How Advances in Tagging Technology Improved Progress in a New Science: Marine Stock Enhancement

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Abstract.—In designing research programs, scientists may constrain development of sequential hypotheses because of perceptions about logistical constraints to using new technologies in monitoring or experimental design. Using trusted, familiar methods can supersede asking which hypotheses would have the greatest impact and what method(s) are required to test them. To help maintain a 'problem-oriented' approach, rather than a 'methods oriented' one, we could strive to remain aware of new innovations and applications in research; this is particularly so for tagging technology, when new methods emerge. Research enabled by recent innovations can be incorporated through collaborations with other scientists or by working directly with vendors to implement and refine new tag technologies and applications. Some tagging studies can be improved by using multiple marking methods (e.g. see recent applications of various tag technologies with common snook Centropomus undecimalis and red drum Sciaenops ocellatus in Florida to evaluate recruitment, mortality, and habitat use of different life stages; Adams et al. 2006; Bennett 2006; Marcinkiewicz, 2007; Brennan et al. 2008; Tringali et al. 2008). Here we consider a few case studies that have implemented a variety of tagging methods to explore poorly understood factors that mediate growth and survival and the effectiveness of hatchery releases to help replenish depleted marine fish stocks.

Introduction

A half century ago, marine hatcheries established in the USA to supplement marine fish stocks were abandoned for lack of evidence of their effectiveness in fisheries management (Richards and Edwards 1986). At that time, success was based on the number of juve-

nile fish stocked rather than on the number of adults added to the catch or spawner biomass. Worldwide declines in coastal fisheries and advances in marine aquaculture have sparked a resurgence of interest in hatchery-based marine stock enhancement (see symposium proceedings edited by Lockwood 1991; Danielssen et al. 1994; Travis et al. 1998; Howell et al. 1999; Leber et al. 2004; and Bell et al. 2008).

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Development of new marine aquaculture technologies coupled with advances in tagging technology, and greater attention to a more cost-effective, conservationorientated, and scientific approach, have fostered a better understanding of whether and how to use marine stock enhancement in fisheries management (Blankenship and Leber 1995; Howell et al. 1999; Leber et al. 2004; Bell et al. 2008; Lorenzen et al. 2010). Contemporary research in this field is more rigorously evaluating and quantifying ecological and biological impacts of hatchery releases and has begun to address critical uncertainties in stock enhancement theory (e.g., Bell et al. 2008; Lorenzen 2008; Lorenzen et al. 2010). Advances in tagging technology have underpinned many of the scientific advances made in this field in the past two decades.

Over 20 years has now passed since the American Fisheries Society convened the June 1988 "International Symposium and Educational Workshop on Fish-Marking Techniques" at the University of Washington in Seattle, which resulted in the useful publication, Fish Marking Techniques (Parker et al. 1990). One of the most lasting impressions from that symposium was the overview talk presented by Ray Hilborn, Carl Walters and Douglas Jester on the "Value of Fish Marking in Fisheries Management" (Hilborn et al. 1990). In this, they made three important observations:

- that World Fisheries Production had leveled off and that total catch would not rise much above 1988 levels (80 million metric tons per year);
- that aquaculture would play an increasingly important role in fish production; and
- that recreational fishing was beginning to exceed commercial fishing in value in North America

Of their five challenges to fisheries managers, one in particular—the use of aquaculture production for enhancement and ocean ranching of fishes—helped stimulate new research on the benefits and risks of marine fisheries enhancement. To highlight the challenges facing those trying to use enhancement effectively, we note that in 1988 there were no previous publications in peer-reviewed journals on the effects or effectiveness of stocking fish that spawn in the sea. Over the next two decades, aided largely by advances made in tagging technology, marine stockenhancement research began to develop an old idea into a new kind of science.

For over a century, two issues had retarded scientific discovery in marine stock enhancement—the small sizes (eggs, yolk sac larvae and postlarvae) of cultured marine organisms being released (e.g., Atlantic cod Gadus morhua; haddock Melanogrammus aeglefinus; Pollock Pollachius virens; flounder Plueronectes americanus, and Atlantic mackerel Scomber scombrus, Richards and Edwards 1986), and lack of adequate marking methods for evaluating the fate of stocked juvenile fishes (Blankenship and Leber 1995). But Keith Jefferts and Pete Bergman had solved the lack of marking technology for small fishes 21/2 decades before this symposium, when they developed the coded wire tag (CWT) system (Jefferts et al. 1963); and both Hager and Noble (1976) and Bilton et al. (1982) had published results of using this system in mark-recapture experiments with salmon. However, lacking a comprehensive reference work on modern tagging methods, most marine aquaculturists and many fishery scientists had no idea how to effectively mark small juvenile fishes. Thus the 1988 AFS symposium in Seattle empowered a generation of field biologists by enhancing awareness of new developments in fish marking methods.

Recognizing this and the need for an update, the conveners of the latest AFS marking

symposium, held in Aukland, New Zealand in 2008, are now helping to stimulate new research by compiling examples of studies aided by the variety of marking technologies available today (this volume). The progress in electronics, computer technology, and genetics over the past twenty years has enabled great advances in fish tagging technology. Examples include genetic tags or markers using microsatellite DNA, antennae systems for passive integrated transponder tags, and archival tags that record temperature, depth, longitude and latitude; acoustic tags have been miniaturized and receivers can be spaced across vast areas for large scale recovery; advances in parental genotyping and automated mass marking machines have enabled millions of hatchery fish to be identified so interactions between hatchery and wild counterparts can be monitored. These tools are providing biologists and managers the technology needed to gain further insight into the biology and ecology, survival, and migratory behavior of our fisheries resources.

This paper highlights some of the research advances that have been made by incorporating various tags and marks in studies of hatchery releases into the sea; moreover, we discuss how combining multiple tag innovations is enabling more rapid progress in understanding how to use an undeveloped fishery management tool—marine stock enhancement

Identifying Optimal Release Strategies

Size-at-release (SAR), release habitat and microhabitat, release season, acclimation, and density dependence are key factors that affect survival of stocked fish and need to be evaluated prior to large scale tests of stock enhancement (Leber 1999). But to do the empirical experiments needed to evaluate these factors, a marking system capable of

providing high information content is needed to identify experimental treatment and control groups of hatchery fish.

The CWT had been used to examine size and season effects on hatchery-released smolt survival in salmonids (Hager and Noble 1976; Bilton et al. 1982), but prior to the 1988 AFS Symposium, biologists working with marine spawners seemed largely unaware of this tag or experienced low and declining tag-retention rates, in part because the tag was not suitable for the small size of individuals being stocked (<50 mm) (e.g., Gibbard and Colura 1980; Bumguardner et al. 1990, 1992). However, immediately following the Seattle symposium, biologists in the USA began to evaluate release strategies with juveniles of several marine fishes including red drum Sciaenops ocellatus (Willis et al. 1995), striped mullet Mugil cephalus (Leber 1994, 1995; Leber et al. 1995), Pacific threadfin Polydactylus sexfilis (Leber et al. 1998) and white seabass Atractoscion nobilis (Kent et al. 1995).

Those researchers enlisted the assistance offered by Washington Department of Fish and Wildlife (WDFW) biologists Lee Blankenship and Ray Buckley, and with their assistance launched a series of tag-retention experiments that resulted in successful adaptation of coded-wire tags (CWTs) to juvenile life stages of all of those fishes. This empowered these research groups to move rapidly into testing release strategies and evaluating the effectiveness of stocking. A series of CWT retention tests using trial-and-error coupled with scanning EM photography was used to select adipose tissue in the snout as the target site for tagging juvenile striped mullet (Oceanic Institute 1990) and cheek muscle as the target site for red drum and white seabass.

In Hawaii, striped mullet juveniles were graded into five size intervals and in summer 1989, only a year after the Seattle symposium; 10,000 were tagged and released into Maunalua Bay, followed by 85,000 more

in 1990 into Maunalua and Kaneohe Bays in Hawaii (Oceanic Institute 1990; Leber 1995). Field sampling followed, using seines and cast nets to monitor the releases, which had been made in open ocean habitats. Recapture results were outstanding. Tag retention averaged 98.6% (97.3-99.6% in five size groups ranging from 45 mm to 130 mm total length [TL]) and biologists recovered 277 tagged hatchery juveniles (4.6% of the mullet collected) in Kaneohe Bay and 456 in Maunalua Bay (15.5% of the mullet collected there) over 46 weeks following the releases in 1990 (Leber 1995). The tag data showed that SAR had a clear and significant effect on recapture rates, with very few of the smaller fish released (45-60 mm TL) recovered after nine weeks at liberty. Critical size for release-recapture experiments in those habitats during summer months was 70 mm TL (Leber 1995).

Thus began a series of factorial, randomized-block, CWT release-recapture experiments designed to gain a better understanding of SAR effects in multiple habitats and the interactive effects of release season and SAR on survival of stocked juvenile striped mullet (Leber et al. 1996, 1997). Based on data recovered from coded wire tags, those experiments showed a clear effect of the seasonal timing of releases on SAR effects, and revealed that pilot-scale releases had actually doubled abundance of striped mullet in nursery habitats. A subsequent evaluation of whether released hatchery mullet were displacing wild mullet at release sites showed that the hatchery mullet were adding to production in nursery habitats, not depressing it (Leber et al. 1995). Subsequent evaluations of the contribution rates of released hatchery fish showed that hatchery fish were providing 15% of the catch in a subsistence mullet fishery in Kaneohe Bay (Leber and Arce 1996). In that study, a sampling program designed to recover cultured mullet from the mullet fishery resulted in the recovery of 214 tagged adult hatchery fish from fishermen. The effects of SAR on recapture frequencies were determined based on CWT information in the recaptured hatchery fish. After teaming with a resource economist, subsequent work with the Leber and Arce (1996) data set revealed that the economically optimal size-at-release, based upon production costs and yields in the fishery of the various SAR groups of hatchery fish released, was 91 mm TL at one release site. 98 mm at another (Leber et al. 2005). The one critical factor we could not evaluate was the effectiveness of releases of large numbers of very small mullet (postlarvae). No marking system with an adequate number of individual codes or batch marks was available at the time for identifying postlarval fishes. Thus, evaluating survival of stocked postlarvae was put on hold for lack of an adequate marking system for distinguishing experimental treatment conditions and replicates in releases of such a small life stage.

Following the development at Oceanic Institute of new aquaculture technology for Pacific threadfin, WDFW biologists helped the Hawaii team adapt CWT technology to this fish and a trial release was conducted with 20,000 tagged juveniles released along the sandy beaches on the Northeast coast of Oahu, Hawaii. In one large experiment, 81,000 Pacific threadfin juveniles were tagged and released to investigate the interactive effects of release season, release habitat, and SAR (Leber et al. 1998). That work provided conclusive evidence that optimal SAR for juvenile Pacific threadfin was dependent upon both release season and release habitat and that habitat alone could cause a complete reversal in size-at-release effects on recapture rate. Subsequent research using CWTs revealed that hatchery-released Pacific threadfin made significant contributions to the fishery on Oahu, HI (Friedlander and Ziemann 2003; Ziemann 2004).

Meanwhile, researchers in Florida and California were also able to successfully apply CWTs in investigations of red drum and white seabass stocking effectiveness and release strategies. Willis et al. (1995) recovered 821 hatchery-released red drum. Results at fixed sampling stations were consistent with the Hawaii results, revealing a large effect of both SAR and release season upon survival of stocked juvenile red drum. The white seabass research in California (Kent et al. 1995; Hervas et al. 2010) revealed similar findings showing a strong relationship between SAR and survival and a clear release-season impact.

The findings in California, Florida, and Hawaii with several species of marine fishes corroborate earlier research results with marine fishes from releases of relatively large juvenile cod marked with external tags in Norway (Svåsand and Kristiansen 1990) and very small juvenile red sea bream Pagrus major in Japan, marked with alizarin complexone (Tsukamoto et al. 1989). However, the later studies also revealed significant interactive effects of release strategies (SAR, release season, release habitat) and that significant contributions of marine hatchery fish could be made to fisheries only by optimizing release strategies, which could not have been done with small juveniles without the aid of a high-information content, benign tag.

As more recent advances have been made in CWT technology, additional applications are now possible, for example mass marking to identify every stocked salmon in a river system, which enables better management of mixed stock fisheries (Vander Haegen et al. 2011, this volume).

Addition of a Visible Tag to the Tagging Toolkit

Clearly, much information can be gained using CWT systems alone as a marking tool, and this became one of the "tags of choice"

for many stock-enhancement researchers in the 1990s and beyond as research on release strategies expanded to other species of marine organisms, e.g. red snapper *Lutjanus campechanus* (Blaylock et al. 2000), swimming crab *Portunus trituberculatus* (Okamoto 2004), barramundi *Lates calcarifer* (Russell et al. 2004), winter flounder *Pseudopleuronectes americanus* (Fairchild et al. 2005), mandarin fish *Simperca chuatsi* (Zhang and Li 2007), and blue crabs *Callinectes sapidus* (Zohar et al. 2008)

Coded wire tags are usually not externally visible and require lethal removal to recover the code (Oven and Blankenship 1993). In Florida, researchers studying hatchery releases of common snook Centropomus undecimalis needed to incorporate an externally visible tag, because the study design required multiple recaptures of small juveniles across seasons and life stages and the ability to distinguish between hatchery fish and wild fish. So laboratory and in situ studies of tag retention were initiated in snook, using CWT and color-coded elastomer implants to identify treatment groups of stocked juveniles (Brennan et al. 2005). Using both tag systems, researchers could evaluate juvenile hatchery snook growth, survival, and interactions with wild stocks across a range of release sites and microhabitats in Sarasota Bay. Brennan et al. (2006) also discovered that caging the released hatchery snook for three days in *in situ* enclosures at release sites actually doubled subsequent survival rates over a 12-months period following release from cages (Brennan et al. 2006). This effectively cut the rearing costs of survivors in half, to the delight of resource economists. Subsequent studies revealed dynamic effects of release habitat on growth and survival (Leber and N.P. Brennan, Mote Marine Laboratory, unpublished data).

Elastomers and CWTs were also used in tandem to test a key question in stock en-

hancement—whether released hatchery fish displace wild fish and thus provide no additive gain in biomass. This analysis found that juvenile hatchery snook were themselves displaced by wild snook, even as surviving hatchery snook contributed to increased production in nursery habitats without affecting densities of wild snook (Brennan et al. 2008), and suggested that a key survival bottleneck had been avoided by size escape from competitive exclusion (the stocked hatchery snook were ~ age 1).

Addition of PIT Tags to the Tagging Tool Kit

To evaluate movement patterns among release sites and microhabitats additional tagging technologies were needed. To monitor movements throughout 24 h, many researchers have implemented transmitting tags and receivers. Juvenile snook are too small at this stage for application of most transmitter tags and acoustic tags. A breakthrough for freshwater researchers working with juvenile fishes came when Zydlewski et al. (2001) documented use of an autonomous antennae system to monitor passive integrated transponder (PIT) tags in Midwestern freshwater systems. After working with the Zydlewski team to learn how to design and build the detector system, Adams et al. (2006) were able to adapt this system for use in brackish water habitats to monitor snook movement patterns in and out of nursery systems in Charlotte Harbor, Florida. Their studies have determined weekly apparent survival rates that will be invaluable in comparing juvenile habitats of different quality (Adams et al. 2006). Snook nursery habitats were small streams and creeks and these systems afforded great locations for implementing the automated PIT tag detection systems.

PIT tags have long been used to evaluate

survival of salmonids, and new applications are constantly emerging (e.g., Bryant et al. 2011, this volume; Ostrand et al. 2011, this volume; van den Broek et al. 2011, this volume).

Addition of Acoustic Tags to the Tagging Toolkit

Following the work on snook in their juvenile nursery habitats, Adams et al. (2009) coupled PIT tags with acoustic transmitters and detectors to study movement patterns and site fidelity of sub-adult and adult snook on their spawning grounds in Charlotte Harbor, Florida, and found very high site fidelity and annual return rates. Also using sonic transmitters and detectors, Pine and colleagues evaluated survival, movement and habitat utilization by snook both inside and outside of their nursery habitats in Sarasota Bay, Florida (Pine et al. 2007; Marcinkiewicz 2007). Their work evaluated habitat use and movement patterns of adult snook in seasons with and without large-scale environmental (red tide blooms) and anthropogenic (dredging) disturbance events. The sonic tags afforded a method for revealing habitat utilization patterns and habitat disturbance effects that could not be approached with other methods. Adams et al. (2009), using sonic tags, PIT tags and external dart tags, found snook homing to spawning ground behavior in Charlotte Harbor at a much smaller scale than ever expected for a broadcast spawner; Pine et al. (2007) and Marcinkiewicz (2007) found variable site fidelity in Sarasota Bay, revealing substantial differences between these two bay systems.

Acoustic tags and receiver arrays have expanded the horizon in studies of fish movement and habitat use patterns, as no fewer than eight papers in the present volume can attest.

Utility of Adding Genetic Fingerprinting to the Tagging Toolkit

For all the answers gained researching snook, additional studies are needed to address many challenging and yet unanswered questions. What about the relative effectiveness of stocking large numbers of small postlarvae or very small juveniles in comparison with stocking larger siblings, an issue that had not previously been examined quantitatively? And what effect might stocking very small fish have upon wild snook at release sites? The tag technology needed to resolve these questions has now emerged in the form of genetic fingerprinting. In collaborations with biologists at Florida Fish and Wildlife Conservation Commission's Fish and Wildlife Research Institute (FWRI), we have used new genetic markers to investigate survival and performance of hatchery-reared postlarval red drum stocked into Tampa Bay, Fl. Through our collaboration with FWRI, genetic fingerprinting is now being adapted for use with snook to evaluate the effects and effectiveness of stocking ontogenetic stages too small for other high-information content tags or marks. Although less flexible than CWTs, which we'd used to identify as many as 80 different batch marks in a single experiment (Leber et al. 1998), genetic fingerprinting is a useful method for batch marking all life stages (eggs through adults) for as many experimental treatment and control conditions as can be accommodated by isolating and rearing siblings in separate tanks or ponds. Through the collaboration with FWRI in Florida, we identified 34 discrete spawning groups, which afforded 34 different experimental treatment conditions in the Tringali et al. (2008) studies of red drum. This afforded identities for fish released at 3 different SAR stages, in 4 different release habitats (river miles) and replicated over 5 years in a randomized-block

design experiment. Based on microsatellite DNA identities of offspring from red drum broodfish matured and spawned in the FWRI hatchery, FWRI biologists have now documented that stocking red drum postlarvae can be as economically efficient as stocking larger (better surviving) juveniles, but only in certain habitats (only one of the two rivers examined) and at certain times of year (Tringali et al. 2008).

Rapid advances are being made in genetic marking systems and one of the latest is close kin matching, which has enabled even tracking of fish across generations (e.g., Bravington and Grewe 2011, this volume).

Discussion

Today's fisheries scientists have tagging tools in their arsenal that now enable research not even feasible in 1988. Tagging systems and innovations have resulted in technologies that are smaller, 'smarter,' more automated, more reliable, and longer-lasting since the 1988 AFS marking symposium. Revolutionary advances in fish tagging and marking technology continue to be made. With each new innovation, seemingly another monitoring breakthrough or logistical constraint to experimentation is solved.

As these innovations occur, it is easy to become attached to the tagging systems that have most enabled research in our own specific arenas. We must not lose sight, though, of the new innovations that are occurring, giving rise to new tag systems. As Platt reminds us in his seminal and still pertinent paper, "Strong Inference" (Platt 1964), we must keep our research focused on 'problem solving;' we must resist becoming 'method oriented' to the point where we miss opportunities to test some of our highest-priority hypotheses. In designing research programs, scientists often and unwittingly constrain development of sequential hypotheses through awareness of lo-

gistical constraints inherent in monitoring or experimental design. It is tempting to use familiar methods we trust and ask "which questions can I solve with this method?" rather than asking "which hypotheses would have the greatest impact in my research program and where can I find the method(s) I need to test them?" Scientific fields of research that stay 'problem oriented' make much more rapid progress than those fields that become more 'method oriented' (Platt 1964). To help maintain a 'problem-oriented' approach in our science, we must strive to stay aware of innovations in tagging methods. By the time most of us realize from a journal article or symposium presentation the hot new research directions enabled by a recent innovation, we have long since planned research hypotheses and written grant proposals based on familiar methods. Awareness of tag innovations is enhanced by collaborations.

In these proceedings, there are many tag technologies available, each tailored to different kinds of applications. Stock enhancement studies can clearly be improved by using multiple marking methods. So, too, can many other fields of research. Integration of CWTs, elastomers, genetic fingerprinting, hydro-acoustic tags and PIT tags were needed in the case studies presented here, to explore poorly understood ecological issues and their effects on movement patterns, site fidelity, release strategies, stocking effectiveness, and density dependence in stock enhancement.

Technological bottlenecks have severely hindered development of the science needed for evaluating effectiveness of hatchery releases over most of the past century. Several of the case studies considered here were enhanced by advances in tag technology. Coded wire tag studies helped resolve many size-atrelease, release-site and release-season issues. But it took the combination of CWTs, PIT tags, sonic tags, and genetic fingerprinting to resolve the other pressing research questions: survival of stocked postlarvae

relative to larger juveniles, density-dependent effects on survival, juvenile habitat effects on movement patterns and site fidelity of spawning populations, and the relative effects of mortality versus dispersal in explaining abundance declines following juvenile recruitment. None of these issues had been examined during nearly a century of stocking fishes, before the development of modern benign tagging technology in the 60s and 70s for evaluating salmonid enhancement. Now, a century after stocking postlarvae began, several critical uncertainties in the field of fisheries enhancement are finally being addressed. including one of the most elusive—survival of postlarval fishes, enabled by advances in tag technologies.

The papers published in these proceedings bring us up to date on the technologies now available for monitoring fish movement, survival and habitat use in their environment. In this paper, we have shown examples of the utility of combining multiple tag systems and stressed the need to keep aware of new technologies as they become available. We note that it was primarily through collaborations and symposia like this one that we gained the insight and experience needed to rapidly incorporate additional tag systems in our research, which enabled much more rapid progress than we would otherwise have made.

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