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# Evaluating Stock Enhancement Strategies: A Multi-Disciplinary Approach

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## Abstract

Stock enhancement, the supplementation of depleted wild fish and invertebrate stocks with individuals reared in aquaculture facilities or captured from other populations, is becoming an increasingly popular method of bolstering heavily fished populations. Although many different aspects of marine stock enhancement have been evaluated for several species of fish and invertebrates, a multidisciplinary approach is often not feasible for many programs. In addition, a systematic, coordinated, comprehensive, monitoring program is not commonly used to test whether stock enhancement efforts actually result in measurable increases in fishery output. In 1999, the Florida Marine Research Institute in St. Petersburg, Florida, and Mote Marine Laboratory in Sarasota, Florida, initiated a multiyear stock enhancement experiment to supplement the red drum (*Sciaenops ocellatus*) population in Tampa Bay, Florida, USA. The original experimental design for releasing aquacultured (hatchery-reared) red drum into Tampa Bay included the following variables: two riverine systems, several sections within each system, two times of the year for release of the fish, and three categories of red drum size-at-release. The ongoing monitoring effort involves the following general categories of activities: breeding and rearing the fish to the stage of growth at which they are designated to be released into the estuaries; developing and using a multigene genetic tag to determine parentage of the hatchery-reared fish and to distinguish those fish from wild fish for many applications; designing and conducting comprehensive, fisheries-dependent and fisheries-independent, field sampling and monitoring programs to obtain information on the survival and dispersal of the hatchery-reared fish after their release, the entry of those fish into the reproductive population, and the contribution of those fish to recreational fishery landings; informing fishermen of the stock enhancement program and soliciting their participation; and monitoring the health of the hatchery-reared fish before and after their release and of the wild fish in the recipient red drum population. Here, we describe the general methodologies and the intergroup coordination used by the research groups charged with developing and executing the Tampa Bay red drum stock enhancement experiment.

## Introduction

Red drum (*Sciaenops ocellatus*) are among the marine species most important to shallow-water and nearshore sportfishers (anglers) in the southeastern USA. Because they are easily available to nearshore-marine and dockside recreational fishermen, they are highly sought in a directed fishery in Florida. Loss or degradation of seagrass habitat, coastal development and associated chronic pollution, and heavy fishing pressure have reduced the number of red drum to a fraction of their former numbers, resulting in severe regulations that limit harvest. In the mid-1980s, the National Marine Fisheries Service declared some red drum stocks to be overfished. Between 1985 and 1987, a series of increasingly restrictive rules governing the red drum fishery were written by the Florida Marine Fisheries Commission, culminating in a rule that indefinitely prohibited all commercial fishing for red drum. Despite these measures, by 1988, the stock appeared to have declined to approximately 5% of its unfished biomass, implying that red drum reproductive potential might be inadequate to sustain local populations (Murphy and Crabtree, 2001). To address this problem and other related issues, staff of the Florida Fish and Wildlife Conservation Commission's Florida Marine Research Institute (FMRI) developed the Stock Enhancement Research Facility (SERF) between 1985 and 1988. In 1999, a multiyear project was initiated to enhance the depleted red drum stock in Tampa Bay, Florida. This project consists of an experimental phase and production phase and is currently in the experimental phase.

The technology now exists to rear large numbers of juvenile red drum in captivity at SERF. However, little is known about when or where to release these fish into Florida's estuarine systems or about the size of red drum that should be released to maximize their survival and to clearly show an increase in adult spawning stocks or fishermen's catches. In the experimental phase of this ongoing stock enhancement project, the influences of location, season of release, and size of release on the short-term and long-term survival of aquacultured (hatchery-reared) red drum stocked into Tampa Bay are being tested.

Here, we describe the methodology for our multi-disciplinary approach to evaluate red drum stocking strategies directed toward enhancing the Tampa Bay red drum population. Through our monitoring effort, we will estimate the short- and long-term survival of the stocked fish, their contribution to the local red drum breeding stock, their contribution to the harvested population, and the long-term genetic impact on wild red drum populations.

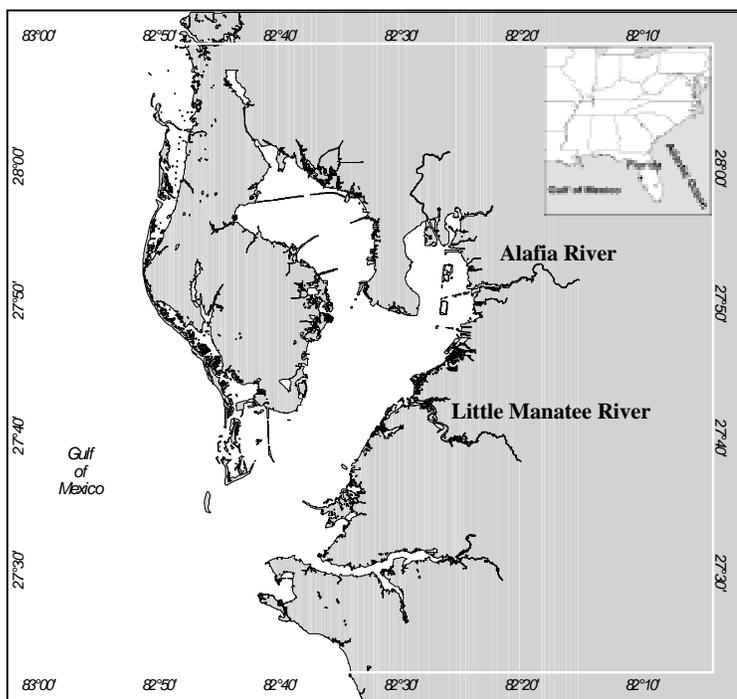
## Methodology

Upon careful examination of various strategies for releasing hatchery-reared red drum into Tampa Bay, we decided on the following protocol. We release the fish into two rivers within the Tampa Bay estuarine system: the Alafia and Little Manatee Rivers (Fig. 1., following page). Both of these rivers are highly productive nursery areas for wild red drum (Peters and McMichael, 1987). We release three size-classes of fish: Phase I (25-45 mm standard length [SL]), Phase II (65-110 mm SL), and Phase III (>135 mm SL).

We release fish at two times of the year: "in-season" (the timing of spawning of wild and hatchery broodstock is approximately the same; thus the size range of the stocked fish closely matches the size range of the same wild-fish cohort) or "out-of-season" (the timing of spawning of that hatchery broodstock is approximately six months after the time of the wild-stock spawning; thus, the size range of the stocked fish differs notably [usually significantly] from that of any wild cohort). We release the fish at different locations along the rivers; these locations are

defined using a grid system in which the rivers are stratified according to distances from their river mouths. The stratification reflects shifts in salinity and temperature regimes from estuarine to marine at the mouths of the rivers.

The complete monitoring program involves the staffs of five separate, but integrated, research programs at FMRI and the staff of the Center for Fisheries Enhancement at Mote Marine Laboratory (MML), a non-profit marine laboratory located in Sarasota, Florida. The FMRI Fisheries Stock Enhancement (FSE) staff breeds adult red drum broodstock, rears their offspring to the appropriate size for release, physically tags all Phase II and Phase III fish with coded-wire tags (CWTs), and participates in all releases in the Alafia and Little Manatee rivers. The FMRI Biochemical Genetics Laboratory (BGL) staff uses a multigene genetic tag to estimate the proportion of hatchery-reared fish in the post-enhancement (admixed) population at various times after the release and at various distances from the release sites. The BGL staff uses a multigene genetic tag to estimate the proportion of hatchery-reared fish in the postenhancement (admixed) population at various times after the release of hatchery-reared fish and at various distances from the release sites, to determine the effective population sizes of the broodstock and the broods, to determine the uniqueness of Phase-I offspring genotypes compared to the genotypic composition of wild-population red drum in the same size cohort, to estimate the proportion of hatchery-reared fish in the Tampa Bay red drum population, and to monitor the long-term genetic impact of the stock enhancement effort on the genetic diversity of the wild red drum population. The FMRI Fishery Independent Monitoring (FIM) and MML staffs routinely and systematically collect red drum from the admixed population; determine the proportion of tagged (CWTs or ultrasound transponders) Phase II or Phase III fish in the size cohorts that could contain those fish; and deliver all fish in the size cohorts that could contain Phase I fish, as well as all unmarked fish in the size cohorts that could contain Phase II or Phase III fish, to the BGL staff for genetic identification. The FMRI Fisheries-Dependent Monitoring (FDM) staff routinely and systematically surveys recreational fishermen to monitor their effort versus catch of red drum, to examine the harvested red drum for presence of CWTs and to obtain tissue samples from all other harvested red drum for genetic identification as wild or hatchery-reared fish. The FMRI Aquatic Health Group (AHG) staff evaluates the health of all hatchery-reared red drum offspring prior to their release and routinely assesses the health status of hatchery-reared and wild red drum captured by the FIM staff in their post-enhancement surveys. The MML staff conducts an



**Figure 1.** Location of the Alafia and Little Manatee rivers in Tampa Bay, Florida, USA. Insert shows location of Florida in the southeastern USA.

extensive advertising campaign geared toward angler awareness and participation in the program, collects fish samples from the Little Manatee River in a manner similar to that used by the FIM staff in the Alafia River, and delivers the appropriate samples to the BGL staff for genetic identification.

The path of a complete enhancement cycle is as follows. The FSE staff, with assistance from others at FMRI, captures wild, adult red drum for potential use as broodstock. The potential broodstock fish are tagged with Passive Integrated Transponder tags (American Veterinary Identification Device Company, Norco, California) and their multigene, genetic-tag genotypes are established by the BGL staff. Selected broodstock individuals are grouped into breeding aggregates and induced to spawn at SERF. Their offspring are reared at SERF to specific size-classes (Phases) and are released into specific sections of the Alafia River or Little Manatee River. Prior to release, the AHG staff evaluates the health of each brood and the FSE tags all Phase II and Phase III fish with CWTs. Both pre- and post-enhancement collections of red drum are obtained from selected locations in Tampa Bay during routine or directed field sampling efforts performed by the FIM and MML staffs. Fish that have CWTs are identified at the time of sampling. All other fish in cohorts that could contain hatchery-reared fish are delivered to the BGL staff for genetic identification. The FDM staff checks red drum harvested by recreational fishermen for the presence of CWTs and, with the permission of the anglers, obtains tissue samples from all fish without CWTs. These tissue samples are also delivered to the BGL staff for genetic identification. The BGL staff assays the fish from the FIM and MML post-enhancement sampling, the FDM recreational-fishermen surveys, and the MML angler-participation endeavor for the multigene genetic tag to ascertain with high probability the origin (hatchery-reared or wild) of these red drum collected from the admixed population.

#### BGL Genetic Identification Procedures

Although logically, broodstock spawning and offspring rearing are the initial steps in any stock enhancement project, we describe the work of the BGL staff first because the genetic identification component of this project is integrated into all other project components.

Central to the genetic monitoring program is a multigene genetic tag composed of a 419 nucleotide-base-pair (bp) region located in the mitochondrial DNA (mtDNA) control region plus nine nuclear-DNA microsatellite loci. In most animals, including red drum, mtDNA is transmitted uniparentally from mother to offspring (Wilson *et al.*, 1985; T. M. Bert and M. Tringali, unpublished data). Typically, the mtDNA control-region nucleotide sequence is highly variable among individuals in populations of marine fishes (Graves, 1998), including red drum (Seyoum *et al.*, 2000). Microsatellites are regions of nuclear DNA composed of sequential repeats of short nucleotide sequences that are typically 2-5 bp in length (Hillis *et al.*, 1996). Microsatellite DNA alleles are inherited from both parents. Allelic polymorphism in microsatellite DNA is measured as the variation in the number of these repeated units and is manifested in genetic assays as DNA fragments of different lengths. Levels of polymorphism and numbers of alleles at microsatellite loci are generally high within species and populations and are therefore useful for parentage analyses and as components of genetic tags.

The BGL staff obtains both the mtDNA and microsatellite genotypes of all female red drum and the microsatellite genotypes of all male red drum held at SERF for potential use as broodstock. In red drum mtDNA maternity studies, which involved >1000 offspring and 14 broodstock mothers, BGL staff found no instances where the mtDNA genetic-tag sequences of the mothers and their offspring differed (unpublished data), nor do they expect to find any in the

future, based on reported mutation rates for the mtDNA control region (Merilä *et al.*, 1997). Thus, because of the unique mode of inheritance of mtDNA, the mtDNA genotypes of the broods are known if the mtDNA genotypes of the female broodstock are known. Red drum from post-enhancement collections whose mtDNA genetic-tag sequences do not match those of any SERF female broodstock individual are highly unlikely to be stocked fish.

The mtDNA control-region sequence data are obtained by using standard Polymerase Chain Reaction (PCR) procedures (Saiki *et al.*, 1988) to amplify (make many copies of) the target mtDNA and an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) to obtain the mtDNA nucleotide sequences. The BGL staff also uses the PCR technique to amplify the microsatellite loci. They multiplex the PCR reactions (make many copies of the alleles from several different microsatellite loci simultaneously) and identify the genotypes using an ABI 310 Genetic Analyzer. Additional details of the laboratory procedures for obtaining the mtDNA control-region genetic-tag nucleotide sequences and the allelic patterns for several of the microsatellite DNA loci, as well as the levels of genetic variation in these DNA segments, are described in Seyoum *et al.* (2000) and Turner *et al.* (1998). The utility of these highly variable DNA segments for genetic tagging and the benefits of using multigene genetic tags in aquaculture are described in detail in Bert *et al.* (2001), Bert *et al.* (2002), and the references therein.

The compound genetic tag is used for a number of purposes in this complex stock-enhancement monitoring program (Bert and Tringali, in preparation). Here we describe use of the tag to distinguish hatchery-reared offspring from wild red drum that are in the same cohort in the post-enhancement red drum samples delivered to the BGL staff.

To determine the baseline level of genetic variation for both the mtDNA and microsatellite DNA components of the genetic tag, the BGL staff analyzed approximately 250 young-of-the-year (YOY) red drum each year from 1998 through 2000 from selected locations in Tampa Bay. To those data they added the genetic-tag genotypes of all fish captured for potential use as broodstock and then characterized the level of variation in this “library” of genetic-tag data. The genetic data library is used in several ways. One use is to estimate the frequency of the mtDNA genetic-tag genotype of each potential broodstock female using wild-population data as the basis for the frequency estimation and to classify each broodstock female based on that frequency. Each female having an mtDNA genotype not previously seen in a surveyed individual is classified as “unique.” Each female possessing an mtDNA genotype previously encountered in one or two other fish is classified as “rare.” Each female possessing an mtDNA genotype previously seen in three or more individuals is classified as “common.”

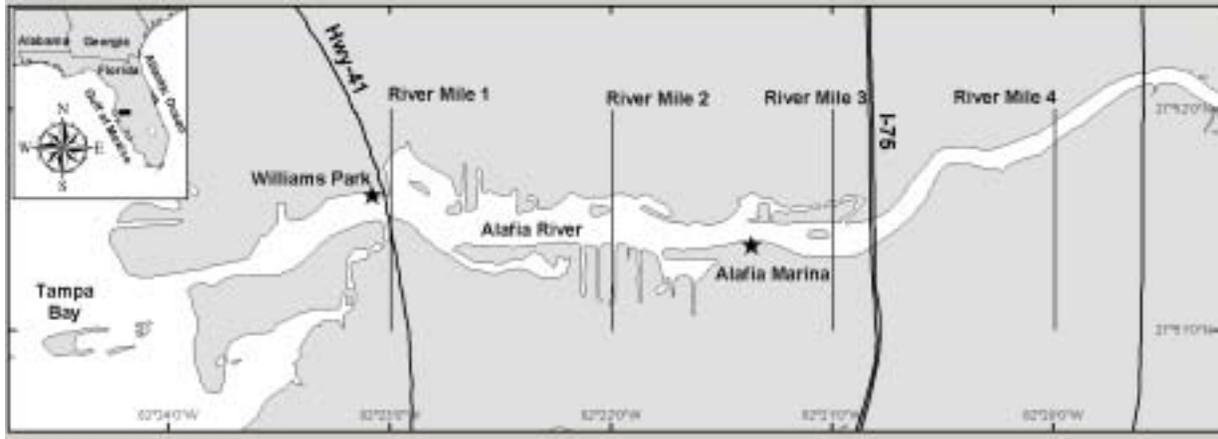
Red drum captured by FIM, MML, or FDM staffs and provided to the BGL staff are first individually assayed for the mtDNA genetic-tag component. Any fish with an mtDNA sequence that matches that of a female broodstock individual is considered to be a “candidate” hatchery-reared fish and is subsequently analyzed for the microsatellite loci. Via parentage analyses, the results from the microsatellite analysis either support or do not support the SERF origin of that red drum. If the microsatellite DNA analysis does not exclude a SERF origin for an individual, the probability (based on the likelihood ratio) that the individual is actually a hatchery-reared fish is computed (Brenner, 1983). These ratios are based on the frequencies of the specific multilocus, microsatellite-DNA genotypes in the red drum analyzed to obtain the baseline data. Typically, these ratios are very small; thus the likelihood of correct positive identifications are very high (> 99.999%).

### FSE Broodstock Spawning, Offspring Rearing, and Release

Over the past fifteen years, the technology has been developed for spawning red drum in captivity by manipulating temperature and photoperiod and for rearing offspring in very large numbers in outdoor man-made ponds (Colura *et al.*, 1976; Arnold *et al.*, 1977; Roberts *et al.*, 1978). In general, adult red drum are collected sporadically throughout the year for use as broodstock. The BGL staff advises the FSE staff on which females to use to produce each brood based on the frequencies of the mtDNA genotypes of those females in the wild population. Their objective is to create broodstock groups that collectively have genetic-tag genotype compositions that distinguish the broods released for each experimental treatment from each other and from the wild population. After the genetic classifications of the potential broodstock females are obtained from the BGL staff, selected females are assigned to broodstock groups at the appropriate times and are placed into 16,000-l circular spawning tanks along with 3-4 males with ripe gonads. Depending on availability, 1-3 unique or rare females are used per broodstock group to produce fish that will be released in Phase I. Phase I red drum broods constitute about 80% of all released fish. There are no constraints on the genetic-tag genotypes or number of females used to produce fish that will be released in Phase II or Phase III. This is because a tenable, nongenetic system of physically tagging Phase I fish (e.g., CWT insertion or oxytetracycline marking of the otoliths) has not yet been developed and the amount of microsatellite DNA analysis required from the BGL staff is reduced. All hatchery-reared red drum that are released as Phase II or Phase III individuals are tagged with CWTs. The CWTs used by FSE are made of stainless steel and measure 1.00-mm long X 0.25-mm wide. Each wire contains a unique decimal code that identifies each fish as being of SERF origin, the broodstock group from which it was spawned, the location and date of release, and the mean size of the brood at the time of release. The genetic assays of Phase II and Phase III individuals not tagged with CWTs serve as a backup to the physical tagging and allow estimation of CWT loss or oversight.

To prepare a broodstock group for spawning, the fish are subjected to appropriate photothermal conditioning to induce gonadal maturation that culminates in spontaneous spawning. When the experimental design requires contemporaneous spawning from several broodstock groups (e.g., to synchronize the rearing of genetically distinct broods for multiple, simultaneous releases), spawning is induced hormonally if the females fail to spawn spontaneously. When hormone induction is necessary, FSE staff implant photothermally conditioned, gravid females with gonadotropin-releasing-hormone time-released pellets or inject them with human chorionic gonadotropin before placing them in tanks with sexually mature males in a prespawning condition. Spawning typically occurs within 30 hours of hormone administration.

After all spawning events, the eggs are harvested from spawning tanks using a 100-l egg collector that skims them from the surface of the water. The eggs are removed, counted volumetrically, and transported to an incubation system for acclimation and hatching. Approximately 60-66 hr after hatching, the larval red drum are transferred to 1-acre outdoor culture ponds. The fish are reared in the ponds until, collectively, they attain the appropriate mean size for their designated phase of release. During the entire rearing process, each brood is maintained separately from other broods. Each brood is also designated a specific site and approximate date for release. Immediately prior to harvesting, samples of fish are provided to the AHG staff for health evaluation and to an independent laboratory for health certification.



**Figure 2a.** Red drum (*Sciaenops ocellatus*) release and sampling domains. Insets show general location of each river in Florida. Release sites were designated as shorelines within each river section where suitable habitat was present. Alafia River; the four river miles delineate the four river grids used in the stock enhancement experiment.

When the red drum reach the appropriate size, the pond is drained to a depth of only a few centimeters and the fish are collected by net. Fish harvested in the Phase I size-class are immediately released. Fish harvested in the Phase II or Phase III size-classes are held in 16,000-liter, indoor, recirculating fiberglass tanks and observed for a recovery period of at least three days. Phase II and Phase III red drum are then tagged with CWTs, which are implanted vertically in the left adductor mandibularis with a Mark IV tagging machine (Northwest Marine Technology, Incorporated [NMT], Shaw Island, Washington). Immediately after being tagged, each red drum is scanned with a Quality Control Device (tube-detector) or Field Sampling Device (V-detector [NMT]) to verify the presence of the tag. The fish are again moved to the holding tanks for another recovery period of at least three days. They are then transported to the release site in a live-fish hauler (mobile tank) at a density of no more than 20 g of fish/l of seawater.

#### FIM and MML Collecting and Validation of Fish Origin

As their component of the stock enhancement experiment, the FIM staff is charged with co-operating with FSE staff in the release of the hatchery-reared red drum into the Alafia River, monitoring the relative abundance of red drum juveniles in the size cohorts that could contain hatchery-reared red drum, examining Phase II and Phase III fish for the presence of CWTs, and providing the BGL staff with tissue samples (usually fin clips) of all red drum that are not tagged but are within the size ranges in which the stocked red drum might occur.

The hatchery-reared red drum destined for release are transferred from the SERF live-fish hauler to the net wells of FIM boats and transported to designated release sites. Using hand-held buckets, FSE and FIM staffs release the fish along shallow-water shorelines that have appropriate habitat. Short-term (24-hr) studies of the survival of red drum in all phases and tag retention of Phase II and Phase III fish are conducted in conjunction with each release event. At each release site, random subsamples of fish (100 Phase I, 50 Phase II, or 30 Phase III, depending on the stage of fish released) are placed separately by phase into cages at the time of release. The cages are checked for fish mortality 6 and 24 hours after release. Percent survival and CWT retention rates are determined and the lengths (SL and total length [TL]) and weights

(g) of all fish are recorded. When the survival rate of the caged fish is low, subsamples are provided to the AHG staff to evaluate possible reasons for the high mortality rate.

The FIM staff samples red drum from the admixed population in two ways. They use a standardized “stratified-random sampling” protocol similar to that used in the FMRI statewide sampling program (McMichael, 2000) and a “directed sampling” protocol in order to maximize the number of red drum captured.

#### *Stratified-Random Sampling*

The Alafia River sampling domain includes the area from the mouth of the river to a distance 4 nautical miles (nm) upriver in waters < 3 m deep, extending 16 m riverward from each shoreline (Fig. 2a). This sampling area is divided into 1-nm-latitude X 1-nm-longitude grids. Each grid is further subdivided into 100 microgrids (0.1 nm X 0.1 nm). The microgrids that incorporate a portion of the shoreline constitute the actual sampling units.

The FIM staff routinely samples this river; 21-m and 61-m haul seines are used to effectively catch red drum ranging in sizes approximately 25-50 mm SL STET 50-300 mm SL, respectively. The 21-m seine is made of 3-mm stretch-mesh nylon, has a center-bag with dimension 1.8-m depth X 1.8-m width X 1.8-m height and is set in water depths up to 1.8 m. The 61-m net is made of 25-mm stretch-mesh nylon, has a center-bag with dimension 3-m depth X 3-m width X 3-m height and is set in water depths up to 2.5 m. These nets are stretched between polypropylene lines that are fitted with evenly spaced lead weights at the bottom and flotation buoys at the top. Both are deployed by boat in a standardized elliptical shape parallel to the shoreline. The wings of each net are then brought together along the shoreline by hand and the bag is retrieved.

To date, red drum releases into the Alafia River have occurred in March-April (Phase II), June-July (Phase III), and December (Phase I) 2000 and in June-July (Phase III) and December (Phase I) 2001. Beginning in January 2000, FIM staff sampled red drum to estimate the relative abundance of wild red drum in the Alafia River prior to the stock-enhancement effort. Since the initiation of the stock enhancement experiment, FIM has sampled within 1-2 weeks after each release and monthly thereafter.

Following standard FIM protocol (McMichael 2000) sampling sites within each grid are randomly selected from the available shoreline microgrids. The sampling gear and number of samples collected each month varies depending on the size of red drum in the river at the time of sampling. For all collections, the sites sampled are evenly distributed among grids and between the north and south shorelines. For example, if 16 samples are collected with the 61-m haul seine, four samples are collected from each grid, and within each grid, two of these samples are collected from north-shoreline microgrids and two are collected from south-shoreline microgrids. When a chosen microgrid cannot be sampled because of gear or habitat constraints (a rare event), an alternate microgrid is selected in a standardized random fashion.

All samples are processed in the field immediately after collection. Most samples are processed according to standard FIM protocols (McMichael, 2000). For each sample, all individuals are identified to the lowest practical taxon and counted. For each taxon, the lengths (mm SL) of 10-40 individuals (depending on the size and total number of individuals in the taxon) are measured. All red drum (up to 100) in the cohorts that could include Phase II and Phase III fish are checked for CWTs by using a NMT V-detector.

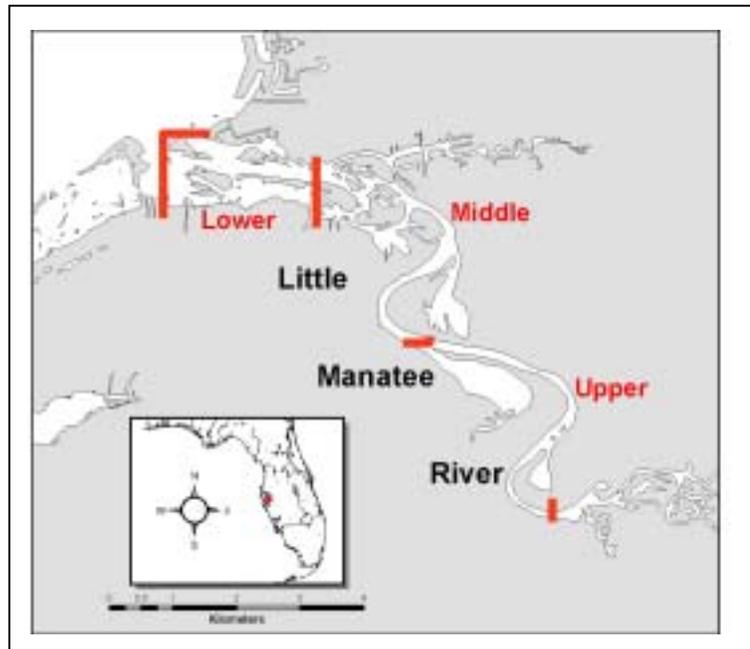
Depending on the number of red drum in the collection, a subsample or the entire sample of red drum is retained from each collection that contains red drum. Each individual is double-checked for the presence of a CWT. Between 10 and 20 red drum per grid per month are delivered to the AHG staff for health evaluation. Fin clips for genetic analysis are taken from all red drum without CWTs. From those fish possessing CWTs, the tags are extracted and later read using a dissecting microscope.

Detailed water quality and habitat information are also recorded at each sampling location. These data include sample equipment identification, location, weather, water quality, habitat, and gear-specific information.

The Little Manatee River sampling domain includes the area from the mouth of the river to a distance approximately 5 nm upriver in water < 1.8 m deep, extending riverward approximately 5 m from each shoreline. The river is divided into lower, middle, and upper sections (Fig. 2b) each subdivided into a 1-nm latitude X 1-nm longitude grid. Both FIM and MML staffs sample this river.

Field sampling protocols are similar to those followed in the Alafia River except for the timing of post-enhancement sampling and the number of collections made each month. Fifty-eight locations per month are sampled for four months after a release. Thereafter, sampling effort is reduced by one-half (29 locations) until the next hatchery release occurs, after which the sampling effort returns to 58 locations per month for the following 4 months.

Thus far, Phase I red drum have been stocked into the Little Manatee River during August 2000 and July, August, and September 2001. Sample-processing protocols also follow those described for the Alafia River monitoring, except for the following. Regardless of the level of monthly sampling effort, only two collections per river section are fully processed (all taxa identified and a subset of each taxon measured). In all other samples, only red drum and economically valuable species are processed. The laboratory procedures are as described for the Alafia River samples except that no red drum are provided to the AHG staff. Water quality and habitat data are also recorded as in the Alafia River sampling.



**Figure 2b.** Red drum (*Sciaenops ocellatus*) release and sampling domains. Insets show general location of each river in Florida. Release sites were designated as shorelines within each river section where suitable habitat was present. Little Manatee River; thick lines delineate the three river sections (lower, middle, upper) used in the stock enhancement experiment.

### *Directed Sampling*

As juvenile red drum attain sizes > 200 mm SL, they migrate into deeper portions of the river or immigrate into other habitats in Tampa Bay (Peters and McMichael, 1987). Little is known of the movements and abundance of these large juvenile and subadult (200-400 mm SL) red drum. Historically it has been difficult to sample red drum in this size-class using standard FIM sampling techniques such as haul seines. To address this problem, FIM staff developed a directed red drum sampling program, which employs specialized gear for the capture of red drum in this size range. This sampling, in combination with the standard sampling described above, allows FIM staff to monitor red drum from the time that they are YOY until they enter the fishery (~400 mm SL).

Directed sampling is conducted monthly in both the Alafia and Little Manatee rivers and adjacent Tampa Bay waters. Sample area and habitat are not restricted. The staffs of FIM and MML search for red drum in a wide variety of habitats and locations using rod-and-reel gear, trammel nets, and ultrasonic telemetry. Sampling sites are selected by locating suitable habitat, by visually sighting red drum, or by using acoustic tracking devices to locate fish tagged with ultrasonic transmitters. The type of sampling gear used depends on the weather, tidal conditions, and accessibility of the habitat to be sampled. The sampling effort depends on the time of year, gear used, and availability of personnel. Directed sampling trips in which rods-and-reels or trammel-nets are used are often scheduled when tide levels allow access to shoreline habitats. Acoustic-tracking trips are conducted weekly following the release of red drum implanted with ultrasonic transmitters (U-tags; Sonitronics, Tucson, AZ). Tracking efforts continue based upon the life of the implanted U-tags and the number of red drum estimated to be in the vicinity. Light-tackle rod-and-reel gear, baited with live bait or artificial lures, is used in a variety of habitats. Trammel nets are used on the mud and grass flats and along some shoreline habitats. Monitoring of U-tagged red drum is conducted in release areas and in adjacent Tampa Bay waters that have habitats suitable for red drum. Additional information on U-tagged fish is provided by anglers who report fish captures.

The trammel nets are approximately 366 m long and 2.4 m deep, are constructed of monofilament netting (70-mm stretch-mesh inner wall and 305-mm stretch-mesh outer wall), and have a leaded bottom line and a floated top line. They are deployed from shallow-draft mullet skiffs such that they encircle and capture the fish. The net is quickly retrieved by hand to reduce fish mortality.

For acoustic tracking, the MML and FSE staffs surgically implant 200- to 550-mm SL red drum with the U-tags. These fish are stocked into the Alafia River at locations that have suitable shoreline habitats; there, they join schools of red drum in the vicinity. The U-tags emit a unique set of “pings” at pre-set frequencies, have a lifespan of approximately 18 months, and have a maximum signal range of approximately 500 m in open water. The means and variances for the six-month survival rate, surgical incision-healing rate, and U-tag retention rate for U-tagged red drum are known. The U-tagged red drum are monitored by using a hydrophone and receiver to listen for signals that help locate the fish. When U-tagged red drum are located, the following is recorded: transmitter code, latitude and longitude, bottom and shoreline habitat data, bottom and surface salinity, water temperature, dissolved oxygen level, and approximate number and size range of the observed fish. After the fish are located, MML and FIM staffs attempt to collect the fish using rods-and-reels and cast nets.

All collected red drum are counted, measured (SL), and checked for CWTs. Fish containing CWTs are taken to the laboratory where the CWTs are extracted and read. Fin clips from the second dorsal fin are removed from all red drum that do not have CWTs and are delivered to the BGL staff for genetic identification.

### FDM and MML Angler Surveys

The FDM staff routinely and systematically interviews anglers to obtain catch (all fish caught) and harvest (fish retained for consumption) information at established locations such as public boat ramps, marinas, bridges, piers, jetties, beaches, and shores throughout Florida. They collect data on the number, size, and species of fish captured; time spent fishing; location of fishing effort; and species targeted. The MML staff supports the FDM efforts through a public-awareness advertising campaign. Because most hatchery-reared red drum are released into the Alafia and Little Manatee rivers without external tags, it is important to inform and enlist anglers to provide fin clips and fish-capture information for all red drum captured in Tampa Bay.

### *Sampling Design*

For the red drum stock enhancement project, the FDM had available approximately 20 years of National Marine Fisheries Service Marine Recreational Fisheries Statistics Survey (MRFSS) data as background information on red drum harvested in Tampa Bay. During the 1.5- to 2.5-year lag between the first release of red drum in spring 2000 and the recruitment of hatchery-reared red drum into the fishery, FDM staff also did the following background work: (1) conducted a baseline assessment of the catch per unit effort (CPUE) of red drum, (2) gathered and analyzed basic statistics (mm SL and weight) on the captured fish prior to the appearance of the hatchery-reared red drum in the collective recreational angler catch (creel), and (3) fine-tuned the sampling protocol.

Initially, FDM examined the MRFSS angler-interview (angler-intercept) data for the Tampa Bay area from 1994 through 1998 to determine sites in Tampa Bay where red drum have been landed. A subset of these sites was selected based on proximity to release areas and the potential for intercepting anglers with red drum catches. The ontogenetically related movements of red drum were also considered in the site selection. As the hatchery-reared red drum increase in size, most of them gradually disperse downstream from their riverine release sites and move toward the open bay. Thus, most angler-intercept locations were selected from the eastern side of Tampa Bay, south of the Alafia and Little Manatee rivers (Fig. 1). However, red drum tagged and released on the eastern side of Tampa Bay have been subsequently captured on the western side of the bay (FMRI, unpublished data). Therefore, selected sites on the western side of the bay were also targeted for monitoring red drum harvested by anglers.

Upon examination of the 1994-1998 MRFSS survey, the FDM staff determined that the protocol for collecting those data was inadequate as the sole methodology for monitoring changes in red drum angler harvest resulting from the stock enhancement effort because insufficient numbers of red drum were being collected. The MRFSS is principally designed to provide catch estimates of all species caught by anglers on a state or regional level (Essig and Holliday, 1991). Therefore, to enable detection of statistical differences in red drum catch rates in the creel, FDM developed a sampling strategy

dedicated to providing a sufficient number of intercepts of those anglers who targeted or captured red drum.

Several problems were considered when designing this directed sampling strategy. Red drum harvest is limited to one fish 46-69 cm (18-27 in) TL per person per day. There are few sampling locations within Tampa Bay where anglers report high red drum catches. Details of the dispersal patterns of juvenile red drum from the Alafia and Little Manatee rivers into Tampa Bay are not well understood; thus, FDM staff would need to make assumptions regarding the proportion of hatchery-reared fish that would eventually be in the Tampa Bay red drum population. Moreover, the FDM 2000-2001 data on anglers that fish in Tampa Bay or use facilities in Tampa Bay to launch and retrieve their boats indicate that approximately 12.5% of these anglers target red drum, 5.0% catch and release red drum, and less than 3.0% harvest the species. Therefore, the estimated number of anglers that would need to be interviewed was daunting.

To improve the estimate of the number of angler intercepts and red drum needed to detect a contribution of hatchery-reared red drum to the creel, the FDM staff consulted MRFSS landings information to establish the proportion of anglers who harvested red drum over the five-year period 1994-1998. They used standard power calculations (Moher *et al.*, 1994; Zar, 1996) to estimate the minimum number of angler intercepts required to detect 10%, 25%, 50%, and 100% differences in red drum catch rates at the 95% confidence level with power levels of 80% and 90%. For example, FDM could be 90% certain of detecting a 50% difference in the catch rate of red drum 95% of the time if hatchery-reared red drum constituted 10% of the Tampa Bay red drum population. The FMD staff routinely uses the power calculations to determine the number and location of intercept sites to target. For this determination, the angler-intercept sites are examined individually and in combination.

To assign sampling days to angler-intercept sites in the directed red drum sampling project, FDM uses a weighted, random-sampling design. The probability of selecting a given site depends on angler activity; busier sites have higher probabilities of being selected than do sites where only a few anglers might be intercepted. To maximize the number of interviews with anglers who catch or harvest red drum, sampling locations are selected weekly to allow timely integration of newly identified sites into the sample pool and the removal of unproductive sites.

FDM staff assumes that the sample pool is dynamic and that fishing activity will change temporally and geographically within the bay. Changes in the number of locations targeted for interviews are done in a manner that minimally disrupts the sampling protocol and optimizes the possibility of gathering data.

An ancillary goal of this sampling activity is to examine the feasibility of this adaptive sampling design, which takes advantage of accrued information to adjust sampling decisions “on the fly” (Oehmke *et al.*, in press; Hardwick and Stout, 1998). Adaptive designs have the potential to produce exact solutions for sample allocation problems inherent in a species-directed fisheries-monitoring project such as the FDM portion of the Tampa Bay red drum project.

### *Sampling Protocol*

For the MRFSS, FDM staff visits approximately 60% of the angler-intercept sites on weekends (Saturday and Sunday) and 40% on weekdays (all other days), reflecting the

increase in fishing pressure during the weekends. In contrast, for the directed red drum sampling, FDM staff visits 50% of the angler-intercept sites on the weekends and 50% on weekdays. Because each FDM staff member can effectively complete only four sampling assignments in a given week and because sites may be assigned only once on any given day, a 50%:50% division of weekend versus weekday angler-intercept site visits represents the most effective use of FDM staff time. If personnel are available, the number of sites visited per week is increased by up to 25%. These additional locations constitute a buffer and are not required to meet the minimum sampling requirements established by the power calculations.

For the directed red drum sampling at each angler-intercept site, an FDM staff member first screens anglers who have completed their fishing trips to determine if they targeted, caught and released, or harvested red drum. Anglers whose trips fall into one or more of these three categories are further questioned about the locations at which they fished, gear types they used, other species they harvested, the duration of their fishing trips, and fishing-group sizes. With their permission, the FDM staff member examines their finfish catches and weighs (kg) and measures (TL) their harvested fish. The harvested red drum are also scanned for the presence of CWTs using the NMT V-detector. If the fish possesses a CWT, the red drum carcass is requested for CWT extraction by FIM staff. From each red drum without a CWT, a fin clip is collected for genetic identification by BGL staff.

To improve the probability that adequate numbers of angler interviews are conducted and to expedite the detection of hatchery-reared red drum in the field, three FDM staff members are devoted to obtaining information on red drum catch in Tampa Bay. In addition, all FDM staff members who survey anglers in the Tampa Bay area in the MRFSS project collect similar data on red drum and angler effort and obtain red drum fin clips for genetic identification when possible.

### *Project Awareness*

Recreational anglers are the principal recipients of the benefits of this project and their participation and involvement are important components of its success. To promote project awareness and engage angler participation, the MML and FSE staffs initiated an outreach campaign. The outreach program includes displaying posters and distributing information and fin-clip kits to bait-and-tackle shops, staging interviews with the media, and directly contacting anglers.

Together, the MML and FSE staffs designed a letter-sized poster advertising the project and soliciting angler assistance. The poster contains a color picture of a red drum, schematic drawings depicting how to take a fin clip from the second dorsal fin, the FWC "Redfish Hotline" phone number, and further instructions on participation. Laminated versions are posted in bait-and-tackle shops, boating supply centers, convenience stores, and marinas and at fishing piers, parks, and boat ramps. Non-laminated and postcard-sized versions are available for anglers to pick up at some of these locations and are provided to angler organizations. The postcard is also included in Fin Clip Kits, which are distributed to bait-and-tackle shops and angler organizations and contain the supplies and instructions needed for anglers to take tissue samples from red drum. Numerous bait-and-tackle shops serve as sites for anglers to obtain Fin Clip Kits and to deliver red drum tissue samples and/or carcasses.

### AHG Fish Health Monitoring

A specific area of technical concern in stock enhancement research and implementation is fish health management (Pruder *et al.*, 1999). Fish encounter more stress in culture conditions than in the wild. Ideally, fish that are reared in aquaculture facilities are exposed to a minimum of pathogens, but they are susceptible to a number of infectious pathogens and parasites that thrive in closed systems (Landsberg, 1989; Landsberg *et al.*, 1994). Releasing diseased or pathogen-carrier fish into natural waters can have serious consequences for indigenous fish stocks (Goede, 1986). Additionally, if hatchery fish are in sub-optimal health before release, they are less likely to survive in the wild after release (Florida Department of Environmental Protection, 1995). Upon release, they may be compromised by parasites commonly found in and on wild fish. When artificial foods constitute a principal component of the diet of hatchery-reared fish, slow adaptation, or a failure to adapt, to the natural prey available in the various release habitats may affect the health or survival of those fish after their release. Any of these factors could influence the survival of the red drum prior to and after release and thereby affect the validity of the experiment. Therefore, the health history of each broodstock individual and hatchery-reared brood must be routinely monitored and documented throughout the breeding and rearing process to ensure with the highest probability possible that the fish are pathogen-free when they are released. Where appropriate, fish are treated prior to release to remove pathogenic parasites and to minimize the risk of parasite transfer to wild stocks (Landsberg *et al.*, 1991). Equally importantly, the admixed population must be routinely monitored before, during, and after release to evaluate any effects that the stock enhancement endeavor may have on the stocked fish or the recipient wild population.

The AHG staff developed an extensive health-screening protocol for evaluating fish. They grossly examine subsamples of each brood for signs of physical abnormalities, mechanical damage or disease. Using compound microscopes, they examine the body surface, gills and internal organs for parasites. They also culture a sample of the posterior kidney for bacteria. The AGH staff applied this protocol to wild red drum from Tampa Bay prior to the initiation of the stock enhancement experiment. They currently apply it to both hatchery-reared and wild red drum in each phase of growth collected from the admixed population by FIM during their post-enhancement sampling. The AHG staff objectives are to presume all fish healthy and pathogen-free at the time of release, to document changes in the health of stocked red drum as they adapt to the wild, and to compare the health and pathogen levels between stocked and wild red drum in the same cohorts and between the admixed and pre-release red drum populations.

#### *Laboratory Protocol*

The AHG staff monitors the ectoparasites *Ambiphyra* sp., *Amyloodinium ocellatum*, *Trichodina* sp., *Trichodinella epizootica*, and *Ergasilus* sp. and the endoparasites *Scolex polymorphus* and *Ceratomyxa* sp. These parasites are common to both wild red drum from Tampa Bay and hatchery-reared red drum from SERF. To determine the presence of gill parasites, the first left gill arch of each fish is excised and examined microscopically. The filaments are then cut from the gill arch and closely examined. Skin scrapes, obtained by scraping a glass coverslip along the length of the body, including the fins, are also examined microscopically for the presence of any external parasites. To examine the internal organs for the presence of parasites, the viscera are removed intact and fresh squash preparations from the liver, kidney, spleen,

anterior and posterior intestine, cecae, and gall bladder are viewed with compound microscopes (Landsberg *et al.*, 1998).

The AHG has identified and routinely monitors the bacterial flora of SERF hatchery-reared red drum both before and after their release and of wild red drum from Tampa Bay, including the Alafia River. To determine the presence and types of bacteria in the fish, a microbiologist using sterile techniques samples the posterior kidney. The presence of bacteria in the kidney is indicative of a systemic bacterial infection.

All tissues and organs evaluated for parasites and bacterial infections are also examined for other types of obvious physical abnormalities, mechanical damage, or disease. The fullness of the gall bladder and color of the bile are noted, as is the relative amount of mesenteric fat. The condition-factor (fish weight [gm] / fish length [mm SL<sup>3</sup>] X 10<sup>5</sup>), hepato-somatic index (liver weight / body weight X 100), and, if appropriate, gonado-somatic index (gonad weight / body weight X 100) are determined because they are indicators of the overall health and robustness of the fish. A portion of the liver is fixed in paraformaldehyde, embedded in plastic, sectioned (3- $\mu$ m thickness), and thionin-stained in the FMRI histology laboratory for evaluation. Comparative percentage concentration of liver lipid is determined by gravimetric assay. The severed head, labeled according to collection-site designation, is provided to FIM staff to check for the presence of a CWT and extract the tag if it is present. Tissue samples or fin clips with appropriate collection data are provided to the BGL for genetic-tag analysis.

#### *Sampling Protocol*

Baseline health information on red drum has been collected during three projects: (1) a long-term AHG fish-health program for all species cultured at SERF, (2) a comprehensive study of the health of the Tampa Bay red drum population conducted in 1992-1993, which resulted in the development of the above protocol, and (3) an intensive sampling effort in the Alafia River for wild red drum, conducted by FIM staff during the two-week period prior to the first release of hatchery-reared red drum in early 2000. In the present stock-enhancement experiment, the FSE staff closely monitors red drum rearing to ensure good health. If problems occur, the AHG staff is immediately notified and investigates potential sources of the problem. One week to ten days prior to harvest, red drum samples are collected for independent health certification by the University of Florida's Institute of Food and Agricultural Sciences Tropical Aquaculture Lab (Ruskin, FL). At that time, a random sample of ten fish from each brood is also collected and evaluated by the AHG staff. The AHG staff evaluates another random sample of ten fish from each brood on the day of harvest and release. If the harvested fish are held longer in tanks for tagging, grading, acclimation, or health reasons, yet another random sample of ten fish is evaluated on the day of that release. The data obtained on that day are compared to previous data to check for changes in the overall health status of the fish during the stressful period of harvesting, tagging, and transportation. Fish that do not meet minimal health criteria are not released. These criteria include evidence of internal or external bacterial infection, detection of levels of parasites higher than normally found in or on wild red drum, low condition-factor, and the presence of external lesions or abrasions. The FSE attempts to correct the health problem and the AHG monitors the health of these fish until the problem is corrected and the fish are released.

Twenty-four hours after the fish are released, the FIM staff examines the fish from the net pens to monitor short-term tag retention and survival of the stocked fish. If mortality exceeds 5%, the AHG staff fully evaluates a sample of both moribund and apparently healthy fish using the laboratory protocol described above.

Shortly after each release of hatchery-reared fish, FIM collects a post-enhancement sample of hatchery-reared and wild red drum from the admixed population in the vicinity of the release area. These fish are evaluated for selected health criteria and to determine if the stocked individuals are more susceptible to wild pathogens and parasite infestations than are the wild fish. The captured fish are maintained alive and the sample from each collection is held in a separate container until they are processed. A maximum of ten red drum per grid, per sampling event (i.e., 40 fish per day) is collected for evaluation. The FIM or BGL staffs inform the AHG staff of the origin of each fish (wild or hatchery-reared) after they test for the presence of a CWT or genetically identify the individual.

The AHG staff uses the baseline red drum health data and the data obtained during this experiment to document the changes in the stocked red drum as they adapt to the local environment and the health effects of the stock enhancement experiment on the admixed red drum population. In the future, red drum that have entered the Tampa Bay fishery and are returned via FIM collections or FDM angler intercepts will be evaluated as is appropriate to assess the long-term health implications of stocking red drum into Tampa Bay.

### **Effort Involved in a Stock Enhancement Experiment**

Perhaps the most unexpected surprise in conducting this stock enhancement experiment has been the tremendous amount of effort and coordination among relatively independent research groups that is necessary. In the 2.5 years since the initiation of this experiment, each group has put forth the effort and obtained the information described below.

In addition to the approximately 600 YOY wild red drum analyzed for both the mtDNA and microsatellite components of the genetic tag, the BGL staff has analyzed the mtDNA component of approximately 2,000 red drum from FIM staff post-enhancement collections and 250 red drum from MML staff post-enhancement collections. Of these individuals, the BGL staff has analyzed, for the battery of microsatellite loci, 230 red drum with mtDNA genetic-tag genotypes that matched those of broodstock mothers. Because most hatchery-reared red drum are released in Phase I, the total number of red drum analyzed for this experiment is expected to double. To reduce the effort and time involved in genetic tag analysis, the BGL staff recently eliminated the mtDNA component and organized the microsatellite analysis in such a way that a preliminary analysis of four loci in one multiplex reaction can be used to screen for the origins of individuals with about 90% accuracy. Those with genotypes consistent with hatchery origin are further evaluated for the remaining five loci, again in multiplex reactions. This strategy eliminates the need to separate broodstock females according to the rarity of their mtDNA haplotypes. Because one goal of this experiment is to measurably increase the average catch of red drum in an angler's creel beyond the pre-enhancement level (i.e., the level that existed when the Tampa Bay fishery was based solely on wild fish), this genetic analysis will continue for a number of years after the stocking component of the experiment is completed.

In seven separate release events during 2000-2001, FSE released approximately 1,242,000 hatchery-reared red drum into the Alafia (334,000 fish) and Little Manatee (910,000 fish) rivers. These fish were spawned from 15 different broodstock groups composed of a total of 28 females and 34 males. Approximately 344,000 and 800,000 fish were released in 2000 and 2001, respectively. Of these, over 1,150,000 were released as Phase-I red drum (242,000 into the Alafia River and 900,000 into the Little Manatee River). Approximately 45,000 of the 62,400 red drum reared to Phase II were released in into the Alafia River in 2000 and nearly 22,700 of the 30,000 red drum reared to Phase III were released into the Alafia River in 2001. The research plan states that 1,360,000 red drum per year will be released, in the ratio of 88% in Phase I, 9% in Phase II, and 3% in Phase III. These are projected numbers of fish. Of course, practical challenges of various types influence the actual number and phase of the fish released.

In fulfilling its obligation to collect an adequate number of red drum from Tampa Bay in pre- and post-release samples, the FIM group has made a total of more than 850 seine hauls in the two rivers targeted as release sites. Of those, approximately 80% were conducted in the Alafia River, one-third with the 21-m haul seine and two-thirds with the 61-m haul seine. The FIM staff has captured a total of approximately 2,000 red drum, almost 150 of which were tagged with CWTs. The FIM directed-sampling program was initiated in 2001, when FIM staff anticipated that stocked hatchery-reared red drum had grown large enough to easily avoid haul seines. In the Alafia River and adjacent Tampa Bay waters during 2001, FIM staff sampled nearly 40 sites using trammel nets and more than 125 sites using rod-and-reel gear. Also during 2001, the FSE and MML staffs U-tagged and released more than 90 sub-adult red drum in three groups of fish. The FIM and MML staffs made nearly 150 field trips to search for these fish. Many of the U-tagged red drum were collected by these scientists and several more were caught by recreational fishermen in several areas of Tampa Bay.

Since October 2001, the MML staff has conducted nearly 200 21-m haul seines as part of their assessment in the Little Manatee River. These samples contained a total of 320 red drum. Similar to the Alafia River collections made by FIM staff, most of the red drum were collected from upriver portions of the study area. From their fisheries-independent sampling effort in the Little Manatee River, MML staff has provided the BGL staff with more than 325 fin clips from captured red drum. Since the initiation of the targeted effort to obtain information on the red drum fishery and find U-tagged fish in anglers' catches, MML staff has made more than 27,000 angler intercepts in the Tampa Bay region and has obtained catch data from 722 anglers. Over 500 red drum were tested for U-tags.

The AHG staff has evaluated nearly 1,000 red drum (more than 300 hatchery-reared fish, 200 stocked fish, and 300 wild fish) for target-parasite prevalence, condition-factor, and hepatosomatic indices. Approximately 9,000 fresh squash preparations of fish tissue have been examined microscopically for parasites and tissue abnormalities. Posterior kidneys from more than 900 fish have been cultured to search for systemic bacteria infections. More than 900 samples of liver from red drum were histologically prepared for microscopic evaluation. Of these, approximately 500 will be evaluated for lipid content.

Of course, a stock-enhancement experiment of this magnitude requires substantial personnel involvement of both full- and part-time employees and, in this case, also recreational anglers. To conduct the genetic analysis and maintain the genetics database, three full-time and three half-time staff members work on various components of the project. To manage the broodstock and rear the broods, 15 full-time FSE staff members are involved. To conduct the pre- and post-enhancement fisheries-independent field collecting and U-tag tracking, the FIM

program uses four full-time staff members. To query anglers and obtain fishery-related information on the red drum harvest in the Tampa Bay region, the FDM program uses the equivalent of four full-time staff members. However, only three of these individuals are dedicated to obtaining information on the red drum fishery. All others obtain information on red drum as a component of general angler-intercept surveys. To monitor the health of the red drum at SERF and evaluate the health status of fish captured in the pre- and post-release samples provided by the FIM staff, four full-time and one half-time AHG staff members work in the laboratory and one full-time staff member works at SERF. In addition to the various hatchery, field, and laboratory personnel, a total of twelve supervisors work part-time to oversee and manage the project.

### **Stock Enhancement Monitoring-Worth the Effort ?**

Wild-population fisheries are seriously declining worldwide (Botsford, *et al.*, 1997; Vitousek, *et al.*, 1997; Pauly, *et al.*, 1998). Three methods are commonly used to attempt the replenishment of depleted stocks: regulating fishing effort, restoring habitats critical to one or more life stages of the stock, and artificially supplementing the reproductive population through restoration or enhancement programs (Leber and Lee, 1997). Stock restoration or enhancement is gaining increased popularity and is practiced at various levels worldwide, but it is generally not closely monitored or evaluated (Welcomme and Bartley, 1998). A major problem in justifying the expense and effort associated with stock enhancement is determining if it is successful. Leber (1999) points out that success has typically been measured by production levels and numbers of fish stocked. However, the success of a stock enhancement endeavor should be evaluated according to the goals of the project. The goals are often defined as the measurable contribution to the fishery or to the reproductive population. Leber (1999) states that in addition to estimating the increase in the size of the enhanced population or the increase in its reproductive output, the focus also should be on determining if the stocked fish are simply replacing the wild fish.

The emphasis on production as the principal measure of success has been maintained because after hatchery-reared fish are released, it is difficult – or, when the stocked fish are released as eggs, larvae, or small fry, it is impossible – to track the stocked fish or to distinguish them from wild fish. Various methods of estimating the success of stock-enhancement efforts have been used. For example, to estimate the contribution of stocked brown trout (*Salmo trutta*), researchers in Denmark used shifts in the frequencies of ‘local’ native mtDNA genotypes over time and comparisons of the frequencies of local versus non-local mtDNA genotypes in rivers undergoing stocking with those frequencies in rivers that had been stocked at different times in the past (Ruzzante *et al.*, 2001). Researchers in Hawaii used increases in the percentage of Pacific threadfin (*Polydactylus sexfilis*) in fishermen’s creels (Friedlander and Ziemann, in press). Japanese researchers used increases in catch statistics versus number of ‘seeds’ released to measure bay scallop (*Argopecten irradians*) stocking success (Kitada and Fujishima, 1997). However, none of these methods unambiguously define the level of contribution that stock-enhancement efforts have made, nor do they demonstrate that the methodology used in releasing the fish provides the hatchery-reared fish the best opportunity for survival.

Release conditions are clearly important in determining the survival of stocked fish. To maximize the probability that stocked fish survive until they contribute to the ultimate objective of the stock-enhancement project, the best release conditions must be known and followed (Leber, 1999). Size-at-release, location of release, and timing of release can each influence

survival of the stocked fish, and these factors can all work synergistically to influence survival (Stoner and Davis, 1994; Leber *et al.*, 1996; Leber *et al.*, 1998; Leber, 1999). The best stocking conditions can be determined only through an experimental approach in the initial stages of the stock enhancement endeavor. In addition, other factors--such as the similarity or dissimilarity between the genetic composition of the stocked fish and the recipient wild-fish population, the health of the stocked fish at the time of release, the degree of handling-induced stress, and the carrying capacity of the environment for the targeted fish species--all have influenced the success of stock enhancement programs (Vea Salvanes *et al.*, 1995; Bell and Gervis, 1999; Kuwada, *et al.*, 2000; Ashford and Danzmann, 2001; Fushimi, 2001; Rasmussen and Geertz-Hansen, 2001).

In the Tampa Bay red drum stock-enhancement program, we have attempted to consider all of these factors in rearing and releasing red drum, and through our genetic-tag- and CWT-based monitoring programs, we should be able to estimate the contribution of stocked red drum to the fishermen's creel. Finally, our detailed accounting of expenditures throughout the rearing process and documentation of the contribution of the stocked fish to the creels of fishermen should enable us to estimate the per-fish cost of this stock-enhancement project. Few marine stocking programs have been monitored for their economic success (Hilborn, 1998).

Studies such as the one described here are huge in scale, are complex, and require the coordination of many diverse research groups. Ideally, all stock enhancement projects would incorporate the research components described here. Obviously, that is impossible for smaller-scale stock-enhancement endeavors. Nevertheless, experimentation with release conditions and attention to the culture conditions, genetic composition, and health of the stocked fish should always benefit a stock-enhancement effort. Because stock enhancement will probably continue to increase in popularity as a remedial method for supplementing depleted fish stocks, this approach could be subjected to increased scrutiny for both its ecological and genetic impacts on wild populations and its economic cost-to-benefit ratio. Thus, the experimental approach to stock enhancement will become increasingly important.

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