

CHAPTER 18

Stock Enhancement of Mugilidae in Hawaii (USA)

Kenneth M. Leber,^{1,*} Cheng-Sheng Lee,² Nathan P. Brennan,¹
Steve M. Arce,² Clyde S. Tamaru,³ H. Lee Blankenship⁴ and
Robert T. Nishimoto⁵

Introduction

Aquaculture-based marine fisheries enhancements have a long history, dating back to the late 19th century when releasing cultured fry into the marine environment was the principal fishery management tool. Stocking fish eggs and larvae was regarded as the way to save what was generally perceived as a declining resource, the causes of which were not well understood. By the early decades of the 20th century, billions of unmarked, newly-hatched fry had been released into the coastal environments (Radonski and Martin 1986). In the United States, Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), pollack (*Pollachius virens*), winter flounder (*Pseudopleuronectes americanus*) and Atlantic mackerel (*Scomber scombrus*) were stocked (Richards and Edwards 1986). No attempt was made to evaluate stocking strategies and success was measured by numbers released rather than numbers surviving. By the early 1930s, after a half century of releases had produced no evidence of an enhancement impact (except for some salmonid stocking programs), stocking programs were largely curtailed in the US and harvest management was established as the principal means to manage marine fisheries. In the 1980s, some states in the US began new stock enhancement programs, following advances in marine fish culture and fish tagging technologies. Most of these new programs were established primarily for research on the efficacy of marine stock enhancement, with a goal of developing more effective stock enhancement strategies.

Efforts to enhance marine fisheries are limited to relatively few marine species. Except for stocking of salmon in the US Pacific Northwest, Japan and China have the largest hatchery-based marine fisheries enhancement programs. Norway began releasing tagged, hatchery-raised Atlantic cod (*Gadus morhua*) in 1983 (Svasand et al. 1990). These efforts were followed in the 1990s and beyond by numerous additional stocking programs around the world, many of which are now chronicled in the proceedings of the International Symposium on Stock Enhancement and Sea Ranching (ISSESR) (see www.SeaRanching.org).

¹ Directorate of Fisheries and Aquaculture, Mote Marine Laboratory, Sarasota, FL 34236 USA.

² The Oceanic Institute of Hawaii Pacific University, Waimanalo, HI 96795 USA.

³ College of Tropical Agriculture and Human Resources, University of Hawaii, Honolulu, HI 96822 USA.

⁴ Director of Biological Services, Northwest Marine Technology, Inc., Shaw Island, WA 98501 USA.

⁵ Hawaii Division of Aquatic Resources, Hilo, HI 96720 USA (Retired).

* Corresponding author: KLeber@mote.org

The science underlying enhancements is relatively recent. There were no published accounts of the fate of stocked fishes until empirical studies of anadromous salmonids appeared in the mid-1970s (Hager and Noble 1976, Bilton et al. 1982), followed by the first published studies of stocked marine invertebrates in 1983 (Appeldoorn and Ballentine 1983) and marine fishes in 1989 and 1990 (Tsukamoto et al. 1989, Svasand et al. 1990). Two universal problems restricted the early development of marine stock enhancement science: 1) lack of a marking method for assessing whether hatchery releases are successful and 2) inability to culture marine fishes through the juvenile (fingerling and larger) life stage. Breakthroughs in marine finfish aquaculture technology and new benign tagging methods have led to resurgence in marine stock enhancement efforts worldwide. Emphasis is now placed on a responsible approach to stocking, emphasizing planning, fisheries management, modeling, genetics, health, pilot experiments to increase survival of released fish, evaluating contributions to wild populations and use of adaptive management (Blankenship and Leber 1995, Walters and Martell 2004, Lorenzen et al. 2010, Sass and Allen 2014). The technology has progressed to the stage where marine stock enhancement is now considered a *bona fide* fisheries management tool (Sass and Allen 2014).

The flathead grey mullet *Mugil cephalus* has a unique role in the modern development of marine fisheries enhancements. *M. cephalus* was the test species chosen for one of the first systematic series of empirical studies to evaluate effectiveness of aquaculture-based marine fisheries enhancements. Beginning in 1988, the Oceanic Institute (OI), located on Oahu, Hawaii (USA) conducted several years of experimental pilot releases with grey mullet and collaborated with the Hawaii Division of Aquatic Resources (DAR) to transfer mullet stock-enhancement technology to the state for implementation in a recreational mullet fishery in Hilo, Hawaii (Leber 1994, Nishimoto et al. 2007).

Grey mullet was also used in the Hawaii studies in the early 1990s to demonstrate the effectiveness of using pilot-release experiments to optimize release strategies; such pilot releases are a fundamental aspect of a 'Responsible Approach' to marine enhancements (Blankenship and Leber 1995, Lorenzen et al. 2010), which was partly inspired by successful results achieved in OI's mullet stock enhancement experiments and by pioneering studies in Japan (Tsukamoto et al. 1989), Norway (Svasand et al. 1990), China (Wang et al. 2006) and the US (Hager and Nobel 1976, Bilton et al. 1982). The Responsible Approach concepts have helped advance this branch of fisheries science (Sass and Allen 2014).

Responsible Approach to Marine Stock Enhancement

The modern generation of marine fisheries enhancement scientists is cultivating an integrative, quantitative and careful approach for developing and managing effective hatchery-based fisheries enhancements. The concepts were originally envisioned by an International Working Group on Stock Enhancement, formed in Torremolinos (Spain) in 1993, and published in 1995 as a platform paper by two members of the Working Group (who with several colleagues later formed the Science Consortium for Ocean Replenishment, SCORE, www.StockEnhancement.org, to help foster and refine the Responsible Approach). The International Working Group and the origin and expansion of these ideas are discussed in Leber (2013).

These concepts are presented in two publications—'A responsible approach to marine stock enhancement' (Blankenship and Leber 1995) and 'Responsible approach to marine stock enhancement: an update' (Lorenzen et al. 2010). The principles for developing, evaluating, and managing marine stock enhancement programs set out in Blankenship and Leber (1995) and Lorenzen et al. (2010) have gained widespread acceptance (Sass and Allen 2014) as a 'responsible approach' to stocking, with basic recommendations for how to make stocking work effectively (and see Cowx 1994, which emphasizes decision-making frameworks for stocking). The 'responsible approach' has been widely cited and provided a key conceptual framework for several subsequent publications (Munro and Bell 1997, Hilborn 1999, Bell et al. 2005, 2006, 2008, Taylor et al. 2005, Zohar et al. 2008). More importantly, it has been used to help guide hatchery development and reform processes in Australia, China, Denmark, Japan, New Caledonia, the Philippines and the USA (Lorenzen et al. 2010). At the same time, there has been a rapid increase in peer-reviewed literature on effects and effectiveness of stocking.

The 10 principles in the original 'responsible approach' (Blankenship and Leber 1995)

- 1) prioritize and select target species for enhancement by applying criteria for species selection; once selected, assess reasons for decline of the wild population
- 2) develop a management plan that identifies how stock enhancement fits with the regional plan for managing stocks
- 3) define quantitative measures of success
- 4) use genetic resource management to avoid deleterious genetic effects on wild stocks
- 5) implement a disease and health management plan
- 6) consider ecological, biological and life-history patterns in forming enhancement objectives and tactics; seek to understand behavioral, biological and ecological requirements of released and wild fish
- 7) identify released hatchery fish and assess stocking effects on fishery and on wild stock abundance
- 8) use an empirical process for defining optimal release strategies
- 9) identify economic objectives and policy guidelines, and educate stakeholders about the need for a responsible approach and the time frame required to develop a successful enhancement program
- 10) use adaptive management to refine production and stocking plans and to control the effectiveness of stocking.

The updated 'responsible approach' (Lorenzen et al. 2010)

Fisheries science and management in general, and many aspects of fisheries enhancement, have developed rapidly since the 'responsible approach' was first formulated. These developments made it necessary to revise the 'responsible approach' to take into account, in particular, the paradigm shift towards analyzing and managing enhancements from a fisheries management perspective (Lorenzen 2005). The developments also provided the tools for implementing the shift.

Most enhancements remain weak in at least four particular areas (Lorenzen et al. 2010):

- 1) Fishery stock assessments and modeling are integral to exploring the potential contribution of stocking to fisheries management goals; yet both are found lacking in most stock enhancement efforts in coastal systems
- 2) Establishing a governance framework for enhancements is largely ignored in stocking programs, thus, diminishing opportunities for integrating enhancement into fishery management
- 3) Involvement of stakeholders in planning and execution of stocking programs is key from the start, but they are rarely made an integral part of program development
- 4) Adaptive management of stocking is not well integrated into enhancement plans, yet is critical to achieving goals, improving efficiencies, and understanding and controlling the effects of stocking on fisheries and on wild stocks.

Lorenzen et al. (2010) expanded on these points and emphasized the importance of their inclusion in the 'responsible approach' (see updated list below). The updated approach is staged in order to ensure that key elements are implemented in the appropriate phases of development or reform processes. In particular, it is important to conduct broad-based and rigorous appraisal of enhancement contributions to fisheries management goals prior to more detailed research and technology development and operational implementation. This basic requirement applies to both development of new and/or reform of existing enhancements.

Stage I: Initial appraisal and goal setting

- 1) Understand the role of enhancement within the fishery system [NEW¹]
- 2) Engage stakeholders and develop a rigorous and accountable decision-making process [NEW¹]

¹ New points added by Lorenzen et al. 2010; not in the original 1995 version.

- 3) Quantitatively assess contributions of enhancement to fisheries management goals
- 4) Prioritize and select target species and stocks for enhancement
- 5) Assess economic and social benefits and costs of enhancement

Stage II: Research and technology development including pilot studies

- 6) Define enhancement system designs suitable for the fishery and management objectives [NEW¹]
- 7) Develop appropriate aquaculture systems and rearing practices [NEW¹]
- 8) Use genetic resource management to avoid deleterious genetic effects
- 9) Use disease and health management
- 10) Ensure that released hatchery fish can be identified
- 11) Use an empirical process for defining optimal release strategies

Stage III: Operational implementation and adaptive management

- 12) Devise effective governance arrangements [NEW¹]
- 13) Define a stock management plan with clear goals, measures of success and decision rules
- 14) Assess and manage ecological impacts
- 15) Use adaptive management

Knowledge gained through research on the kinds of issues presented here is now being used and expanded upon by scientists in this field worldwide to evaluate marine fisheries enhancements in fundamentally different habitats and conditions and at different spatial and temporal scales. Since 1990, science and knowledge in this field have expanded exponentially. Collectively, this work has begun to demonstrate how and under what conditions marine fisheries enhancements can complement current approaches to sustaining, restoring, conserving and enhancing marine and estuarine fisheries and fish (and invertebrate) populations.

Aquaculture-Based Fisheries Enhancement Terminology

Confusion about the terms used in this field reflect one of the signs of a new science—lack of consensus on terminology. Stock enhancement has often been used as a generic term referring to all forms of hatchery-based fisheries enhancement. Bell et al. (2008) and Lorenzen et al. (2010) classified the intent of stocking cultured organisms in aquatic ecosystems into various basic objectives. Together, they considered five basic types, listed here from the most production-oriented to the most conservation-oriented:

1. *Sea ranching/Lake ranching.* Recurring release of cultured juveniles into marine, estuarine and lacustrine environments for harvest at a larger size in ‘put, grow, and take’ operations. The intent here is to maximize production for commercial or recreational fisheries.
2. *Stock enhancement.* Recurring release of cultured juveniles into wild population(s) to augment the natural supply of juveniles and optimize harvests by overcoming recruitment limitation in the face of intensive exploitation and/or habitat degradation.
3. *Re-stocking.* Time-limited release of cultured juveniles into wild population(s) to restore severely depleted spawning biomass to a level where it can once again provide regular, substantial yields (Bell et al. 2005).
4. *Supplementation.* Moderate releases of cultured fish into very small and declining populations, with the aim of reducing extinction risk and conserving genetic diversity (Hedrick et al. 2000, Hilderbrand 2002).
5. *Re-introduction.* Temporary releases with the aim of re-establishing a locally extinct population (Reisenbichler et al. 2003).

Capture-Based Enhancement of Mullet Fisheries

In many countries, mullet fry and fingerlings are captured from the sea and stocked in inland lakes and reservoirs as a form of fisheries enhancement (Lovatelli and Holthus 2008). Wild caught post-larvae and

fingerling *M. cephalus* and other mullets have long been used to create fishpond, lagoon and lake fisheries, dating back to ancient Roman civilization in the Mediterranean region (Basurco and Lovatelli 2003). They have been stocked into inland water lakes of the El Fayyum area of Egypt since the 1920s, and into the Black Sea and Caspian Sea regions of Russia since 1930 (FAO 2006). Modern examples include capture based mullet fisheries in some countries in the Mediterranean region, Asia, and in Hawaii, USA (Ellis 1968, FAO 2006, Nishimoto et al. 2007, Saleh 2008, Snovsky and Ostrovsky 2014).

Stocking wild-caught mullet in inland lakes has been known in Egypt for more than eight decades. The importance of wild seed collection increased with recent aquaculture developments. In 2005, 69.4 million mullet fry were collected for both aquaculture and lake ranching and 156,400 tonnes of mullet were produced in lakes, semi-intensive ponds and coastal net pens (20% of Egypt's annual aquaculture production). As aquaculture of mullet has become more profitable, pressure on wild-caught fry has increased. The high cost of hatchery produced mullet seed has limited expansion of hatcheries in Egypt. The effect of capture-based fisheries on wild stocks of mullet is not well studied and this has become a subject of debate between aquaculture farmers and capture fisheries communities (Saleh 2008).

In Israel, two species of mullets, *Liza ramada* and *Mugil cephalus*, are stocked each year in Lake Kinneret. Neither of these reproduces in the lake. Commercial catch rates show that 27–28% of introduced fish of each species are landed. *M. cephalus* has greater impact per introduced fish than *L. ramada* (Snovsky and Ostrovsky 2014). Regular stocking programs were initiated in the 1950s. These stocking programs and fisheries on Lake Kinneret are controlled by the Israel Water Authority and by the Israel Fisheries Department of the Ministry of Agriculture. Grey mullets have the highest value of any commercial fish caught in Lake Kinneret. After collapse of the tilapia fishery in 2008, grey mullets comprised the primary income-generating commercial catch. Currently, the number of stocked fry is limited to one million fingerlings of these two species combined per year (Ostrovsky et al. 2014).

In the 1960–80s, *Mugil cephalus* and *Liza ramada* fry were used to stock the volcanic freshwater lakes of Central Italy which did not have any direct connection with the sea and were the base of a specific fishery, using the ‘*cefalare*’ (nets especially designed for mullets - *cefali* in Italian) when adult mullets would gather in large schools (Cataudella and Monaco 1983).

There are smaller capture-based fisheries in some areas of Greece and Italy, where extensive culture systems are used to farm grey mullet in more or less confined brackish coastal lagoons, relying on wild fry that are collected and grown naturally each year. Wild caught grey mullet are also stocked in enclosed areas in Korea, Hong Kong, Taiwan and Singapore (Lovatelli and Holthus 2008).

Ancient Hawaiian people built and operated fish ponds along the shores of all the principal Hawaiian Islands. These ponds were stocked with a variety of marine species. Today, some of these ancient Hawaiian fishponds, once destroyed by natural causes, have been rebuilt and many are stocked with grey mullet (Nishimoto et al. 2007).

Looking to the future: can wild fry support expansion of capture-based mullet fisheries? Clearly, concerns about overfishing wild mullet stocks are already starting to limit current capture-based mullet fisheries. For example, in response to fisheries declines, some countries in the Mediterranean are already considering restrictions on collections of wild mullet juveniles stocked to support lake fisheries (Vasilakopoulos et al. 2014, A. Tandler, pers. comm.); what is needed is commercial-scale hatchery production of *M. cephalus* fry for grow-out and for supporting lake ranching and marine fisheries enhancements. In 2007, FAO held an “international workshop on technical guidelines for the responsible use of wild fish and fishery resources for capture-based aquaculture production” in Viet Nam and produced technical guidelines on capture-based aquaculture (Lovatelli and Holthus 2008).

Aquaculture-Based Enhancement of Mullet Fisheries

While capture-based fisheries for mullets have supported subsistence fisheries in Asia, Hawaii and the Mediterranean region for centuries, reliance on wild-caught fry cannot continue at the current pace, much less expand to meet demand (Saleh 2008). Thus, several countries are considering adding culture-based fisheries enhancements to their mullet fisheries management strategy. Following the first induced spawn

in captivity (Tang et al. 1964) in Taiwan, and early larval-rearing successes in Taiwan (Liao et al. 1971, Liao 1975), attempts to close the life cycle with levels of fry production large enough to enable commercial scale aquaculture of *M. cephalus* made great progress in the 1980s and '90s (Nash and Shehadeh 1980, Lee et al. 1988, 1992, Tamaru et al. 1991, 1993, 1994, Liu and Kelley 1994, Lee and Ostrowski 2001). These developments in striped mullet aquaculture technology enabled our studies of the effectiveness of aquaculture-based mullet stock enhancement in Hawaii.

Case Study: Research in Hawaii to Develop Flathead Grey Mullet Stock Enhancement Technologies

Despite well over a century of stocking marine organisms into the sea to enhance fishery stocks, prior to the 1990s, very little effort had been allocated to assessing the effectiveness of stocking programs (Leber 2013). However, in the late 1980s, systematic studies began to develop and assess marine stock enhancement in Norway, Japan, China and the US. One of these efforts was a program launched in the US in Hawaii, which initially used flathead mullet *M. cephalus* as a test species. This work was one of the pioneering efforts worldwide to evaluate the potential of marine fisheries enhancement. The Hawaii project has been the only stock-enhancement assessment project conducted with *M. cephalus* that has employed experimental pilot releases and adaptive management to improve the outcome of stocking. Thus, the Hawaii work is presented here as a case study of fundamental aspects of conducting effective culture-based marine fisheries enhancement with *M. cephalus*.

Beginning in 1988, the Oceanic Institute (OI) began to receive federal funding from NOAA-Fisheries (US Department of Commerce) to examine the feasibility of replenishing declining marine fish populations in Hawaii using releases of cultured fish. OI's Stock Enhancement research was focused on developing effective stock enhancement strategies and transferring that technology to the state of Hawaii. The NMFS project, titled Stock Enhancement of Marine Fish in the State of Hawaii (SEMFISH), funded the primary research to develop and test enhancement strategies. In 1990, the Hawaii Division of Aquatic Resources (DAR) funded a collaborative project with OI to enable training and transfer of OI's marine stock enhancement technology to DAR. DAR aimed to restore to former abundance species whose numbers had become depleted, at least in part, by loss or degradation of natural spawning and nursery habitats. The OI and DAR projects were eventually curtailed in the early 2000s, owing to funding constraints.

Selection of Flathead Grey Mullet *M. cephalus* as the Top Candidate for Stock Enhancement Research in Hawaii

Initially, the Hawaii researchers convened a series of public workshops to identify species that were potential candidates for stock enhancement research in Hawaii (Leber 1994). A formal, semi-quantitative decision-making process was used to develop criteria and rank species. Based on the results of two workshops to prioritize species *M. cephalus* was selected, along with Pacific threadfin *Polydactylus sexfilis*, for a multi-year study to assess the potential to create culture-based fisheries in the sea.

Production of *M. cephalus* Fry for Stock Enhancement Research on Oahu, Hawaii

Successful stock enhancement is dependent on appropriate numbers and sizes of healthy, fingerlings being available for release at the appropriate time of year. This requires careful planning of production goals several months in advance of releases, and monitoring abundances and growth rates of cultured fish throughout the production process. In addition, specific measures must be taken in the hatchery to prevent disease and parasite outbreaks, and to ensure that genetic integrity of wild stocks is not degraded by hatchery releases (Tringali and Leber 1999, Tringali et al. 2007, Lorenzen et al. 2012).

Production of grey mullet for the Hawaii stock enhancement research followed protocols developed by OI for mullet broodstock acquisition, maturation and spawning, larval rearing, and nursery (Liu and Kelley 1994). Production of fingerlings for stock enhancement releases also required close

attention to production parameters (growth rate, size distribution and population size), disease management, and genetic protocols for minimizing negative interactions between hatchery and wild fish (Blankenship and Leber 1995, Lorenzen et al. 2010). A detailed description of the rearing process is found in Tamaru et al. (1993) and Liu and Kelley's (1994) mullet culture manual.

Planning fish production for stock enhancement releases should begin with clear objectives about the intent of stocking and the numbers and sizes of fingerlings needed to be released into specific nursery habitats at specific times of the year (see below). In addition, recommended genetic protocols for hatchery releases require that sufficient mature broodstock from each habitat in which releases are to take place are available for spawning (Blankenship and Leber 1995, Lorenzen et al. 2010, Tringali et al. 2007). Because fish production levels need to satisfy requirements for hatchery releases, release numbers should be set with a 'window' (i.e., a range in which release numbers can fall and still meet release goals to allow for potential losses of fish from disease or parasitic outbreaks, or unexpected malfunctions in the culture system). In Hawaii, the intent of stocking was to develop and evaluate effective stock enhancement strategies. Rearing ~ 80,000 fingerlings per year for pilot release experiments was the target for aquaculture production.

Identifying Released *M. cephalus* to Enable Evaluation of Stocking Impact

Selecting a high-information content tag to identify hatchery reared fish to quantify success or failure of stocking is one of the most critical components of any enhancement efforts (Blankenship and Leber 1995, Lorenzen et al. 2010, Leber and Blankenship 2012, Leber 2013). Without some form of assessment, one has no idea of the success of a particular approach. Natural fluctuations in wild fish abundance can mask successes and failures and further necessitate a proper monitoring and evaluation system coupled with adaptive management (Walters and Hilborn 1978, Leber 2013).

Tagging technology provides the basis for quantitatively assessing survival, growth and dispersal of released fish, and their contribution to wild populations. Recaptures of tagged, hatchery-raised fish through regular sampling enables fishery managers to evaluate and refine release strategies, giving them control over the impact of hatchery releases on the fishery.

Selecting a Tagging System

Several methods are available for tagging fish including the Coded Wire Tag (CWT), Visible Implant Elastomer (VIE), Visible Implant Alpha (VIA), Passive Integrated Transponder (PIT) tags, acoustic tags and genetic fingerprinting. The CWT is considered the most suitable tagging method for stock enhancement programs, as it enables high capacity tagging of large numbers of small fish, high information control and reasonable cost considerations (Leber and Blankenship 2012).

Coded-wire tagging is done by implanting a single micro tag (1 mm long x 0.25 mm diameter) into fish tissue (usually nose or cheek tissue) beneath the skin using a technique that has no negative effect on fish health or behavior or human health. The CWT is a stainless steel tiny wire, marked with rows of numbers denoting codes of batches of fish and individuals. The tagging is done using an injector in a professional way. The tagging of large numbers of fish for stock enhancement programs requires an automatic injector and qualified operator. In addition, detectors that sense small changes in the magnetic field caused by the CWT while passing next to it are used to detect the presence of a CWT in tagged fish. Reading the codes on the tag is performed with a typical dissecting microscope.

CWTs appear to have negligible effects on tagged animals, and they are relatively cost-efficient for large-scale tagging programs (Nielsen 1992). Tags can remain in the animals indefinitely, enabling scientists to identify hatchery-raised fish at any stage of their life cycle. One of the greatest advantages of CWTs is the high information content provided by an almost unlimited number of possible codes.

CWTs have a numerical code etched onto the surface of each tag. For stocking programs, the code is often used to identify batches of fish, such as a release lot, but it is also possible to use sequentially coded CWTs to identify individual fish. These tags are automatically magnetized before insertion into cartilaginous, connective or muscular tissue. CWTs can be injected into the snout of grey mullet using head molds specifically designed by OI and Northwest Marine Technology, Inc. biologists for various

sizes of juvenile grey mullet (45–130 mm Total Length [TL]). The head molds enable rapid tagging (~ 800 to 1,000 fish per hour by an experienced tagger) and are critical for correct placement of the tag. CWTs are typically implanted in the snout region of grey mullet and thus accurate placement from head molds prevents the tag from being injected into sinus cavities and eventually ejected from the fish. Because of the small size of the tags, minimal tissue damage occurs during tag insertion, and insertion wounds heal rapidly. Thus, CWTs can be used effectively with small juvenile fish (as small as 45 to 60 mm TL; Leber et al. 1996). Testing CWTs on 27 different genera of fish has shown tissue interaction to be minimal (Bergman et al. 1968, Fletcher et al. 1987).

For grey mullet, CWT retention rates averaged at least 97% over a period of several years (Leber 1995, unpubl. data). Initially, the Hawaii researchers had very poor tag retention in the snout region of grey mullet. The problem was solved by sectioning tagged fish and identifying tag placement using scanning electronic microscopy. This revealed that the tags were being injected into sinus cavities. Acceptable CWT retention rates were achieved with grey mullet after redesigning head molds to specifically target cartilaginous tissue in the head region.

CWTs are detected electronically by their magnetic field using a tag-detecting wand (NMT, Inc.). Tagged fish are returned to the laboratory where tags are dissected from fish using a binary search. Codes are read using a standard binocular microscope. CWTs injected into hatchery-released fingerlings have been recovered years later from adult mullet captured in Hawaii's mullet fishery (Leber and Arce 1996).

Evaluation of Release-Strategy Effects on Success of Mullet Stocking

Pilot releases should always be conducted prior to launching full-scale enhancement programs (Blankenship and Leber 1995, Lorenzen et al. 2010, Leber 2013). Experiments to optimize release strategies, by understanding the interactive effects of stocking variables (size at release, release habitat, release season, release magnitude) on survival of cultured fish released into the wild, are a critical step in identifying enhancement capabilities and limitations and in determining effective release strategies (Leber 1995, 2013, Leber et al. 1995, 1996, 1997, 2005). Pilot releases also provide the empirical data needed to plan enhancement objectives, test assumptions about survival and cost-effectiveness, and improve models predictions of enhancement potential.

To design effective pilot releases, critical variables that could affect survival of hatchery-released fish in the wild should be identified and then tested using an appropriate experimental design. Stocking variables that typically impact survival of stocked fish include release habitat, size-at-release, release season, release magnitude; these variables also have interactive effects (Fig. 18.1; Leber 1995, Leber et al. 1995, 1996, 1997). Acclimation and acclimatization prior to release should also be tested to determine impact on post-release survival of stocked fish (Brennan et al. 2006).



Figure 18.1. Key release variables that can strongly affect survival of released fingerlings. Evaluating the effects of these was the primary focus of the stock enhancement research in Hawaii.

Following pilot releases, sampling should be conducted to monitor survival of released hatchery fish and the effects of the chosen release strategies. Early indicators of stock enhancement effect include recovery rates, density of cultured and wild fish captured in samples and proportion of hatchery fish in collections (release contribution). Sequential pilot releases can be used to maximize enhancement benefits in a full-scale enhancement program.

Evaluation of Size-at-Release Effects on Success of Mullet Stocking

Several Size-At-Release (SAR) intervals should be evaluated during initial pilot experiments to identify optimal SAR (SAR resulting in the highest survival to production cost ratio; Leber et al. 2005) prior to large-scale hatchery releases. In the pilot experiments with grey mullet in Hawaii, a range of SAR groups were tagged and released into prime nursery habitats (Leber 1995, Leber et al. 1995, 1996, 1997). A range of fish sizes were produced by rearing eggs from several spawns, each spawn being about six weeks apart.

During summer 1990, 85,848 juvenile mullet were graded into five size groups (ranging from 45 to 130 mm total length), identified with binary-coded wire tags, and released into two estuaries (2 x 5 factorial design) on Oahu, Hawaii as part of an OI experiment to evaluate size-at-release and release habitat impacts on recruitment and survival of hatchery-released mullet (Leber 1995). Forty two thousand eight hundred and twenty two of the tagged fish were released into Kaneohe Bay on the east (windward) coast of Oahu and 43,026 were released simultaneously into Maunalua Bay on Oahu's drier south shore. To replicate experimental treatment groups, releases were blocked in time across five release lots.

To evaluate effects of size-at-release on and survival rates of released mullet, both bay systems were sampled monthly with cast nets over a 10 month period after releases. Researchers captured 733 tagged grey mullet, 277 from Kaneohe Bay and 456 from Maunalua Bay. Within six weeks after releases, recapture frequencies were clearly skewed in favor of fish that were larger at the time of release. Fish smaller than 70 mm when released were rare or absent in collections within 15 weeks after their release into Maunalua Bay, and within 25 weeks in Kaneohe Bay. This study confirmed results of a smaller-scale 1989 pilot study in Maunalua Bay and showed that fish size-at-release can have a critical impact on survival of cultured mullet in the wild. Pilot studies to identify minimum size-at-release should be conducted at each site targeted for marine hatchery releases.

Interactive Effects of Size-at-Release and Release Season

In a series of pilot releases over three years, Leber et al. (1995, 1996, 1997) showed the effectiveness of the adaptive management process at work, increasing the effectiveness of stocking by over 400% by modifying release strategies based on results from the pilot releases. Such 'active' adaptive management needs to be put into practice in existing stocking programs (Leber 2013).

Size-at-release (SAR) markedly impacted survival of stocked mullet (Leber 1995). Release season is another important variable to evaluate in pilot release experiments prior to conducting large-scale hatchery releases. Releases should be conducted during the natural recruitment time for the target species and those results compared with releases in other seasons. The Hawaii studies revealed that release season can clearly impact survival of grey mullet released into the wild by affecting size-at-release effects (Leber et al. 1997).

Hatchery-raised grey mullet were released into Kaneohe Bay, Hawaii during the spring and summer of 1991 as part of a pilot experiment to evaluate the impact of release season on recapture rates of released fish (Leber et al. 1997). Ninety thousand eight hundred and seventeen cultured grey mullet fingerlings were tagged and released into two replicate nursery habitats (Kahaluu stream and Kaneohe stream). During each season, three replicate lots of five size intervals (ranging from 45 to 130-mm total length) were released at both nursery habitats (3-way factorial design). Released fish were identified with binary-coded wire tags. Close attention was paid to releasing roughly identical numbers of fish among release lots for each season-SAR-site combination.

Survival, movement, and growth of released fish were monitored monthly over 45 weeks with a sampling program established at six nursery habitats in Kaneohe Bay. Results showed that survival and growth of released mullet were directly affected by the interactive effects of release season and size-at-

release. Recapture frequencies, based on the number of individuals released within treatment groups each season, revealed an obvious and direct relationship between size-at-release and recapture rate (Fig. 18.2); when fish were released in the summer, recapture frequencies were directly proportional to size-at-release within a month after release. In contrast, size-at-release had little effect on recapture frequencies for fish released 10 weeks earlier, in the spring (Fig. 18.2). Spring was the only season tested in which 45–60 mm grey mullet have contributed significantly to abundances in the wild. However, larger fingerlings apparently had better survival rates in the wild when held in the hatchery until summer.

These data highlight how futile it would be to conduct summer releases in Kaneohe Bay of individuals that were smaller than 60 mm. As hypothesized by Leber et al. (1997), in habitats where survival of released fish is strongly impacted by fish size-at-release, survival of grey mullet will be greater when releases are timed so that fish size-at-release coincides with modes in population size structures of wild stocks. A corollary to this is the fewer wild fish in a particular size interval, the lower survival will be of released fish in that size interval.

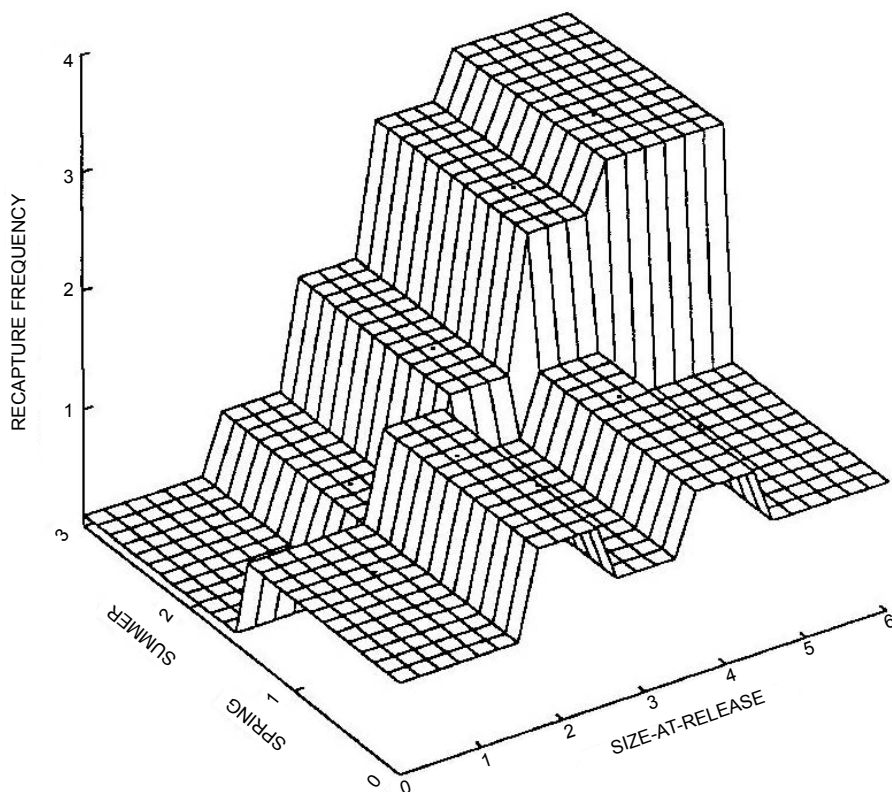


Figure 18.2. Percent of tagged, hatchery-released grey mullet recaptured in cast net samples following spring and summer releases, given for each of five size classes released (1 = 40 to 60 mm total length; 2 = 60 to 70 mm; 3 = 70 to 85 mm; 4 = 85 to 110 mm; and 5 = 110 to 130 mm total length). Data are percent recaptured of total fish released per treatment group.

Release Microhabitat Effects on Success

Mullet stocking programs should question the choice of release microhabitats carefully. In the study below (Leber 1995, Leber and Arce 1996), the Hawaii team realized the apparent loss of 30,000 *M. cephalus* fingerlings by stocking them in the ‘wrong’ habitat.

Pilot experiments with grey mullet reveal the importance of evaluating the effect of release habitat on survival of hatchery fish in the wild (Leber 1995, Leber and Arce 1996). In 1990, habitat preferences

for juvenile grey mullet in Hawaii were not well understood. The literature describes *Mugil cephalus* as euryhaline, catadromous fish, well adapted to low salinities in rivers and estuaries (Blaber 1987). It seemed reasonable in 1990 to expect that releases anywhere in Kaneohe Bay would result in comparable survival, but this was not the case.

In 1990, only around 12,000 grey mullet were released in Kaneohe Bay at Kahaluu stream, but over 31,000 grey mullet were also released that year along the shoreline near the Hawaii Institute of Marine Biology pier in south Kaneohe Bay (HIMB) (Leber 1995). In 1991, 45,000 fish were released near the inlets at Kahaluu stream and an equal number at Kaneohe stream in the southern portion of the bay (Leber et al. 1997; Fig. 18.3).

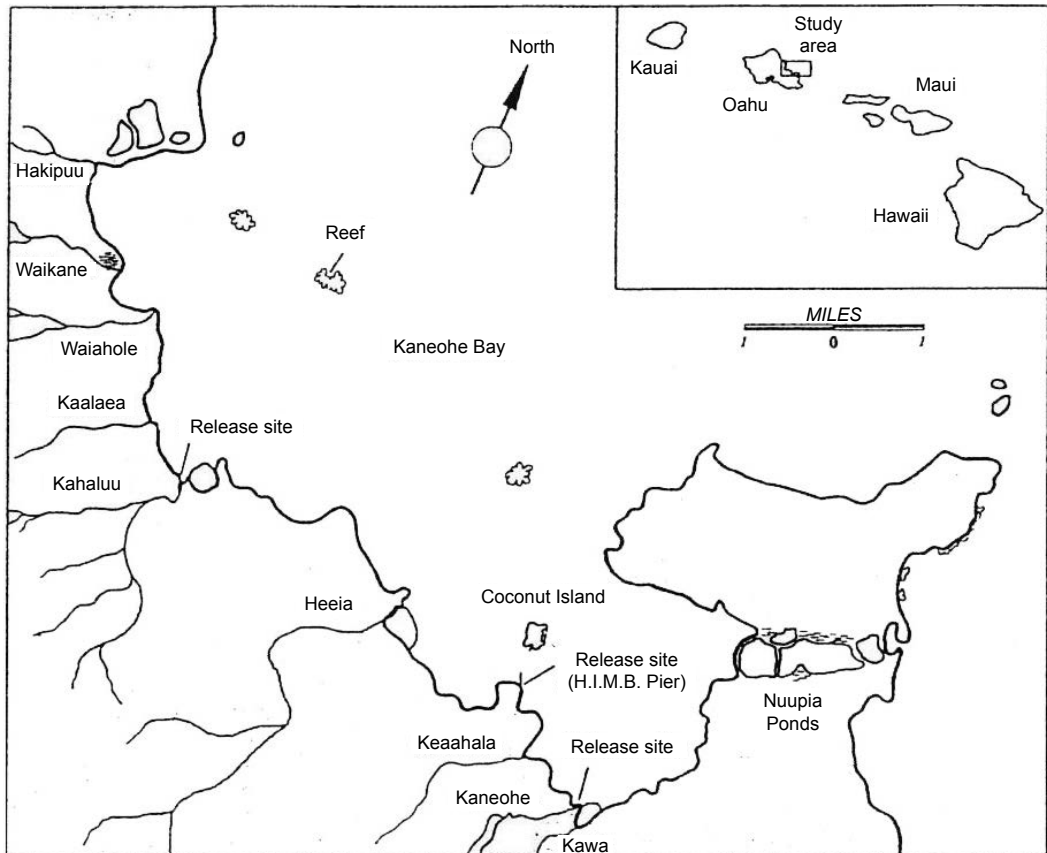


Figure 18.3. Map of Kaneohe Bay, showing release sites (from Leber and Arce 1996).

Fish released in the vicinity of streams showed similar performance in 1990 and 1991. But the 1990 release of 31,000 fish near HIMB resulted in few fish recaptured after week 16. None of the 31,000 fish released in 1990 near HIMB have been retrieved from the commercial mullet fishery in Kaneohe Bay, yet at least 20 individuals from the 1990 release at Kahaluu were recovered from that fishery during contact interviews with fishermen (Leber and Arce 1996).

Clearly, to optimize stocking success, each habitat targeted as a release site should be evaluated with pilot tag-release-recapture trials prior to large-scale hatchery releases. Initially, hatchery fish should be released into at least two to three different sites to compare differential effects of these habitats on fish survival.

Release site assessments should be conducted prior to selecting release sites, to locate primary nursery habitats containing ample food and other necessary resources, where juvenile wild fish of the target species occur naturally. Information on preferred nursery habitats for a target species can be obtained from the literature, from scientists who may have access to unpublished data, and from fishermen and others knowledgeable about the target species' ecology. Net sampling at potential nursery habitats may also be necessary before pilot releases begin, to obtain data on the target species' distribution and abundance in the wild, to clarify preferred nursery habitats. In addition, other release-site considerations include whether the site is accessible to release equipment (a truck or trailer with live fish hauling tank, hoses, etc.), and whether or not the site is frequented by fishermen, as (illegal) fishing pressure on released juveniles can threaten enhancement efforts.

Leber and Arce (1996) hypothesized that refuge from predators afforded by mangroves and other shoreline vegetation in the north end of Kaneohe Bay accounted for better survival of mullet released at Kahaluu inlet than of those released near HIMB. Mangroves are extensive along the northern shoreline of the bay from Kahaluu stream to Waiahole stream, whereas much of the shoreline in the southern portion of the bay near HIMB lacks mangroves. Also, the shoreline near HIMB is largely fronted by seawalls and mudflat-coral rubble habitat. Leber et al. (1996, 1997) showed relatively good survival following releases at Kahaluu stream, regardless of any subsequent movement along the shoreline towards adjacent streams.

Other factors besides ecological characteristics at a particular release site can impact fish survival and should be considered in determining optimal release habitat. For example, whether or not fishing regulations are enforced at a site can significantly impact survival of released fingerlings.

Test of Concept: M. cephalus Stocking Impact on Juvenile Recruitment

In the Hawaii pilot experiments with flathead grey mullet, hatchery-release variables were steadily refined to maximize grey mullet enhancement potential. Based on results from two years of pilot hatchery releases in Kaneohe Bay, a pilot experiment was designed to incorporate improved release strategies in a test of the marine stock enhancement concept (Leber et al. 1996). This study employed release strategies that had been steadily refined through the adaptive management process (in this case, with information learned through the pilot releases) to evaluate the real potential to use hatchery releases to significantly increase juvenile grey mullet recruitment in Kaneohe Bay, the largest estuary in Hawaii.

Essentially, this experiment evaluated the first assumption of the marine stock-enhancement concept: that cultured fishes released into coastal waters actually survive, grow and contribute substantially to recruitment. The criteria for success were: (1) cultured fish released in this study comprise at least 20% of the juvenile grey mullet in net samples four months after release; (2) cultured fish persist in net samples throughout the study; and (3) growth of cultured fish is comparable with measured rates in wild juveniles. If these criteria were met, it was reasonable to assume that cultured fish had substantially affected juvenile recruitment at the study site.

Eighty thousand five hundred and seven cultured grey mullet were tagged with coded wire tags and released during spring and summer into the Kahaluu stream, the principal mullet nursery in Kaneohe Bay (Fig. 18.4). For each release season-SAR combination, the experiment was replicated with three release lots at each of two release locations at Kahaluu stream (stream mouth and upper stream lagoon). SAR determinations were based on the 1991 study that revealed a strong effect between release season effects and SAR effects on survival (Leber et al. 1997). The seasonal timing of releases (spring and summer) was based on results from the previous pilot releases (Leber 1995, Leber et al. 1997). In the test-of-concept study, all five SAR intervals were released in spring. Only the three largest size groups were released in the summer (no fish smaller than 70 mm TL).

Recapture rate was six-fold greater than recapture rates had been after initial releases in Kaneohe Bay. The ~ 600% increase was a direct result of modifying release habitat and size-at-release (SAR) protocol based on recapture rates in pilot releases (releases in this experiment were confined to the vicinity of freshwater streams, and a minimum size of 70 mm total length was used during summer releases).

After 11 months, cultured fish comprised 50% of the grey mullet in collections at the release site, 20% in a nursery habitat 1 km to the north, and 10% in a nursery 3 km north of the release site. The location

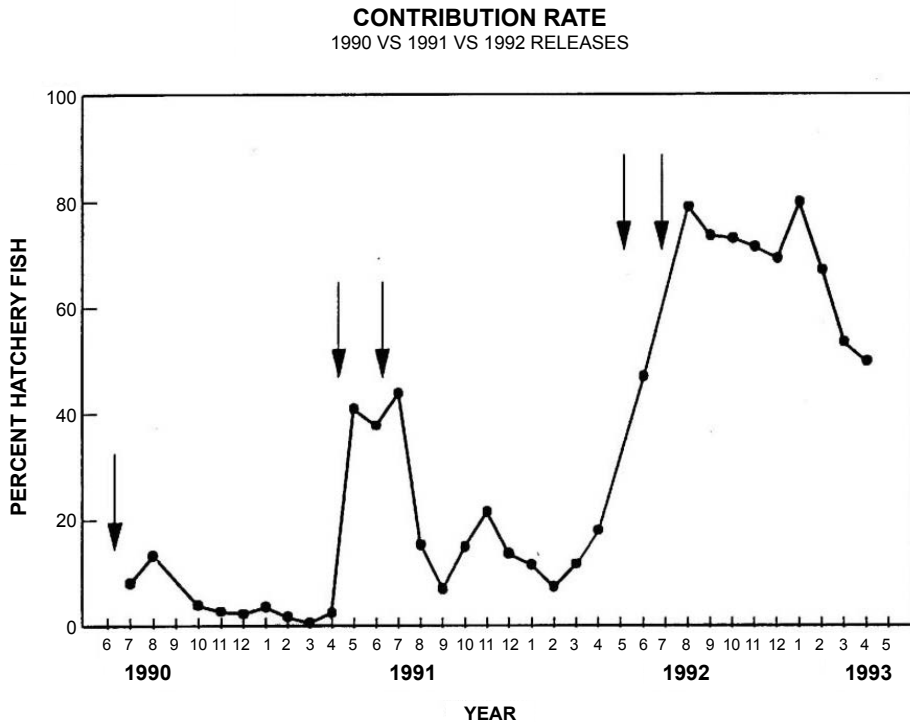


Figure 18.4. Contribution of juvenile cultured grey mullet to wild stock abundance in cast-net samples over the three year period of pilot release experiments in Kaneohe Bay. Arrows identify stocking events, showing a cumulative effect of adaptive management of release strategies—an increase of ~600% in the contribution of stocked mullet to overall mullet abundance in mullet nursery habitats in Kaneohe Bay (from Leber et al. 1996).

of releases at Kahaluu (stream mouth vs. upstream lagoon) significantly affected post-release dispersal patterns of cultured fish, but not growth or relative survival. SAR effects on recapture rates corroborated earlier results showing that the smallest (45 to 60 mm) fish released could survive spring releases better than summer releases. There was also a trend towards better survival of larger individuals when they were released in the summer.

Stocking effect on mullet abundances in nursery habitats was remarkable after adjusting release strategy to incorporate findings from pilot releases in Kaneohe Bay. There was a substantially greater impact on juvenile abundances in Kaneohe Bay following the 1992 releases than after pilot releases in 1990 (Leber 1995) and in 1991 (Leber et al. 1997). Proportions of cultured fish at Kahaluu 10 months after releases increased from around 3% following 1990 releases, to around 10% after 1991 releases, to around 50% in this study (Fig. 18.4).

Clearly, pilot experiments are crucial for managing enhancement impact. What is clear here is that the results realized from the 1990 releases, prior to any adaptive management, pale in comparison to what was achieved following the releases in 1992. The results from the 1992 releases were made possible by steady refinement in release strategies, based on posing hypotheses with each pilot release, monitoring the results, then making appropriate changes in release strategies based on the results accumulated from successive pilot releases.

Cost-Effectiveness of Size-at-Release of Hatchery Fish Recovered in the Mullet Fishery

How should one decide what is the optimal size hatchery fish to release? For mullet, hatchery costs to rear *M. cephalus* to various fingerling sizes in Hawaii were evaluated and compared with relative yields in the

fishery of the various sizes of *M. cephalus* stocked in Kaneohe Bay. Those results were used by Leber et al. (2005) to select optimal size-at-release.

To determine unit cost to produce the various size-at-release groups, a bioeconomic model, originally developed to evaluate shrimp aquaculture production (Leung and Rowland 1989), was adapted to grey mullet production. The model specified costs associated with using existing facilities, established culture methods, and following hatchery guidelines needed to prevent deleterious genetic effects in the hatchery, as recommended by Shaklee et al. (1993), Kapuscinski and Jacobson (1987) and Busack and Currens (1995). The model determined the operating costs to produce and rear around 90,000 grey mullet to the median size within each of the five SAR intervals used in pilot release experiments in Kaneohe Bay.

Fishery contribution rates and production costs were determined for cultured fish released in 1990–1992 pilot studies that were subsequently landed in the commercial mullet fishery in Kaneohe Bay (Leber and Arce 1996). Recovery in the fishery of fish that were smaller than 60 mm when released was very poor relative to recovery from larger SAR intervals, particularly when releases were conducted in summer (Fig. 18.5).

To identify the most cost-effective (optimal) size of mullet to release, Leber et al. (2005) developed a simple mathematical model to determine the optimal SAR. The production-related cost of an enhancement effect (dollars spent in the hatchery to achieve a hatchery fish contribution to the fishery) was least for fish that were 85–110 mm TL when stocked. Although the cheapest fish to rear among the size intervals that were produced were those in the 45–60 mm interval, these results revealed that releasing larger mullet can result in greater cost-efficiency when the increase in yield (because of the increase in survival afforded by releasing larger fish) more than offsets the increase in production costs of rearing larger mullet. In Kaneohe Bay, stocked mullet afforded a greater fishery contribution per dollar spent on production when intermediate-size, not small, grey mullet fingerlings were stocked. These results do not suggest that intermediate size fingerlings should always be stocked by stocking programs; the point was that one should identify the most cost-effective size to stock, which may vary among systems based on local environmental and ecological conditions at release sites. The optimal SAR may be small fish in some systems (e.g., Tringali et al. 2008) and larger fish in others.

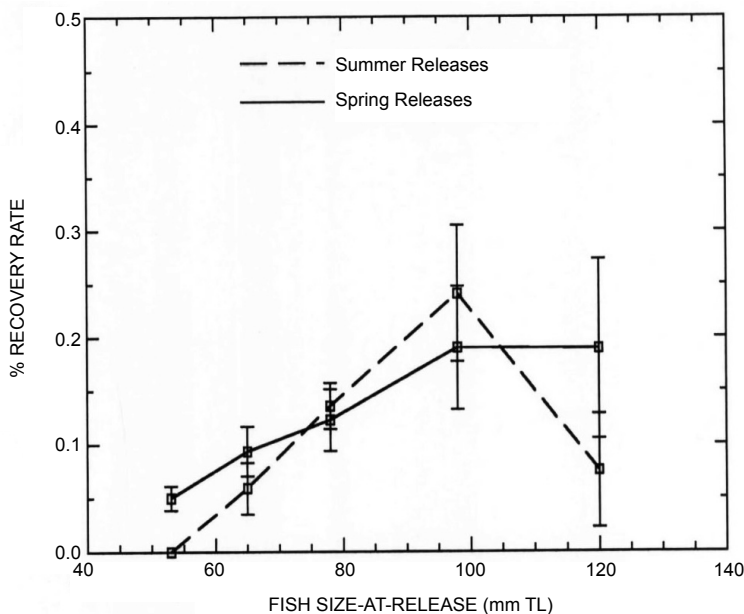


Figure 18.5. Relationship between mean percent recovery rate ([number recaptured/number released] \times 100) and fish size at release (SAR) for 214 cultured grey mullet recovered from the fishery in Kaneohe Bay, Hawaii (Leber and Arce 1996, Leber et al. 2005).

Production Cost per Recruit. Using the production and cost data from this study, fish production costs in the hatchery were distributed across fishery recruitment levels for hatchery fish, assuming a release of 91,286 individuals in the optimal SAR interval, 85–110 mm TL (Fig. 18.6). This models hatchery production cost per fish landed for fishery recovery values ranging from 2 to 100% (for 91,286 fish produced and subsequently caught in a fishery). With this model, total hatchery production costs averaged over the number of landed hatchery fish decreased logarithmically from around US\$30 (in 1993 dollars) per hatchery fish landed if only 2% of the released grey mullet are caught in the fishery, to US\$12 if 5% are caught, US\$6 if 10% are caught, US\$3 if 20% are caught, US\$1.20 if 50% are caught, and US\$0.60 if 100% are caught.

Thus, pilot-release studies that reveal ways to maximize survival of stocked fishes without necessarily increasing rearing costs can improve cost efficiency in stocking programs. A primary concern for cost efficiency of enhancement should be “how do stocking variables affect optimal size at release”; for example, how does timing of releases (seasonal, tidal, time of day), release habitat (and microhabitat), stocking magnitude, and acclimation prior to release affect post-release survival and optimum size-at-release? These factors can all be examined in ongoing stocking programs by adopting an adaptive management approach.

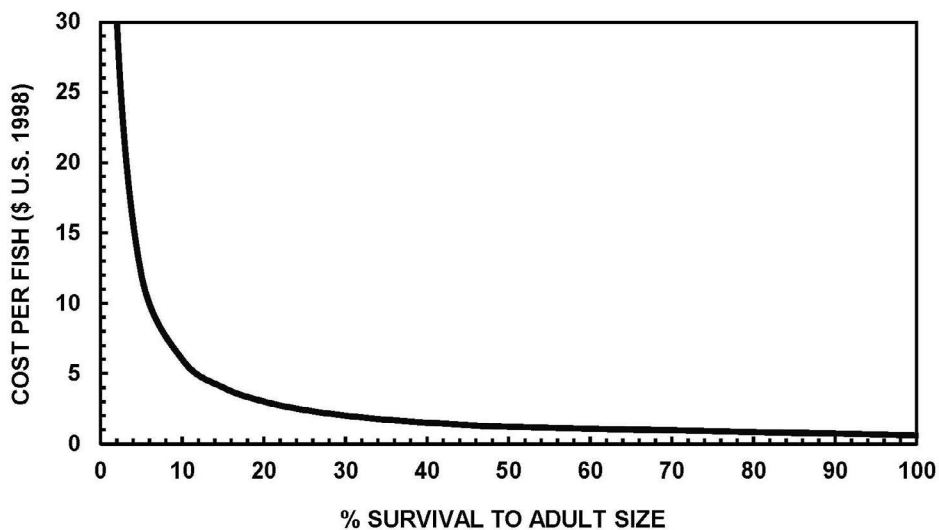


Figure 18.6. Unit production costs apportioned over simulated hatchery grey mullet landings. Production cost per hatchery recruit in the fishery is for 85–110-mm TL fingerlings stocked into Kaneohe Bay (Leber et al. 2005).

Does Stocking Cultured *M. cephalus* Enhance or Displace Wild *M. cephalus*?

Assessment of release impact should go farther than evaluation of survival and contribution rates of hatchery fish. Evaluation of hatchery fish interactions with wild stocks is also critical. Such an evaluation can address the second corollary of the marine stock enhancement concept: that hatchery fish enhance rather than displace wild stocks.

The first empirical stocking study to evaluate effect of stocking magnitude on hatchery-wild fish interactions was conducted by the Hawaii project, which documented that released mullet could indeed increase abundances of juvenile recruits in a principal nursery habitat in Hawaii without displacing wild individuals (Leber et al. 1995). In the summer of 1993, 6,000 wild mullet fingerlings were captured, tagged, and released at two of the most productive nursery habitats in Kaneohe Bay (Kaneohe stream and Kahaluu tributary). The release of wild fish established a pre-treatment condition to gather baseline data on the dispersal patterns of wild fish. A month after the wild releases, 30,000 hatchery fish were released into the Kahaluu tributary to establish the primary treatment condition, leaving Kaneohe stream as the control site.

Hawaiian researchers evaluated the hatchery impact by comparing dispersal patterns for wild fish released at the treatment site (Kahaluu tributary) with dispersal patterns of wild fish released at the control site (Kaneohe stream). Results show that recapture rates for wild fish were nearly identical between treatment and control sites, indicating that wild fish were not displaced from their natural nursery sites by hatchery releases (Fig. 18.7).

Initial dispersal patterns of the cultured fish released into Kaneohe Bay showed greater movement of cultured fish out of the release habitat than was expected, based on results from previous smaller scale releases at that site.

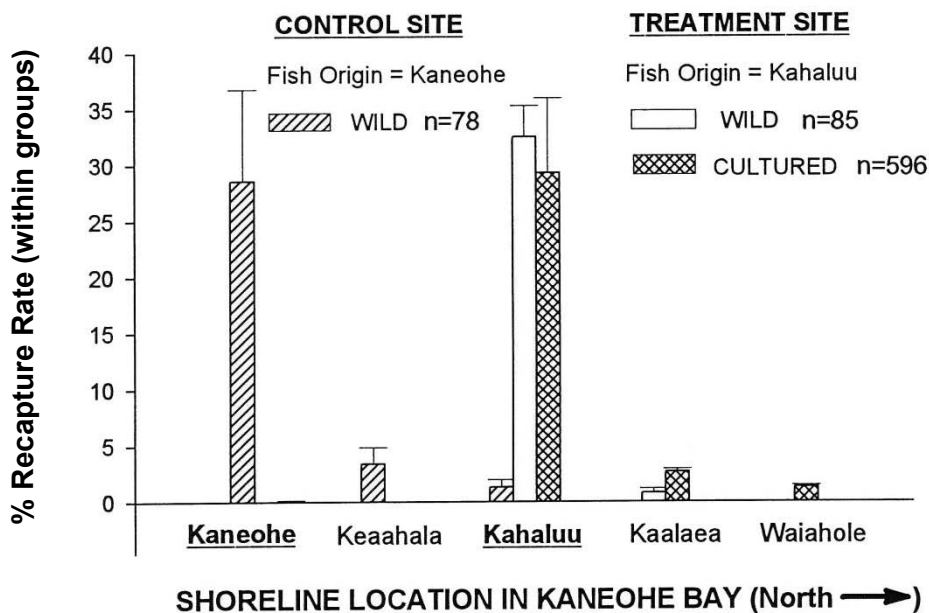


Figure 18.7. Post-stocking dispersal patterns of wild and cultured mullet from release sites, with (treatment site) and without (control site) hatchery releases of cultured mullet. Mean percent recaptured (within treatment groups) is shown with standard errors for wild fish tagged and released at the control site, wild fish released at the treatment site, and cultured fish released at the treatment site (from Leber et al. 1995).

Putting Culture-Based Mullet Enhancement into Practice in Hilo Hawaii

In Hawaii, mullet was prized as a food fish for royalty, and in modern times, it is also targeted by the recreational fishery, particularly in Hilo Harbor on Hawaii Island. Today, pole-and-line fishing for mullet is becoming a dying art (Nishimoto et al. 2007). Mullet fishing, once easily recognized by the numerous, small wooden platforms, called stilt chairs, dotting the tidal flats (Hosaka 1944) in Kaneohe Bay and Ala Wai Canal on Oahu Island, is gone. These platforms are now prohibited because of environmental regulations. Rather, small skiffs now replace the platforms for mullet fishermen. Hilo Harbor, especially the Waiākea Public Fishing Area (PFA), is one of the last strongholds of this type of mullet fishing (Fig. 18.8). Fishers use a system of a delicately balanced bobber and tandem hooks baited with algae, primarily the chain diatom, *Melosira tropicalis* (Julius et al. 2002). Fishing for grey mullet *M. cephalus* in Hilo is the only fishery in the world where diatoms are used as bait (Nishimoto et al. 2007).

In 1990, the Hawaii Division of Aquatic Resources (DAR) and Oceanic Institute (OI) partnered to develop a collaborative project to help restore the declining coastal mullet stocks using hatchery-based fisheries enhancement. Mullet fingerlings were cultured at OI (and later at DAR's hatchery facility in Hilo

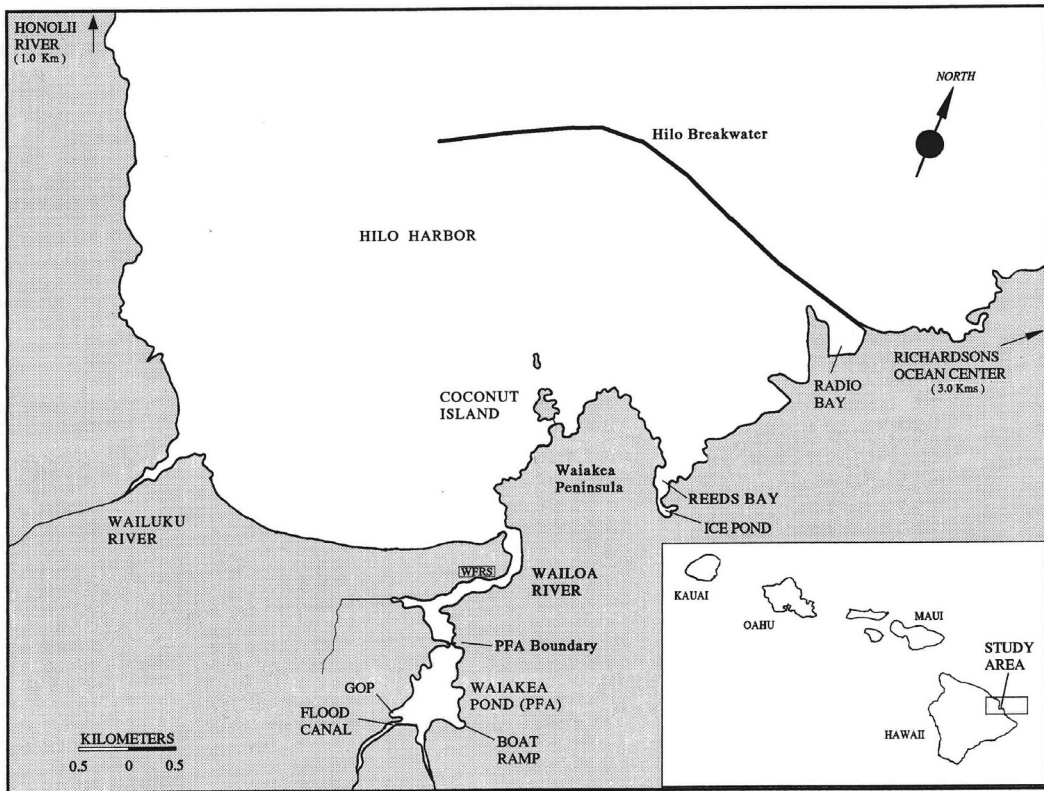


Figure 18.8. Hilo Harbor, Wailoa River Estuary and Waiākea pond.

Harbor) and shipped to the State Fisheries Research Station in Hilo for growout. Fingerlings of various sizes were batch tagged with internal Coded Wire Tags (CWT). Tagged fishes were kept for several days to allow recuperation from tagging stress.

A total of 268,228 CWT mullet fingerlings were released at various locations in Hilo Bay from August 1990 to September 2000, except for 1996 when none were released. Hatchery release impact was assessed by creel sampling the recreational fisheries (starting in 1991) and by conducting bimonthly cast-net sampling (starting in 1990) at fixed stations in Waiākea Pond, Wailoa River, and Reeds Bay, all located within Hilo Harbor (Fig. 18.8).

The results were significant: (1) The prototype marine stock enhancement experiment demonstrated that even small-scale releases can have a significant impact on wild stock abundance in the mullet fishery in Hilo; (2) The number of mullet entering the fishery was significant and was achieved annually; and (3) The Wailoa River Estuary, especially the boat launching ramp, was found to be an excellent release site (Nishimoto et al. 2007).

The number of CWT identified hatchery-released mullet in the fisher's creel ranged from a low of 3.9% in 2003 to as high as 61.1% during 1999 (Nishimoto et al. 2007). The overall average increase in the recreational mullet fishery after nine years of releasing hatchery-raised mullet was 21.7%.

The Hilo mullet project verified the potential of stock enhancement as an effective tool to replenish diminishing stocks. Based on the results of this project, several management measures were implemented to further DAR's mission of replenishing and conserving native fish stocks (Nishimoto et al. 2007).

Conclusions

The *M. cephalus* fisheries enhancement studies in Hawaii showed that recovery rates identified during the juvenile phase of the life cycle were a reasonably good indicator of the effects of release strategies on post-release survival patterns of hatchery fish caught in the fishery. Consistent with the results from studies of juveniles, the studies of hatchery mullet caught in local fisheries showed: (1) a direct relationship between size-at-release and recapture rate after summer releases; (2) higher recovery of individuals > 70 mm when released in the spring, rather than summer, and zero recovery of fish < 60 mm if released in summer; (3) that release habitat had an important effect (especially when fish were released away from the vicinity of their freshwater nursery habitats)—for example, shoreline releases in Kaneohe Bay near HIMB pier resulted in very poor (zero) recovery of any of the five size ranges of hatchery fish stocked, whereas releases within documented *M. cephalus* nursery habitats always resulted in recaptures when fish size was timed to coincide with size modes of wild mullet.

Such information from pilot experiments, about how release strategies affect survival and recruitment of cultured fish to nursery habitats and eventually to the fishery, is clearly needed to plan effective stock enhancement programs.

Marine fisheries enhancement appears to have high potential as one of the tools in the Hawaii fishery-management toolbox, if used responsibly and with a focus on managing the stocking program to achieve the stated goals of stocking and with ample attention to all of the factors that need to be considered in managing enhancement programs for success (Blankenship and Leber 1995, Lorenzen et al. 2010, Leber 2013, Sass and Allen 2014).

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