ASSESSING THE FEASIBILITY OF WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS) STOCK ENHANCEMENT

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ABSTRACT

Progress to date has been good. We have either completed, or made progress on, 6 of the 9 objectives, and have also expanded the research to include additional studies. Results to date indicate that winter flounder stock enhancement is feasible if done correctly. Apart from some disease problems, perhaps associated with over-crowding, the production of juveniles has proceeded as scheduled, and we believe that the mass production of winter flounder juveniles is possible. We have found that cultured fish grow as well as their wild counterparts, and that they make the transition from formulated diets to wild prey within a matter of days. We have also found that all flounders less than 20mm total length are vulnerable to green crab predators, but that cultured fish of this size may be more vulnerable than their wild counterparts. Preliminary results from bird predation studies indicate that avian predators should certainly be considered in any stocking program. Gulls in particular appear to be voracious predators on young flounders, and there doesn't seem to be any preference for lab-reared or wild caught fish. Fish predators also consume small flounders, and results of preliminary lab studies indicate that lab-reared fish (light colored) may be more vulnerable than dark colored wild fish. The first pilot scale release has been made, and we will be evaluating the success of this over the next several months. Lastly, we have initiated an extensive, and unplanned, series of field experiments designed to evaluate different release locations and times. We have also begun an experiment to study the effect of stocking density on the growth and survival of juvenile winter flounders.

RATIONALE

The primary goal of this research is to assess the feasibility of winter flounder, Pleuronectes americanus, stock enhancement. While the enhancement of numerous freshwater fish species (e.g., trout) through hatchery production and stocking programs is widely practiced, marine fish stock enhancement has been slow to develop. Declines of many marine species, combined with improved abilities to rear large numbers of fish, have caused a renewed interest in marine fish stock enhancement. In this, fish are raised in captivity, and then released to the wild to increase abundance, promote or accelerate recovery, and/or to ensure the survival of stocks threatened by extinction. Over the last 20 years, enhancement programs for dozens of marine fin fish species, including cod, Japanese flounder, turbot, plaice, red drum, striped mullet, white seabass, Pacific threadfin, and ocean perch have been initiated. Because many of these have achieved their intended purpose, the recent interest in marine stock enhancement continues to grow.

The winter flounder, Pleuronectes americanus, is widely distributed along the east coast of North America, ranging from Labrador to Georgia. While it supports important commercial and recreational fisheries throughout its range, it is most abundant in the Gulf of Maine. As with most groundfish species, catches have declined precipitously in recent years. Combined US commercial and recreational landings in 1979 were over 7,000 metric tons (mt), but landings declined by more than 87% over the next twelve years. 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. Fisheries regulations designed to reverse the trend of declining catch include increased codend mesh sizes, reductions in fishing effort, minimum fish size, and area closures. 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 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. Winter flounder were among the species strongly suggested for further study. We believe 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 techniques for culturing them on a small (laboratory) scale have been developed. Given these techniques, and the wealth of information on commercial scale production techniques that have been developed for other flounder species including turbot, Japanese flounder, and summer flounder, it is likely that a minimal amount of additional research would result in the ability to grow winter flounder on a scale sufficient for stock enhancement and/or commercial aquaculture. 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, living in shallow sand and silt areas, and making short tidal excursions. Further, studies 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. The non-migratory nature of the young fish ensures their prolonged availability for discrete field studies. Another reason for the selection of winter flounder is that their biology and ecology are very well known relative to most fish species. 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.

The proposed research precisely fits the criteria listed in the Sea Grant planning document entitled "Sustaining A Sea Beside the Sea." In particular, the section related to the management and development of living marine resources, states that: "The primary goal of the UM/UNH Sea Grant College Program is to provide scientifically based information that will contribute to the development and continuation of effective stock enhancement efforts, and to a significant, sustainable aquaculture industry in northern New England and the nation". The proposed research is specifically designed to contribute to the development of effective stock enhancement. As importantly, another aspect of the research (juvenile production)
proposes to rear winter flounder on a scale larger than any in history. Results of this component of the research will result in the development and demonstration of commercial production techniques for winter flounder, and evaluate the performance characteristics of this species under intensive conditions. As such, the research will contribute to the development of a new aquaculture industry in northern New England based on winter flounder.

The proposed research builds upon a previous Sea Grant project designed to develop commercially viable aquaculture industries in New England based on cod and haddock, and other projects designed to increase the commercial production of summer flounder. We will benefit from the knowledge gained in those studies, and extend the research into yet another species that should be strongly considered for stock enhancement, and as the basis for an aquaculture industry in New England. The user community that will benefit from the research is large and diverse. Finfish culturists will benefit from the techniques developed to rear winter flounder on a large (commercial) scale, and from the ability to view the production facility. The field data collected as part of this study will provide fisheries biologists and managers, both state and federal, with additional insights into winter flounder ecology, recruitment mechanisms, and population dynamics. Additionally, both estuarine and fisheries ecologists will benefit from the data collected in our field studies. Our collection data will also supplement those gathered by the New Hampshire Dept. of Fish and Game. Since the project will directly involve commercial fishermen in broodstock collection, and both recreational and commercial fishermen in helping to assess the success of the enhancement program, both user groups will be familiar with the research. In the long run, if enhancement proves effective, both groups will benefit from rebuilding of the fisheries.

OBJECTIVES

The overall objective of this research is to assess the feasibility of winter flounder, *Pleuronectes americanus*, stock enhancement. Our proposed work is associated with the development of optimum release strategies, and the performance of released fish. To examine potential differences between laboratory-reared and wild juvenile winter flounder, we propose to test the following hypotheses:

1) $H_0$: There is no difference in growth, survival, and diet between lab-reared and wild young-of-the-year (YOY) winter flounder.

2) $H_0$: There is no difference in behavior of lab-reared and wild YOY winter flounder when exposed to cues from a potential predator.

3) $H_0$: There is no difference in predation rate by green crabs on YOY winter flounder of five different sizes.

4) $H_0$: There are no differences in rates of predation by a fish predator on "naive" and "experienced" lab-reared YOY winter flounder.

5) $H_0$: There is no difference in predation rate by a visually feeding fish predator on light-pigmented and dark-pigmented YOY winter flounder on a dark substrate.

6) $H_0$: There is no difference in predation rate by a visually feeding fish predator on dark-pigmented lab-reared and dark-pigmented wild YOY winter flounder on a dark substrate.
To examine the performance of hatchery reared winter flounder juveniles, we plan to conduct small, pilot scale releases of fish, and to collect data from these fish through a field sampling program. Hypotheses to be tested by this release and recapture program include:

7) $H_0$: There is no difference in the growth rates of hatchery-reared and wild winter flounder juveniles.

8) $H_0$: There is no difference in the survival of hatchery-reared and wild winter flounder juveniles.

**PROGRESS TO DATE**

Progress to date has been good. A variety of laboratory and field experiments have either been completed or are presently being conducted, techniques to raise sufficient numbers of juveniles have been largely developed, and the first experimental release of juveniles has taken place. Perhaps not unexpectedly, the research to date has led to additional objectives and hypotheses, and we plan to pursue them as time allows. This will be facilitated by the addition of three undergraduates who are conducting their senior thesis work in conjunction with the project. We do not anticipate any problems completing the objectives set forth in the original proposal. Lastly, the research has attracted the attention of others investigating stock enhancement, and we have collectively submitted a proposal to establish a national consortium on this subject.

**Null Hypothesis 1**

There is no difference in growth, survival and diet between lab-reared and wild young-of-the-year (YOY) winter flounder.

A preliminary experiment was conducted in the autumn of 1998 to test this hypothesis. In this, wild and lab-reared winter flounder juveniles were stocked together into 6, one cubic meter, enclosed pens. Wild and lab-reared fish were tagged differentially so that we could track growth, survival and diet of fish from both origins. Stocking occurred in September, and at biweekly intervals over the next 8 weeks, all fish from each pen were enumerated and measured. On each sampling date we also removed and sacrificed representative fish to determine gut contents. Additionally, core samples were made both in and around the pens so that we could track the abundance of natural benthic prey organisms. Results from this preliminary experiment showed that cultured fish switched to wild food quickly, and there were no significant differences in growth, diet or survival between lab-reared and wild juveniles. While these results were encouraging from a stock enhancement perspective, we note that growth was poor for all fish. The likely reason for this was the rapidly cooling water temperatures at this coastal site. Aspects of this experiment were repeated again this summer, except that pen deployment occurred in a variety of locations. This work is described later in the additional research section.

**Null Hypothesis 2**

There is no difference in the behavior of lab-reared and wild YOY winter flounder when exposed to cues from a potential predator.

We have not yet begun this study, but anticipate that one or more of the senior thesis students will begin this work within the next several weeks.
Null Hypothesis 3

There is no difference in predation rate by green crabs on YOY winter flounder of five different size classes.

As young-of-year (YOY) flatfish grow, they achieve size refuges in which they are no longer vulnerable to a series of predators. It has been shown, for example, that from settlement to 20 mm TL, YOY winter flounder, *Pseudopleuronectes americanus*, are vulnerable to predation by the sevenspine bay shrimp, *Crangon septemspinosa*. Once beyond this period of shrimp predation, it has been suggested that the fish enter into another period in which they are susceptible to predation by green crabs, *Carcinus maenas*.

To examine this predator-prey size relationship, a 2x6x6 factorial experiment was conducted in which both cultured and wild winter flounder were tested. Six winter flounder size class treatments (11-20, 21-30, 31-40, 41-50, 51-60, 61-70 mm total length) were tested against six green crab size class treatments (11-20, 21-30, 31-40, 41-50, 51-60, 61-70 mm carapace width). Five replicate trials were conducted for each combination and a control treatment of flounder only (no crabs) ensured all mortality was predator-related. Trials were conducted in plastic aquaria that measured 38L x 27W x 4H, containing sediment sieved to a diameter of 68-500 microns so that even the smallest fish would be able to bury. Trials began when one crab was introduced into each tank and allowed to acclimate for 24 hours. After the acclimation period, 10 flounder from a single size class and fish type (cultured or wild) were stocked into each tank. After 24 hours, all crabs were removed and the surviving fish were counted and removed.

A total of 269 trials were conducted - 86 using wild fish and 183 with cultured fish. Results showed that flounder of all size classes were preyed on by all size classes of green crabs, however, as expected, mortality was highest in large crab-small fish combinations. Mortality was significantly higher to cultured winter flounder < 20 mm compared to all other larger fish size classes. Due to unavailability, wild fish sizes 11-30 mm were not tested. Overall, crabs consumed twice as many wild fish (14%) than cultured fish (7%). This was most likely an artifact due to the stress the wild fish experienced being captured and brought into the lab.

Null Hypothesis 4

Survival of lab-reared YOY winter flounder in the wild is not dependent on size.

We had proposed to test this hypothesis by stocking variously sized flounder into small field enclosures, examining their survival rates, and determining if differences existed among size classes. To date we have not done this experiment. Based on the work done to test hypothesis 3 (above), we are quite confident that size at release would, in fact, have a dramatic effect on survival rate, i.e. we would reject the null hypothesis. This, combined with results from other U.S. stock enhancement studies currently underway, and the logistical difficulty of doing this experiment, has caused us to move this experiment to a lower priority. Our expectation is that we will do the experiment, but not before we work on what we now perceive as more important aspects of the research.

Null Hypothesis 5

There are no differences in rates of predation by a fish predator on "naïve" and "experienced" lab-reared YOY and wild YOY winter flounder.

We have not yet begun this study, but anticipate that one or more of the senior thesis students will begin this work within the next several weeks.
**Null Hypothesis 6**

There is no difference in predation rate by a visually feeding fish predator on light-pigmented and dark-pigmented YOY winter flounder on a dark substrate.

Because the cultured fish may have a different coloration than wild fish and the release area substrate, it is important to discern whether the fish are more conspicuous to their predators. The primary visually hunting predators of YOY winter flounder are other fish and birds. Work associated with hypothesis 7 (following), using a sea raven for a predator, it was determined that light-colored, cultured winter flounder were not preyed upon any more than dark-colored, cultured winter flounder on a dark substrate. It is valuable to an enhancement program to determine if this is also true for avian predators, and whether predator rates are based solely on coloration or whether differences exist between the cultured and wild fish.

These questions are being answered through a new predation experiment in the field that are designed to study avian predation. Floating, open-topped wooden pens containing a 2 cm layer of sediment on the bottom are being used. These are attached to a floating dock away from any disturbance. In each trial run, a total of ten flounder (5 light and 5 dark) are stocked out into each pen and made available to birds for a 5 hour period. At the end of the trial, all remaining fish are removed and identified (by tags and color) to identify the survivors. A stationary motion detector camera focused on the pens photographs movement caused by the birds. Survival data is analyzed using Chi-square goodness of fit test.

To date, 8 trials have been run in which more light-colored than dark-colored flounder have been eaten. Statistical analyses have not been completed since the experiment is still ongoing. The main avian predators, documented photographically, are herring and great black-backed gulls. There has also been some cormorant activity. Future trials will incorporate dark wild fish too, in an effort to determine if differences exist between dark cultured and dark wild winter flounder.

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**Null Hypothesis 7**

There is no difference in predation rate by a visual predatory fish (sea raven) on light-pigmented and dark-pigmented YOY winter flounder on a dark substrate.

Differences in pigmentation between hatchery-reared and wild winter flounder could lead to higher predation rates of the lab fish in the naturally dark, mud/sand environment. The lab-raised winter flounder are much lighter in color than the wild fish, due to the light color of the rearing tanks. In the wild, these fish should change their pigmentation to match that of the substrate. However, since we know from our preliminary experiments that this change is gradual, lab-reared fish may be prime targets for visual predators.

To compare the rates of fish predation on dark and light pigmented winter flounder, starved sea ravens were used as the visually hunting predator. Trials were conducted in a 1.8-m diameter, round fiberglass tank filled to a 0.5 m depth and contained 1-2 cm layer of dark sediment on the bottom. Prior to each trial, one sea raven was introduced into the experimental tanks and allowed to acclimate for at least 12 hours. The trial began with the introduction of one light and one dark colored winter flounder released simultaneously. Behavioral displays, time to capture first flounder, and first flounder captured (light or dark) were recorded. After the first flounder was captured and ingested by the raven, the trial was terminated. Data were analyzed using Chi-square goodness of fit test.

Forty trials were conducted using eight different sea ravens. The ravens ate twenty light-colored and 10 dark-colored winter flounder. In the remaining 10 trials, neither
flounder was captured by the ravens. These trials terminated on average after 63 minutes (+/- 31 minutes; 1 s.d.) when the raven had not moved for at least 10 minutes. Although there was no statistical difference in predation on the flounder types (p=0.068), the results were very suggestive (0.05<p<0.10) that sea ravens selected light colored winter flounder over dark colored winter flounder. Planned additional trials should increase sample size, and thus statistical power, enough for a definitive conclusion.

**Null Hypothesis 8**

There is no difference in the growth rates of hatchery-reared and wild winter flounder juveniles.

**Null Hypothesis 9**

There is no difference in the survival of hatchery-reared and wild winter flounder juveniles.

Testing of these two hypotheses will be done in association with the pilot scale releases, scheduled for each year. Because the first batch of fish for this year’s pilot scale release just occurred (Sept. 20th), we have not been able to test these hypotheses as yet. We will, however, begin the extensive field work necessary for this in the days and weeks ahead, and anticipate no difficulties in meeting these objectives. The number of fish in this year’s pilot scale release will be 1900. The first batch already released (September) included 855 fish, released at 3 separate study sites (285 per site). Fish released at each of the 3 sites were differentially tagged (color-coded elastomer) so release site could be identified for recaptured fish. An identical release will occur in October, but here the fish will be tagged with still other colors that we can identify release location as well as date. Ninety tagged fish are being held in lab to monitor tag retention and any tag related mortality.

**Juvenile Production**

Production was slightly less than anticipated, both at the Coastal Marine Lab and at Great Bay Aquafarms. Part of the reason for this was a severe, and rapid disease outbreak, to which we lost many fish before treatment could be administered. Our preliminary thoughts are that the disease problem was exacerbated by high stocking densities, and this led us to set up a separate experiment to study this problem (see below).

**Work Done in Addition to that Proposed**

Seeking the answers to certain questions, such as those we initially set forth, often leads to new questions. Such has been the case with this research project. For example, we initially proposed to release our fish at a single location. Results of the in-situ pen experiments conducted at the proposed release site, which included low growth rates, led us to question the wisdom of this. Thus, with the assistance of a dedicated senior thesis student, we developed, and have begun to test, the following null hypothesis:

There is no difference in growth and survival of cultured YOY winter flounder in pens in 3 sites in the wild.

To pick the optimal release site, growth rates of penned, cultured, juvenile winter flounder were monitored at three possible release sites during July – August 1999. Each site contained nine replicate pens placed in groups of three. Five elastomer tagged fish were stocked into each one cubic meter, open-bottom pen. Each fish was weighed and measured weekly for 8 weeks. Growth data were analyzed using a nested ANOVA with subsites nested within sites. Initial and final core sediment samples were taken within and outside
each pen to account for food availability, and to make sure the flounder did not graze down the benthic fauna. Additional core samples were taken within each pen for a substrate analysis to determine percent gravel, sand, silt, and clay composition.

Because this experiment just ended, final statistical analyses are not yet complete. It is clear, however, that all fish in all subsites grew both in length and weight, and that growth may be better in the upper estuarine location, perhaps because of warmer water temperatures in September. A complete analysis of growth, food availability, and substrate type will be completed in the next several weeks.

In addition to the experimental work described above, we have initiated an extensive trawling and benthic coring field program designed to determine the optimal release season for a stock enhancement program. We feel strongly that it is imperative that basic ecological interactions in the release area are understood. Determining the distribution and abundance of the wild fish and their main predators and primary prey are of paramount importance. Beginning in July 1999, trawling has been conducted biweekly in the Great Bay Estuary in the three release sites selected for a pilot scale release of winter flounder. Using a 1-meter beam trawl and a sediment corer, numerical abundance and size frequency distributions are being recorded for all juvenile winter flounder caught. The numerical abundance of their primary prey, Gammarid amphipods, is being determined as well. In addition, numerical abundance and size frequency distributions are being recorded for all green crabs (Crangon spp.), and potential predatory fish caught. Based on these data, the optimal time of release for cultured winter flounder will be determined as the period in time when prey are most abundant but predators and wild competitors are less prevalent.

Lastly, we have initiated an experiment to test the following null hypothesis:

There is no difference in growth and survival of YOY winter flounder in four different density treatments.

The four treatments being tested, each with 3 replicates, are 50, 100, 200 and 300% stocking density. In this, stocking density is measured as percentage of the available substrate (tank bottom) relative to the combined ventral surface area of the fish in the replicate. For example, fish stocked in a 50% replicate cover only half of the tank bottom, while those at 200% would theoretically form a double layer of fish on the bottom. This experiment has been running for 8 weeks to date, and will continue for another 4. Upon completion, we will do a complete statistical analysis of the data.

**BUDGET JUSTIFICATION**

Personnel include H. Howell, who will spend a minimum of one summer month each year coordinating and supervising the research, and E. Fairchild, who will be using aspects of this study for her doctoral thesis. An annual stipend and tuition are sought to support her participation. As the study is labor intensive (culturing fish, conducting experiments, assessing pilot scale releases), we seek funds to pay undergraduate students. To leverage the minimal funds we've requested for this purpose, we will seek to hire students eligible for work-study. Supply funds will be used for fish feed, misc. plumbing parts, tagging supplies, etc. Funds are also requested for Great Bay Aquafarms who will be producing most of the juvenile fish. These monies will be used to pay for extra utility costs, tank space rental, and the extra production of microalgae and live feed (rotifers and brine shrimp). It is important to note that Great Bay Aquafarms will be producing fish for this grant at approximately $1 each. This will probably be less than their actual cost of raising the fish, and the difference should be considered as matching funds.