. P. McVey (eds) *Responsible Marine Aquaculture* CABI Publishing, New York.

Leber, K. M. In Press. Marine Stock Enhancement in the USA: Status, trends and needs. *In* Leber, K.M., S. Kitada, H.L. Blankenship and T. Svåsand (eds) *Stock Enhancement and Sea Ranching II*. Blackwell Scientific Publications, Oxford.

Leber, K. M. and R. N. Cantrell. In Review. Effects of fish size-at-release on the relative cost to enhance striped mullet stocks in Hawaii.

Conference Abstracts

(~60 contributed papers, invited papers and Keynote talks given. Abstracts provided upon request)