# The Role of Genetics in Managing Declining Fisheries Resources

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## ABSTRACT

With increasing human population, rising consumer demand, and intensification of industrial fishing, many valued fisheries are in decline. This review and synthesis explores how genetics informs classical fisheries management programs, especially in the context of managing declining fisheries resources. Discussing underlying principles and illustrative case studies drawn as possible from Southeast Asia, I focus upon application of genetics to: (1) define biologically based management units, (2) monitor the impacts of fisheries and fishery management actions, and (3) guide and evaluate fisheries restoration activities. Because overexploitation and declining fisheries arise from issues of economics, sociology, and politics, an effective approach to their solution must itself be interdisciplinary; application of genetic principles provides a valuable addition to such a holistic fisheries management program. Increasingly, progressive fisheries management agencies have in-house capabilities for genetic assessment and monitoring functions. Against this background, developing countries might seek to build their capacity for applied population genetics, either within fisheries management agencies or via scientific collaboration with research-oriented universities. While much progress has been achieved, the task of applying genetics to the effective management of declining fisheries is large and mostly before us.

**Keywords:** Fisheries science, fishery management, management unit, evolutionary significant unit, impact assessment, fishery supplementation

## INTRODUCTION

Wild populations of fish and invertebrates are targeted in important commercial and recreational fisheries. With increasing human population, increasing affluence driving rising consumer demand, and intensification of industrial fishing, many valued fisheries are in decline. The declines of particular fisheries are evident in terms of annual harvest exceeding maximum sustainable yields and declining catches. For example, catch of Chilean jack mackeral *Trachurus murphyi* has decreased in recent years due to depletion and reduced catch quota, northwest Atlantic cod *Gadus morhua* is considered overfished, and among the seven principal tuna species, one-third of stocks are estimated as fished above sustainable levels (UNFAO, 2014). At the global level, of the world's marine fisheries tracked by the Food and Agriculture Organization of the United Nations

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(2014), 9.9% were underfished, 61.3% were fully fished, and 28.8% were overfished in 2011. Responding effectively to the crisis in the world's fisheries (McGoodwin, 1990) poses a major challenge to fisheries managers. Effective response will depend upon mobilizing the expertise not only of classical fisheries scientists such as statisticians and population dynamics modelers, but also of fisheries economists, sociologists, policymakers, and aquaculturists. Genetics plays an increasingly important role in the management of fisheries. The goal of this review and synthesis is to explore how genetics informs classical fisheries management programs, especially in the context of managing declining fisheries resources. Discussing underlying principles and illustrative case studies drawn as possible from Southeast Asia, I focus upon application of genetics to: (1) define biologically based management units, (2) monitor the impacts of fisheries and fishery management actions, and (3) guide and evaluate fisheries restoration activities. My intent is to spark thinking on how application of the principles and tools of genetics might strengthen management of fisheries of particular interest to readers.

## Identifying units of management

The threshold issue for managing a fishery is defining it. Most often, this issue is approached on a spatial basis. While for administrative reasons, it might be most straightforward to define a fishery in terms of political units (e.g., the Indo-Pacific king mackerel *Scomberomorus guttatus* fishery of Thailand), such a unit may not have a defensible biological basis. That is, managing a fishery on the basis of political boundaries may not include all key spawning, recruitment, and fishing areas, leading to inaccurate estimation of stock size, stock-recruitment relationships, and total allowable catch. Indeed, management units for many major fisheries are not appropriately defined biologically on the basis of stock structure (Reiss *et al*., 2009). Further, many fisheries are comprised of mixtures of contributing populations, and harvest of mixed stocks can lead to overexploitation of demographically weak contributing populations. For example, coastal fisheries for Chinook salmon off the coast of California in the United States led to harvest of protected populations; recognition of this harvest through use of coded-wire tags and genetic markers led to restrictions of non-selective ocean fisheries (reviewed by Winans *et al*., 2001). These case studies support the critical point that not only demography, but also genetics is important for defining biologically meaningful management units.

*Genetic principles*. – Management units (MUs) are defined as populations that are demographically independent of one another (Allendorf and Luikart, 2007), meaning that their population dynamics depend mostly on local birth and death rates, and not on genetically effective migration from other spawning assemblages. Identification of MUs – similar to "stocks" widely referred to in the fisheries management literature – is useful for short-term management, such as managing habitat, delineating fishing areas, setting harvest rates, and monitoring population status. Fish populations often are structured at hierarchical levels, such as rivers (which each may contain an MU) that are nested within a watershed (which collectively may contain an evolutionary significant unit, or ESU, as defined below). MUs generally do not show long-term independent evolution or strong adaptive

variation. Critically, MUs represent populations that are important for the long-term persistence of the ESU to which they belong. Offering an operational definition, Moritz (1994) suggested that MUs are populations that have substantially divergent allele frequencies at many loci. One possible limitation of this approach, however, is that allele frequency differentiation cannot be interpreted directly as evidence for demographic independence (Allendorf and Luikart, 2007). A related issue is determining whether migration from nearby spawning assemblages would be sufficient to reestablish an MU should it become overharvested or extinct. Palsboll *et al*. (2007) proposed that the identification of MUs from population genetic data be based upon the amount of genetic divergence at which populations become demographically independent; MU status would be assigned when the observed estimate of genetic divergence is significantly greater than a pre-defined threshold value.

To illustrate the application of the MU concept, I consider a case study involving sockeye salmon *Oncorhynchus nerka*. Fisheries managers manage local populations separately so that an adequate number of individuals from each local population escapes catch and reproduces in order to ensure the persistence of the local populations (i.e., the MUs) that make up a fished salmon stock. Ramstad *et al*. (2004) analyzed approximately 100 sockeye salmon from 11 spawning sites throughout the Lake Clark drainage of the Bristol Bay system in Alaska at 11 microsatellite DNA loci in order to determine whether these spawning assemblages are demographically isolated. The effective population size, $N_e$, for each of the Lake Clark spawning sites was ~1000 or slightly greater. Using the criterion of at least 10% exchange (Hastings, 1993), groups spawning at these sites would be demographically isolated if they exchanged fewer than about 100 adults, which corresponds to genetic differentiation ($F_{ST}$) of 0.0025 under a classical Wright–Fisher island model of migration-drift equilibrium. Therefore, the fish at these spawning sites would constitute separate MUs if their genetic divergence, $F_{ST}$, exceeded 0.0025. The overall value of $F_{ST}$ among these sites excluding one outlier was ~0.007 (95% CI of 0.004–0.010). This being greater than the threshold of 0.0025, the authors concluded that these 11 spawning aggregations were demographically isolated and should be considered separate MUs.

MUs are more or less frequently nested within higher-order units, recognition of which is critical for conservation purposes. An evolutionary significant unit (ESU) can be defined as a population or group of populations that merits priority for conservation and separate management because of high genetic and ecological distinctiveness from other such units (Allendorf and Luikart, 2007). Different principles underlie different definitions of ESU, leading to somewhat different criteria for defining ESUs. Ryder (1986) was the first to use the term, defining ESUs as populations that represent significant adaptive variation based on concordance between sets of data derived from different techniques. The concept of an ESU came into wide discussion during deliberations regarding protection of salmonid stocks under the U.S. Endangered Species Act. Waples (1991) proposed that a population warrants protection as an evolutionarily significant unit if it satisfies two conditions: (1) it must be reproductively isolated from other conspecific units, although it does not have

to be absolutely isolated, and (2) it must be an important component of the evolutionary legacy of the species. To be considered an important component of the evolutionary legacy, a population must satisfy at least one of the following criteria: (1) the population is genetically distinct, (2) the population occupies unique habitat, (3) the population exhibits unique adaptation to its environment, or (4) if it went extinct, it would be a significant loss to the ecological or genetic diversity of the species. Dizon *et al*. (1992) regarded ESUs as populations that are distinctive based on morphology, geographic distribution, population demographic parameters, and genetic variation. Taking a phylogenetic viewpoint, Moritz (1994) defined ESUs as populations that are reciprocally monophyletic for mitochondrial DNA variation and show significant divergence of allele frequencies at nuclear loci, an approach that defines practical criteria for recognizing ESUs but does not consider adaptive variation. Focusing on adaptive variation, Crandall *et al*. (2000) regarded ESUs as populations that lack: (1) "ecological exchangeability", i.e., that have different adaptations or selection pressures (resulting in different life histories, morphologies, quantitative trait variation, habitat, or predators) and different ecological roles within a community, and (2) "genetic exchangeability", e.g., no recent gene flow, and show concordance between phylogenetic and geographic discontinuities. The concept and implementation of the ESU framework have been the subject of much discussion within the conservation and fisheries management communities (Nielsen, 1995). Differences in underlying principles and ESU criteria have led to controversy in practical conservation in some cases. Nonetheless, substantial overlap in criteria regarding reproductive isolation, adaptive differentiation and concordance across multiple data types is such that there is consensus that application of the ESU concept can support rational decision making regarding conservation of populations or groups of populations.

Perhaps the best-developed applications of the ESU concept within the aquatic sector pertain to Pacific salmonids, and I discuss such a case study. In 1994, the U.S. National Maine Fisheries Service (NMFS) initiated a Pacific coast-wide status review of sockeye salmon. The biological review team examined genetic, life history, biogeographic, geologic, and environmental information in the process of identifying ESUs. In particular, genetic data; physical, chemical, and biological characteristics of nursery lakes; sockeye salmon river entry and spawn timing; and smolt outmigration timing were found to be most informative characters for this process. Based on this examination, Gustafson *et al*. (1997) identified six sockeye salmon ESUs and one provisional ESU; I present illustrative descriptions of two. The Okanogan River ESU within the Columbia River drainage of Washington is differentiated from other such groupings because of: 1) use of a very eutrophic lake-rearing environment, Lake Osoyoos, which is unusual for sockeye salmon, 2) the tendency for a relatively large percentage of the Okanogan River sockeye salmon population to return as 3-year-olds, 3) juvenile outmigration-timing differences between Okanogan River and Lake Wenatchee-origin fish, 4) adaptation of Okanogan River sockeye salmon to much higher temperatures during adult migration in the Okanogan River, and 5) allozyme data indicating that this population is genetically distinct from other sockeye salmon in the Columbia River

drainage. The Quinault Lake ESU includes all sockeye salmon that spawn in the Quinault River drainage and develop as juveniles in Quinault Lake. Early river-entry timing, protracted adult run timing, extended lake residence prior to spawning, unusually lengthy spawn timing, unusual skin pigmentation of spawners, and genetic differences from other coastal Washington sockeye salmon are important factors characterizing this ESU.

Against the background of these two case studies involving sockeye salmon, I note that definition of ESUs remains before us for most commercially fished species. Nonetheless, once the biological units for conservation, the MUs and ESUs, have been identified, management then turns toward addressing any threats to these units, in our context, most often overfishing.

*Applications of genetic principles.* – The principles and tools of population genetics have been applied to research and practical management of several Southeast Asian fisheries, including studies aimed at identification of management units. Among studies of freshwater fishes were several on the allozyme variation of *Clarias* catfishes in Thailand. Na-Nakorn *et al*. (1998) surveyed variation at 19 isozyme loci in broadhead catfish *Clarias macrocephalus* from different parts of Thailand. Differentiation was noted among the Chiangrai-Prachinburi populations of northern Thailand and the Pattani-Yala populations of southern Thailand, with greater genetic variation in the northern group of populations. The results advanced understanding of differentiation among broadhead catfish populations within and among the respective regions and posed implications for regulation of translocations of stocks. Microsatellite loci showed high genetic variation ($H_o$ = 0.62-0.71) in these four populations (Na-Nakorn *et al*., 1999).

Na-Nakorn *et al*. (2004) examined allozyme variation in 25 natural populations of walking catfish *Clarias batrachus* collected throughout Thailand, including 12 populations from Chaophraya River basin in the center of the country, five from the Mekong River basin, one from the east, and seven from the south, as well as one population of hatchery origin. Genetic variability in terms of number of alleles per locus, average polymorphism, and individual polymorphism were significantly higher in collections from the Chaophraya River basin than from the Mekong, east, and south. The hatchery population also had relatively high genetic variation. Analysis of variation showed two differentiated groups, one from the southern provinces including a population from the east, and one from Chaophraya and Mekong river basins. Alleles peculiar to the African catfish *C. gariepinus* genome were observed in 12 of the natural populations and the hatchery population, providing evidence of genetic introgression which probably has persisted for several generations.

The economically important catfishes of the genus *Clarias* – *C. batrachus* and *C. macrocephalus* – are just two of the five clariids native to Thailand. Na-Nakorn *et al*. (2002) examined isozyme variation among four species of the catfish genus *Clarias* and found that native Thai species and the introduced *C. gariepinus* can be distinguished by horizontal starch gel electrophoresis of allozymes. An undescribed species of *Clarias* was collected from Thailand that exhibits several fixed allelic differences as

well as distinct meristic and morphological traits compared to previously described *Clarias* species.

The Mekong giant catfish *Pangasianodon gigas*, endemic to the Mekong River basin, is one of the largest freshwater fishes of the world, weighing up to 300 kg. Several species of the family Pangasiidae are important food fishes in Southeast Asia. Populations of the Mekong giant catfish and other closely related species have declined markedly due to overfishing and habitat destruction caused by anthropogenic activities. Mekong giant catfish is considered Critically Endangered by the International Union for the Conservation of Nature. Na-Nakorn *et al*. (2006) assessed the level of genetic diversity of nine pangasiid catfish species using DNA sequences of the mitochondrial 16S rRNA gene. Approximately 570 base pairs (bp) were sequenced from 672 individuals of the nine species. In general, low levels of intraspecific variation were observed, not only in the critically endangered Mekong giant catfish but also in other closely related species that are presently common and abundant. Four haplotypes were detected among 16 samples from natural populations of the critically endangered Mekong giant catfish. In spite of the limited sample size for some species investigated, the results indicated that the level of genetic variation in wild populations of the Mekong giant catfish is similar to that of some related species, which suggests that wild populations of the Mekong giant catfish might be more robust than currently thought, or that contemporary populations carry a genetic signature of historically larger population size (as discussed in the following section). The authors noted that most conservation efforts have concentrated on the Mekong giant catfish alone and little attention has been paid to its relatives. With respect to the nine pangasiid catfish species, information concerning population structure is currently lacking, especially on a finer scale, for example, between upstream and downstream locations and between tributaries in each river system.

Against this background, Ngamsiri *et al*. (2007) examined the genetic diversity of wild populations of *P. gigas* collected from Thailand and Cambodia using microsatellite DNA and 384 nucleotides of the mitochondrial DNA control region. Observed and expected heterozygosity values were relatively low in comparison with those of non-endangered freshwater fishes. The two populations from Thailand and Cambodia showed similar levels of genetic diversity at the mtDNA control region, with 13 haplotypes. The pairwise $F_{ST}$ value between the two populations based on genotype frequencies of microsatellite and mtDNA markers suggested a close genetic relationship. Although the sampling sites in this study were approximately 1200 km apart, variability of microsatellite and mtDNA markers indicated non-significant heterogeneity between the two sample sites. This finding is supported by knowledge that the Mekong giant catfish is a migratory fish; it has been hypothesized that the fish migrates north for spawning in northern Thailand, after which the fingerlings drift downstream to the Tonle Sap Great Lake, where they remain until maturity. Results of this study suggested that Mekong giant catfish formed a single population in the Mekong River, which has no natural barriers blocking its migration. The data did not support an earlier hypothesis that there are

two distinct populations of Mekong giant catfish. The authors recommended screening more samples to further test the findings of this study.

The principles of population genetics and the tools or molecular genetics also have been applied in studies identifying stock structure of Southeast Asian marine fishes and invertebrates. Orange-spotted grouper *Epinephelus coioides* contributes significantly to capture fisheries. Little is known of its population genetic structure. Antoro *et al*. (2006) quantified genetic diversity at four microsatellite loci in six *E. coioides* populations throughout their range in Thai and Indonesian waters; 250 individuals were collected from two locations in Thailand (Nakornsrithammarat and Trang) and four in Indonesia (Sibolga, Lampung, Jepara and Flores). Genetic variation was relatively low in terms of observed heterozygosities and average numbers of alleles per locus. Population structuring was significant ($F_{ST}$ = 0.074). Genetic distances between populations ranged between 0.016 and 0.086. Results of Mantel's test showed no correlation between genetic distance and geographical distance. The neighbor-joining tree clearly separated the Nakornsrithammarat population in the Gulf of Thailand from the others, which comprised two subgroups, the Trang and Sibolga populations in the Indian Ocean and the three populations in the Java Sea. The findings are useful for fishery management, i.e., genetic differentiation suggests that these populations should be managed separately. Recent bottlenecks were inferred for the Flores, Jepara, Lampung and Nakornsrithammarat populations; hence, fisheries management should be aimed at avoiding or minimizing anthropogenic threats due to overexploitation.

The Asian moon scallop *Amusium pleuronectes* occurs throughout most Indo-Pacific coastal areas. The species is of significant commercial value in Thailand, although catch statistics showed declines through the early 2000s. Mahidol *et al*. (2007) conducted a baseline survey of seven populations of *A. pleuronectes* along the coasts of Thailand using a partial nucleotide sequence of the mitochondrial 16S rRNA gene. A total of 16 unique haplotypes were detected among 174 individuals, with 27 variable sites among 534 bp sequenced. Low levels of intra-population variation were observed. The mitochondrial haplotypes clustered into two distinct groups (estimated to differ by 2.62-2.99% nucleotide divergence) respectively from the Gulf of Thailand and the Andaman Sea. Analysis of molecular variance revealed a high $F_{ST}$ value (0.765) and showed that the majority of the total genetic variance (76.03%) occurred among the Andaman Sea and Gulf of Thailand groups, with little variance among populations within groups (0.52%) or within populations (23.45%). The existence of two distinct lineages is the result of ancient, geographically driven divergence in *A. pleuronectes*. The estimated time since Gulf of Thailand and Andaman Sea populations shared an ancestral haplotype was estimated to be 1.3 -1.5 million years ago. Accordingly, the authors recommended managing the Gulf of Thailand and the Andaman Sea populations separately, discouraging transfer of individuals between the respective areas.

Aquaculture of green mussel *Perna viridis* is rapidly expanding. Culture of green mussel on the Gulf of Thailand depends on natural spat, which are believed to originate from spawning grounds adjacent to the

mouths of six main rivers. Populations of green mussel may be depleted due to various causes, including heavy exploitation and mass mortality. Prakoon *et al*. (2010) examined spatial and temporal variation at five microsatellite loci in 11 populations of green mussel in the Gulf of Thailand. The results showed moderate genetic variation of all populations in terms of average number of alleles per locus, effective number of alleles per locus, mean allelic richness, and observed and expected heterozygosity, without significant differences among populations. No indication of bottleneck or genetic disequilibrium was observed. The magnitude of genetic variation within populations suggested that culture of green mussels in the Gulf of Thailand is potentially sustainable. Genetic differentiation among spatially defined populations was low ($F_{ST}$ = 0.0046 for samples collected in January 2007, and $F_{ST}$ = 0.0088 for samples collected in July 2007), according with expectations due to its planktonic early life history and the small geographic distance between populations. The 3-4-week planktonic stage enhances gene flow among local populations by dispersal mediated by sea currents. Interestingly, analysis of molecular variance revealed significant temporal variation, and multidimensional scaling separated temporal population groups. Assignment test results showed that most recruits were from other populations. The genetic structure of green mussel was inconsistent with the stepping-stone genetic model, in which genetic distance correlates with geographic distance; rather, it exhibited "chaotic genetic patchiness" (Hellberg *et al*., 2002), where genetic differentiation among spatial populations is very low and genetic differentiation among cohorts is significant.

The surf clam *Paphia undulata* has supported an offshore fishery in Thailand since the 1970s. Most fishing sites have experienced declines in production over the past two decades, and overexploitation and low levels of genetic variation have been suggested as responsible for the low productivity of the species. Donrung *et al*. (2011) screened 330 inter-simple sequence repeat (ISSR) markers to assess the genetic diversity of 500 surf clams sampled from four fishing areas in the Gulf of Thailand and one location in the Andaman Sea. Three neighboring populations (Samut Prakan, Samut Sakorn and Samut Songkram) in the upper Gulf of Thailand exhibited moderate genetic variation, while populations from the lower Gulf of Thailand (Surat Thani) and the Andaman Sea (Satun) had relatively low genetic variability. Results of various analyses of population genetic differentiation revealed high levels of population structuring, implying that gene flow may occur between stocks in the upper Gulf of Thailand, while the other populations are more geographically isolated. The authors suggested that the relatively low amount of genetic diversity in the Surat Thani and Satun stocks may constrain their ability to survive in a changing environment. The authors suggested that reduction of fishing effort, augmentation of wild stocks, and development of aquaculture should be considered to sustain the surf clam fishery.

While illustrative, the case studies for Southeast Asian fisheries described above are not exhaustive; for example, other studies have assessed genetic variation in giant tiger prawn *Penaeus monodon* (Klinbunga *et al*., 2001) and ass'-ear abalone *Haliotis asinine* (Klinbunga *et al*., 2003). The key point is

that while notable inroads have been made, the body of work on Southeast Asian species has not defined MUs for all ecologically and economically important species. Application of the ESU concept is likewise incomplete. With much key work yet to be executed, I predict that the spatial extent of ESUs might relate closely to the major water bodies of the region – the Andaman Sea, Gulf of Thailand, and South China Sea and their tributary watersheds, a hypothesis that will need to be tested with the body of work yet before us.

**Using genetic diversity to infer demographic decline**

*Genetic principles*. – The decline of fisheries, while generally construed in terms of demographic decline, if sufficiently severe also poses genetic impacts in terms of reduced effective population size $N_e$ and associated loss of genetic variation. While several different concepts of $N_e$ have been developed, in our context, we are most concerned with the amount of allele frequency drift, and hence with the variance effective population number. Among mechanisms that decrease $N_e$ to less than census $N$ are three that are particularly common: unequal numbers of male and female breeders, high variance in family size, and genetic bottlenecks (i.e., small numbers of breeders in one or more generations). The data on breeding success required for applying demographic approaches for estimating $N_e$ frequently restrict their usefulness for practical application. Several molecular genetics-based approaches have been developed for estimating $N_e$ which are of greater practical utility in our context.

Some such models estimate $N_e$ using inferences based on the correlation of genetic variation with population size. Large populations generally will exhibit greater genetic variation, and alleles at unlinked loci will be randomly associated. Smaller populations will exhibit less variation, and because of the limited number of allelic combinations occurring in a small population, some unlinked alleles will be inherited together by chance alone. Applying this approach, Hill (1981) developed a method to estimate $N_e$ by assessing linkage disequilibrium ($D$) and correlation of alleles at different loci ($r$) in a sample drawn from a population. The correlation among alleles, $r$, is estimated as $r = D / (p(1-p) + q(1-q))^{½}$, where $p$ = frequency of allele A at locus 1, $q$ = frequency of allele B at locus 2, and $D$ = Burrow's composite measure of equilibrium, a measure of linkage. A matrix of such correlation values is constructed. $N_e$ ($D$), the $N_e$ as estimated from linkage disequilibrium data, is estimated as $N_e(D) = 1 / (3(r^2-1/s))$, where $s$ = sample size. This yields an estimate of $N_e(D)$ for each polymorphic locus; to obtain a single value of $N_e$ ($D$), the arithmetic mean value of the $r^2s$ and the harmonic mean of the $s$'s are calculated and substituted into the equation. Equations are presented by Bartley *et al*. (1992) for calculating confidence intervals and the variance of $N_e(D)$. Both $D$ and $r$ will be zero in an ideal, infinite, randomly-mating population, but will depart from zero in real, finite populations due to drift, migration, selection, and linkage. Bartley *et al*. (1992) demonstrated use of Hill's approach to wild and cultured populations of fish using isozyme marker data, and an example of application of that approach is

presented below. Waples and Do (2009) used simulated data to evaluate how highly polymorphic molecular markers affect precision and bias in the single-sample, linkage disequilibrium method of Laurie-Ahlberg and Weir (1979) and Hill (1981). Low-frequency alleles upwardly biased $N_e$, but a simple rule could reduce bias to about 10% without sacrificing much precision. With datasets routinely available (10–20 loci with 10 alleles; 50 individuals), precise estimates could be obtained for relatively small populations ($N_e < 200$), and small populations would not likely be mistaken for large ones. However, it was difficult to obtain reliable $N_e$ estimates for large populations. With microsatellite DNA data, the linkage disequilibrium method has greater precision than the temporal method (described below), unless the latter is based on samples taken many generations apart.

A second approach is embodied in models (e.g., Nei and Tajima, 1981; Pollack, 1983) using temporal variation in allele frequencies to estimate an average $N_e$ per generation over the time interval of interest. That is, these models use direct measurement of the effects of random drift to estimate what population size would have caused the observed change in allele frequencies. The approach is particularly robust over intervals of two to ten generations and when $N_e$ is small (Waples, 1989). The disadvantage of the temporal variance approach is that two temporally separated samples of the population (at least two generations apart) are needed.

A third approach to estimating $N_e$ quantifies heterozygosity excess (Pudovkin *et al*., 1996; Cornuet and Luikart, 1996). The approach is based on the observation that when populations are small, binomial sampling error produces genotype frequency differences between males and females, resulting in an excess of heterozygotes in their progeny relative to Hardy-Weinberg expectations. By quantifying the magnitude of heterozygote excess in their progeny, one can estimate $N_e$. A primary advantage of this approach is that only one sample is needed to estimate $N_e$. However, the method may be valid only for breeding systems with random union of gametes, as in broadcast spawners, and confidence intervals about $N_e$ estimates are very large. However, Luikart and Cornuet (1999) evaluated the accuracy and precision of the heterozygote excess method using simulated and empirical data for monogamous, polygynous, and polygamous mating systems using realistic sample sizes of 15-120 individuals and 5-30 markers with varying levels of polymorphism. Estimates of the effective number of breeders, $N_{eb}$, were nearly unbiased for all mating systems. However, confidence intervals were acceptably small only for polygynous and polygamous systems with fewer than 10 effective breeders unless samples included over 60 individuals and 20 multiallelic marker loci.

A fourth approach is based on coalescent theory, which grew from the classical population genetics concept of neutral evolution. In its most common application, coalescent theory is used to work retrospectively to trace all alleles of a gene exhibited by the members of a population to a single ancestral copy. Using an estimate of the mutation rate for the gene in question, coalescence-based methods can be applied to allow researchers to estimate the timing of population bottlenecks, which can provide insight into both natural and anthropogenic processes impacting aquatic or marine populations. Phylogenetic and

coalescent theory have provided a number of estimators for inbreeding effective population numbers based upon nucleotide sequence and microsatellite data (Felsenstein, 1992; Fu, 1994a,b; Kuhner *et al*., 1995; Beerli and Felsenstein, 1999). However, inbreeding effective population number is a more retrospective statistic, while variance effective population number reflects more recent demographic and population genetics processes (Crandall *et al*., 1999). A recent bottleneck would result in a large inbreeding $N_e$ and small variance $N_e$, while rapid increase in population size would result in small inbreeding $N_e$ and large variance $N_e$. Relating to our context, phylogenetic and coalescence-based methods are appropriate for estimating $N_e$ over an evolutionary time-scale (hundreds of generations). First applied in terrestrial species, coalescent approaches have been applied more recently to aquatic and marine species – examples are presented below.

A detailed consideration of the theory underlying the respective methods for estimating contemporary and historical $N_e$ is beyond the scope of this review. Interested readers may consult an excellent treatment of the topic by Wang (2005). Comparing among gametic disequilibrium, temporal variance, and heterozygote excess methods for estimating $N_e$, Schwartz *et al*. (1998) regarded the temporal variance method the most promising because it provides increasingly precise estimates of $N_e$ as the number of alleles per locus increases. They noted, however, that the performance of the three methods had not been compared under the same conditions. Schwartz *et al*. (1999) noted that coalescent, maximum likelihood, and Bayesian statistics (Nielsen *et al*., 1998, O'Ryan *et al*., 1998) can be used to estimate current $N_e$ from changes in allele frequencies assuming a known divergence time from a common ancestral populations.

Estimation of $N_e$ is eased with the availability of useful software packages. For example, NeEstimator v. 2 (Do *et al*., 2014) produces estimates of contemporary effective population size using several different methods and a single input file. These methods include three single-sample estimators (updated versions of the linkage disequilibrium and heterozygote-excess methods, and a new method based on molecular coancestry), as well as the two-sample temporal method.

*Application of genetic principles*. – A rich and growing body of literature addresses estimation of effective population size in wild populations of fish and aquatic or marine invertebrates. Indeed, studies of such populations drove much of the development of the underlying theory.

Among the four case studies considered by Bartley *et al*. (1992) using the correlation of alleles approach is one involving a declining wild Chinook salmon *Oncorhynchus tshawytscha* population. Isozyme variation at 20 loci was surveyed in a sample of 94 juvenile salmon collected as they began to descend the Sacramento River in California in the western United States. Among those fish, the estimated $N_e$ ($D$) was 85.5, i.e., considerably lower than the estimated census number $N$ approximately 2000 fish in the run. While $N_e$ ($D$) should be taken as a rough estimate, the implication is that the genetic resource of the winter-run Chinook salmon in the Sacramento River may indeed be threatened.

Modifications of models applying temporal variance approaches for estimating effective sizes of salmonid populations were made by Waples (1989, 1990), Waples and Teel (1990), and Tajima (1992). Jorde and Ryman (1995) showed how to estimate $N_e$ for brown trout populations with overlapping generations. Temporal variation models have been shown useful in estimating $N_e$ in hatchery populations of salmon (Waples and Teel, 1990) and shellfish (Hedgecock and Sly, 1990; Hedgecock *et al*., 1992, Appleyard and Ward, 2006). Exemplifying application of the temporal method to fish populations, Miller and Kapuscinski (1997) examined historical genetic changes at seven microsatellite DNA loci in a natural population of northern pike *Esox lucius* over a period of 32 years by using PCR to amplify DNA from the epithelial cells adhering to archived fish scales. Estimates of $N_e$ for the time intervals 1961-1977 and 1977-1993 were 35 and 72, respectively. For the entire interval, 1961-1993, the estimate of $N_e$ was 48 when based on a weighted mean derived from the above two estimates or 125 when calculated from 1961 and 1993 samples only. The corresponding ratios of effective size to adult census size ranged from 0.03 to 0.14. An $N_e$ of 48 over a 32-year period would imply that this population lost as much as 8% of its heterozygosity in that time. More generally in our context, the results show how genetic methods can be applied to assess historical trends in $N_e$ for a focal population.

Known by a number of common names, including Australasian snapper and silver seabream, *Pagrus auratus* is a porgie found in coastal waters of Australia, China, Indonesia, Japan, New Zealand, Philippines and Taiwan; its distribution in the Northern and Southern hemispheres are disjunct. Hauser *et al*. (2002) performed microsatellite analysis of a time series of archived scales for Australasian snapper through its history of exploitation. Their results showed loss of allelic diversity (decline in heterozygosity $H$, and temporal fluctuations in allele frequencies (Figure 1). The estimated $N_e/N$ of $\sim 10^{-5}$, while low, is not unknown for marine organisms with "sweepstakes" recruitment in which but a few spawners contribute most of the subsequent recruitment. The authors predicted that loss of genetic diversity could affect the productivity, persistence, and adaptability of New Zealand snapper.

The European plaice *Pleuronectes platessa* is a commercially important flatfish in the North Atlantic Ocean. Hoarau *et al*. (2005) used DNA from archived otoliths (1924-1972) and fresh material from 2002 collections to estimate $N_e$ in plaice. Their results showed an estimated $N_e$ of 20,000 in the North Sea and 2,000 in Iceland, and an estimated $N_e/N$ ratio of $\sim 10^{-5}$. The authors noted that while populations from 1924-1960 were in Hardy-Weinberg equilibrium, populations after 1970 were not. Genotyping errors and Wahlund effects due to mixing of differentiated populations were ruled out, and the authors attributed the observed heterozygosity deficit to inbreeding. The emergence of inbreeding coincided with increase in fishing mortality after World War II (Figure 2). The apparent inbreeding in populations as large as those of plaice is surprising to most population geneticists; clearly, we need to advance our understanding of social and mating behaviors in the species.

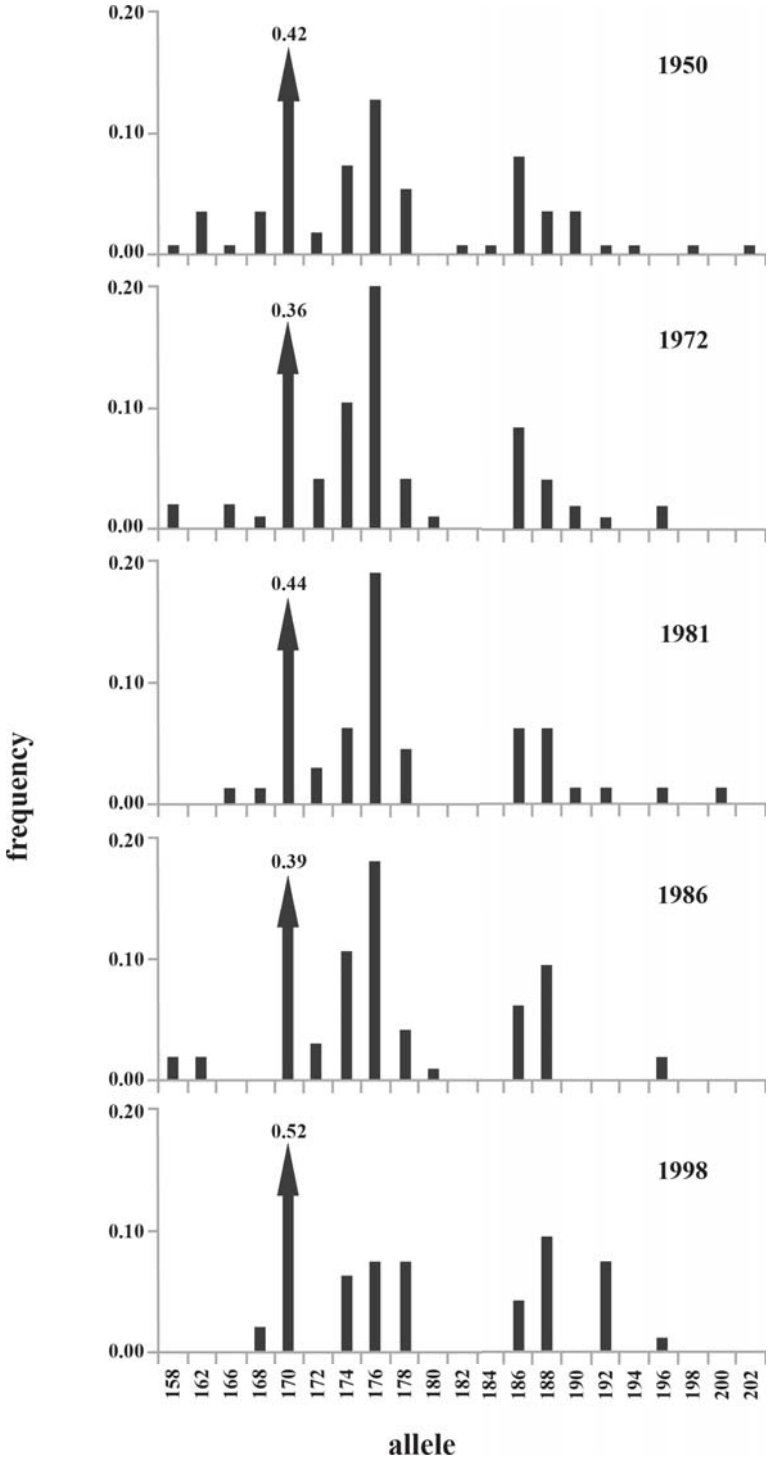


Figure 1. Allele frequencies at the *GA2B* locus in Tasman Bay, New Zealand snapper *Pagrus auratus*, showing the effects of random genetic drift and consequent loss of microsatellite alleles (Hauser *et al*., 2002). The frequency of the most common allele (170 bp) is indicated above the arrow.

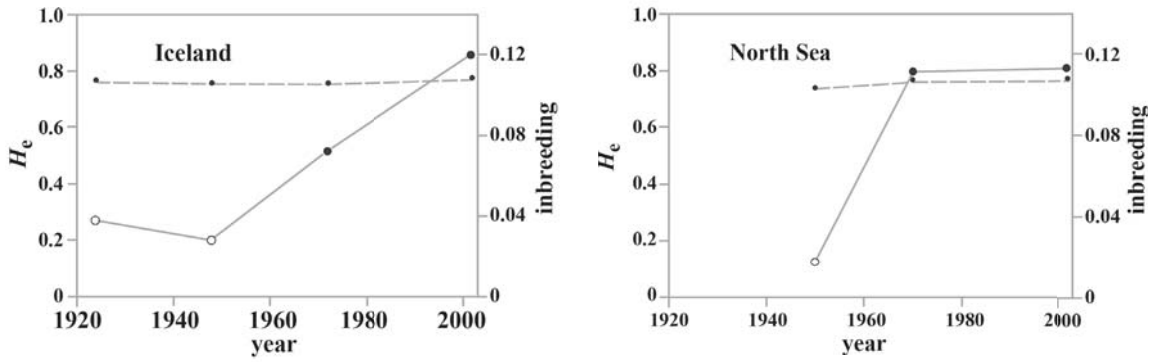


Figure 2. Temporal changes in genetic diversity $H_e$ (dashed line) and inbreeding (solid line) in North Sea plaice, *Pleuronectes platessa* (Hoarau *et al*., 2005). Open circles: not significant; filled circles: significant at $p < 0.001$. Inbreeding is defined as the excess of homozygotes $(MLH_{obs} - MLH_{exp})/MLH_{exp}$.

O'Leary *et al*. (2013) estimated the effective number of breeders for winter flounder *Pseudopleuronectes americanus*, a formerly abundant and heavily fished species, in six estuaries in New York on the U.S. east coast. Screening 11 microsatellite markers with 6–56 alleles per locus, the estimated effective number of breeders for 1–2 years was remarkably small, with point estimates ranging from 65–289 individuals. Excess homozygosity was detected at 10 loci in all bays ($F_{IS}$ = 0.169-0.283), and individuals exhibited high average internal relatedness (mean IR = 0.226). After testing for and ruling out alternative explanations, such as technical and sampling artifacts, the authors suggested that inbreeding is very common in all bays sampled. Results of this study support the hypothesis that even historically common marine fishes can be prone to inbreeding, an issue that should be considered in fisheries management and conservation plans.

The American horseshoe crab *Limulus polyphemus* is a large marine invertebrate that is native to the Atlantic and Gulf coasts of the United States and to the Yucatan Peninsula of southern Mexico. The dynamics of individual populations of horseshoe crabs likely has been affected by the last Ice Age, a more recent climatic fluctuation termed the Little Ice Age, and overexploitation in historical fertilizer, bait and biomedical fisheries. Faurby *et al*. (2009) screened variation at 12 microsatellite DNA loci in 1218 crabs from 28 localities, analyzing the variation using Bayesian coalescent methods. Results of their analyses (Table 1) showed strong declines in population sizes throughout the species distribution, except for the isolated population on the Yucatan Peninsula, where an increase in population size was inferred. Demographic bottlenecks in populations in the core of the North American range were variously attributed to colonization of newly opened habitat after the Ice Age, effects of the Little Ice Age from 1450-1600 AD, and overharvest in historic times. The results of this study indicate the importance of considering both historic climatic changes and ongoing anthropogenic effects in efforts to understand population dynamics of declining fisheries.

Table 1. Estimated effective population sizes (and associated 95% confidence limits) for horseshoe crab *Limulus polyphemus* estimated by Faurby *et al*. (2009): $N_O$ = estimated mode current effective population size (confidence limits), $N_A$ = estimated mode ancestral effective population size, $X_a$ = estimated mode time between current and past population size in generations, and T = point estimate of year of initial change in population size (sampling year 2005AD, generation time = 13 years). Results are from five replicate runs from the program MSVAR1.3 using the exponential model of Storz and Beaumont (2002).

| **Region** | $N_O$ | $N_A$ | $X_a$ **(generations)** | **T** |
|---|---|---|---|---|
| Hog Bay, Maine | 20 (0-123,026) | 100 (0-6,607) | 22 (0-7,307) | 1719AD |
| Upper New England | 63 (1-2,535) | 398 (6-15,885) | 28 (0-1,884) | 1641AD |
| Mid and South Atlantic | 5,620 (29-219,834) | 43,820 (7,215-91,834) | 5 (0-155) | 1940AD |
| Mid Atlantic | 3,939 (28-143,546) | 23,452 (12-37,564) | 4 (0-84) | 1953AD |
| South Atlantic | 1,012 (0-18,732) | 1,841 (0-71,453) | 8 (0-193) | 1901AD |
| Florida Atlantic | 398 (3-125,895) | 2,511 (12-63,533) | 3 (0-184) | 1966AD |
| Florida Gulf | 126 (10-11,220) | 251 (13-15,849) | 11 (0-180) | 1862AD |
| Mexico | 3,162 (13-158,483) | 25 (0-324) | 27 (7-1,217) | 1654AD |

Estimates of genetically effective population size have been made for several Southeast Asian species, leading to interesting insights into natural history, the effects of fisheries upon populations of fish, and the vagaries of our tools of inferrence. Among freshwater fishes, although fishery data show a significant decline in catches of pangasiid catfishes, analysis of mitochondrial 16S rRNA sequence data by Na-Nakorn *et al*. (2006) suggested demographic expansion of populations of all species. A possible explanation for these conflicting observations is that the genetic data may reflect the signature of past populations but not their present status. For example, the unexpected, relatively high number of haplotypes observed in the present population of Mekong giant catfish could reflection large historical population size. The estimated times of expansion for the Pangasiid catfish populations range from 0.08 to 1.44 million years ago, in the early to mid-Pleistocene. Focusing on the Mekong giant catfish, Ngamsiri *et al*. (2007) estimated an $N_e$ of 2245 in Thailand and Cambodian populations. The census number $N$ for the population is not known. Annual yields of the fish caught at Chiang Khong, Thailand, the northernmost collection site, had declined since 1983. The genetic variability suggested that the effective population size may have been reduced. The relatively low genetic variation in orange-spotted grouper observed by Antoro *et al*. (2005) could have been a result of population isolation, small population size, or historical population bottleneck. The evidence for the occurrence of a bottleneck in their samples was inconclusive, however, because the results varied among the four tests that they applied: the sign test, standard difference test, Wilcoxon sign-rank test, and the mode-shift test. Results of the standard difference test indicted recent bottlenecks in the Lampung, Jepara, and

Flores populations, and of the Wilcoxon test bottleneck for the Nakornsrithammarat population. The Wilcoxon test provided relatively high power, while the standard difference test may have suffered from the low number of loci used in the study. The sign test showed no evidence of a recent bottleneck. Observation of conflicting results among tests and models of mutation are not particularly uncommon in studies of this type. The authors noted that the allele frequency distribution was approximately L-shaped for all populations, which implied that no recent bottlenecks occurred in these populations.

**Using genetics to infer mechanism for fishery decline**

*Genetic principles*. – A key goal for management of declining fisheries is to address the cause (s) of decline so that the fishery can most effectively be rebuilt. While the principal cause of decline is often overfishing, which can be addressed by reducing fisheries-related mortality (i.e., by reducing fishing effort, raising minimum size limits, or protecting spawning by limiting the harvest spatially or temporally), in some cases the decline may be driven by mechanisms not related to overharvest. Knowing the factor driving the decline may suggest management options, or indicate the impracticability of options under consideration. For example, while recruitment failure often drives the decline of fisheries, the causes of that failure may be difficult to infer. Did outbreak of disease impose excessing natural mortality? Was there a toxic spill at a key nursery site? At a fundamental level, how can fisheries scientists draw a linkage between decline of a fishery and a causal mechanism? Several approaches might be adopted to characterize the response of fish or invertebrates to a putative environmental stressor. For example, an individual exposed to a stressor may respond by changes in gene expression, or through biochemical or immunological pathways. Focusing on genetic pathways, gene expression assays developed over the past decade have enabled us to screen the expression of up to thousands of genes simultaneously.

DNA microarrays contain thousands of synthetic DNA sequences immobilized on a solid surface, often glass (Figure 3a). Each such sequence corresponds to a transcript for a gene expressed by the species for which that array was designed. Fundamentals of design, construction and screening of microarrays are discussed by Peatman and Liu (2007) and Zhang *et al*. (2009). Briefly, to screen a microarray, a small number of individuals are exposed to a stimulus (the treatment group) or not (the control group). mRNA is collected from each group and labelled with a fluorescent dye particular to that group. A solution of the labelled transcript then is applied to the microarray under precisely controlled conditions. The solution is removed and each DNA feature spotted on the microarray is screened for fluorescence using a laser and a light collector. Comparison of the level of fluorescence of the two respective dyes yields a quantitative estimate of the degree to which expression of a given gene was up- or down- regulated in treatment individuals relative to control individuals. The key point in our context is that using microarrays, fisheries scientists can identify the molecular and biochemical pathways through which animals respond to environmental stressors, including temperature, salinity, nutrient limitation, disease, toxins and other

stressors. Fisheries scientists might identify the molecular genetic pathways through which animals respond to environmental cues for life-cycle events such as migration, gonadal and gametic maturation, and spawning. Limiting the utility of the approach, however, microarrays have been developed for only a limited number of species of interest to fisheries scientists, including zebrafish *Danio rerio* (Ton *et al*., 2002) and salmonids (Rise *et al*., 2007).

A microarray experiment might identify scores to hundreds of genes whose expression was induced in response to a stimulus or stressor. Such microarray results should be validated by quantitative RT-PCR or other methods (Rise *et al*., 2007). For each candidate gene, both technical replicates (each reaction run in triplicate) and biological replicates (10 individuals for each condition) should be run to determine the degree to which the putative change in gene expression is consistent. The biochemical pathway within which the gene is expressed should be identified to assess its relevance to the stimulus or stressor. Once a candidate gene is linked to an environmental driver, the qRT-PCR assay might be applied to monitor gene expression in the field. A body of ongoing research is aimed at identifying a battery of gene expression assays for given organisms for use in biological monitoring for classes of environmental stressors, e.g., freshwater mussels potentially vulnerable to toxic chemical spills.

*Application of genetic principles.* – Since their development in the late 1990s, microarrays have been applied to a widening array of questions (Rise, 2007), some pertinent to this review. The emerging field of toxicogenomics uses genomics approaches to determine how exposure to toxic substances affects molecular pathways and biological processes in individuals and populations, for example, for rainbow trout *Oncorhynchus mykiss* (Koskinen *et al*., 2004; Krasnov *et al*., 2005). Study of host transcriptome response to infection provides basic information on immune response pathways (Rise *et al*., 2004; Ewart *et al*., 2005), and may inform development of vaccines or therapeutics. A growing effort addresses response of fish to changing environmental conditions, for example hypoxia (Ju *et al.,* 2007).

The use of microarrays to approach practical issues facing fisheries management is exemplified well by a study of sockeye salmon in Canada. Sockeye salmon productivity in the Fraser River of British Columbia declined to the point that 2009 returns were less than the demographic replacement rate. Contributing to the collapse were massive (40-95%) mortalities of adult sockeye salmon before spawning, both in the Fraser River *en route* to spawning areas and on spawning grounds. The causal mechanisms of this mortality were unknown. Miller *et al*. (2011) undertook a functional genomics study in an attempt to advance mechanistic understanding of the role of salmon condition (before mortality events occur) on migration and spawning success in the river. The authors took non-lethal biopsies from ocean- and river-caught salmon and did watershed-scale biotelemetry to follow the fate of tagged fish migrating upstream. Functional genomics and tracking of individuals were used to correlate physiological profiles with failed migrations and reproduction. Gene expression was profiled in gill tissue using a small microarray with 6000 features and a larger one with

32,000 features. Expression profiles were compared between fish that arrived at spawning areas (successful migrants) with those that perished *en route*.

Levels of expression of key genes, with high levels shown as "warm" colors and low levels shown as "cold" colors in Figure 3b, differed among ocean-tagged fish (panel A), freshwater-tagged fish (panel B), and spawners (panel C), as well as among successful and unsuccessful migrants or spawners. The functions of particular genes had been annotated, i.e., characterized by comparison of their DNA sequences to those of genes of known function; cryptic names for those genes are indicated on the right side of the figure. Because the patterns of up- or down-regulation were complex, the authors applied principal components analysis to compare and contrast gene expression profiles among individuals that were or were not successful in migration to freshwater, migration up-river to spawning grounds, and spawning.

Contrasting patterns of gene expression were associated with successful or unsuccessful migration to freshwater; principal component 1 (PC1 in panel A of Figure 3b) explained 12% of the variance. Upper river mortalities were twice as common in fish showing the PC1 "negative" profile of gene expression and three times less common in the fish showing the PC1 "positive" profile. The difference corresponded to an odds ratio of 13.5; i.e., those ocean-tagged fish showing a mortality-related genomic signature (the PC1-negative profile) had a 13.5-fold greater chance of dying *en route*. The authors noted that while in seawater over 200 km from the river, up to 60% of the fish contained a gene expression signature that was predictive of subsequent in-river survival. In river-tagged fish migrating in freshwater to the spawning grounds, the same PC1 genomic signature was associated with a 50% increase in mortality before reaching the spawning grounds in one of the three stocks tested. At the spawning grounds, the same genomic signature was associated with 3.7-fold greater odds of dying without spawning.

Relating the raw findings to mechanisms driving the decline of sockeye salmon that motivated the study, the authors noted that 25 biological processes were differentially affected in the ocean, 34 in freshwater, and 47 at spawning. Critically, 65% of the affected biological processes were consistent with responses to viral infections. These findings indicate that Fraser River sockeye salmon containing the genomic signature correlated with elevated mortality may have been responding to viral infection. The key point in our context is that Miller *et al*. (2011) showed a mechanistic signature associated with premature mortality of salmon measurable over a month to less than one week ahead of death and throughout the Fraser River. They hypothesized that the genomic signal associated with elevated mortality was in response to a virus infecting fish before river entry and that persisted to the spawning areas.

Evolved resistance to infectious disease (including viruses, bacteria, myxozoans, and some fungi) may reduce susceptibility, but as barriers to pathogen or parasite distributions are weakened and as environments become more stressful, previously unexposed populations may be impacted and pathogenicity enhanced. Miller *et al*. (2014) reviewed the evolutionary and ecological impacts of

Figure 3a

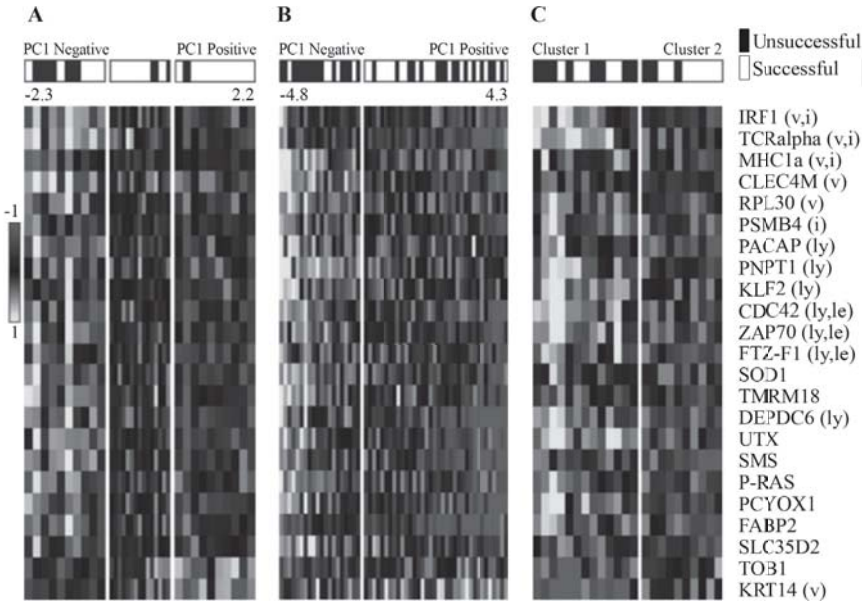


Figure 3b

Figure 3. A. Insertion of a DNA microarray into a reader. The microarray itself is the 1-cm square in the center of the holding cassette. B. Heatmaps for 23 annotated genes significantly associated with survivorship in all three studies of Miller *et al*. (2011). For (A) ocean-tagging and (B) freshwater-tagging studies, heatmaps reflect the ranking of individuals along the PC1 axis (rotational values shown above the heatmap), in which the associations with fate were strongest at the ends of the PC1 distribution, which are demarcated by white blocks. Migration success, depicted in the top bar, was reduced at the PC1-negative end of the axis for both studies. (C) For the spawning study, the heatmap reflects the relationships depicted by clustering significant genes from the freshwater PC1-based *t* test, with the white block differentiating the two emergent clusters and the black/white bar reflecting unsuccessful and successful spawners, respectively. (Left) Expression levels are indicated by the color scale ranging from (up-regulated) yellow to (down-regulated) blue. Missing values are shown in light gray. (Right) Literature associations of genes with viruses (v), immune response (i), lymphocytes (ly), and leukemia (le) are depicted with letter codes in parentheses next to gene names.

infectious diseases in wild salmon and suggested ways in which recently developed technologies can identify the pathogens and parasites of greatest potential importance. They presented four case studies regarding pathogen impacts on adult salmon migration success, impact of river warming on parasite replication, and infection status on susceptibility to predation. They argued that the future health of wild salmon populations should be considered in a holistic context that includes the cumulative or synergistic impacts of multiple stressors. These approaches will identify populations at greatest risk and most critically in need of management.

Fisheries scientists had long observed that after migration from the open ocean to the coast, Fraser River sockeye salmon would mill about just off shore for 3–6 weeks prior to migrating up the Fraser River. This milling behavior changed abruptly starting in 1995, decreasing to only a few days in some years; this early migration had dramatic consequences that necessitated risk-averse management strategies. Early-migrating fish consistently suffered the high mortality noted above. Hinch *et al*. (2012) synthesized the results of multidisciplinary research aimed at understanding early migration, associated high mortality, and utilization of the scientific results by fisheries managers. Early migrants are more reproductively advanced and ill-prepared for osmoregulatory transition upon their entry into freshwater. Gene expression profiles observed using microarrays indicated that many early migrants were also immunocompromised and stressed, exhibiting a profile consistent with viral infection. Early migration brings the salmon into the river when it is 3-6$^{o}$C warmer than historical norms, which for some late-run populations approaches or exceeds critical maxima, leading to the collapse of metabolic and cardiac scope, and to mortality. As peak spawning dates have not changed, the surviving early migrants tend to mill in warm lakes near to spawning areas; this results in the accumulation of many more thermal units and longer exposures to freshwater diseases and parasites compared to fish that delay freshwater entry by milling in the cool ocean environment. Experiments have confirmed that thermally driven processes are a primary cause of mortality for early-entry migrants. The Fraser River late-run sockeye salmon early migration case study illustrates the linkages between salmonid gene expression, physiology, behavior, and environment, as well as the contribution of genetics to the multidisciplinary effort to understand such phenomena.

As noted at the outset of this case study, annual population-specific estimates of in-river loss for Fraser River sockeye salmon have fluctuated dramatically. Before drawing linkages between disease, river entry timing and survival, lack of knowledge about the causes of mortality and limited capacity to predict run size forced managers to take a precautionary approach, restricting harvests in attempts to compensate for subsequent in-river mortality. Reduced harvests in years when fish were relatively healthy then were viewed as unnecessarily restrictive by some stakeholder groups. More recently, improved predictability and greater understanding of causal mechanisms enabled fisheries managers to achieve a better balance between achieving conservation goals and maintaining harvest opportunities (Cooke *et al*., 2012). While predictive tools still remain somewhat empirical, by documenting a sound physiological basis for the variation

in mortality, conservation physiology research has driven a shift in management approach. Future management may be more directly linked to fish physiology. Cooke *et al*. (2012) suggested that screening for biomarkers that predict in-river fate in marine test fisheries could inform management of potential survival of a migrating population and that adjustments to harvest could be applied proactively to marine and lower river fisheries, although they noted that such 'real-time' management approaches have yet to be realized.

Gene expression profiling studies have led to insights into causes of mortality in other systems as well, increasing our understanding of those systems. Mass mortality in bivalve communities occurs with varying severity and temporal irregularity. The causes are of interest because bivalves often are used as sentinels of ecosystem quality, and because mass mortality events can impact ecological communities and fisheries dependent upon them. Mass mortality in Pacific oyster *Crassostrea gigas* is believed to be linked to environmental factors, especially high water temperature. Chaney and Gracey (2011) sought to identify gene expression markers differentiating between oysters that would survive or die during a mortality event. Identification of such biomarkers could offer insights into the pathophysiology of mortality, as well as providing tools to investigate the timing of mortality events with regard to prevailing environmental conditions. The authors took haemolymph samples nonlethally from individual oysters at intervals throughout the summer, creating a temporal series of samples for each individual. Mortality was monitored concurrently. Using a microarray, the authors identified a signature of gene expression that distinguished oysters that survived the summer from individuals that suffered mortality. Near-mortality oysters could be distinguished from longer-lived oysters by the elevated expression of genes associated with cell death, lysosomal proteolysis, and cellular assembly and organization. The signature was validated by comparing biomarkers generated across two separate mortality events. The gene expression pattern that was observed in hematocytes provided biomarkers of deteriorating health, but did not offer significant insights into what caused mortality or the source of the primary lesion. The authors suggested that clues to the primary cause of mortality probably lie in a tissue or cell type other than haemolymph, which will inevitably require lethal sampling of animals.

Gene expression profiling has emerged as a powerful discovery tool in ocean change biology (Evans and Hofmann, 2012), increasing mechanistic understanding of ecologically significant processes. The ocean's uptake of anthropogenic $CO_2$ drives chemical reactions that alter seawater chemistry, increasing the concentration of hydrogen ion ($H^+$) and decreasing the abundance of carbonate ion ($CO_3^{2-}$). Marine organisms vary considerably in their response to ocean acidification. Evans *et al*. (2013) monitored natural pH dynamics within an intertidal habitat in the Northeast Pacific, demonstrating that upwelling exposes resident species to pH regimes not predicted to occur elsewhere until 2100. They cultured the progeny of adult purple sea urchins *Strongylocentrotus purpuratus* collected from this region in $CO_2$- acidified seawater representing present-day and near-future ocean scenarios and monitored gene expression using a microarray with complete coverage of the *S. purpuratus* transcriptome (28,036 genes). The gene expression patterns observed

showed that *S. purpuratus* larvae may alter the bioavailability of calcium and adjust skeletogenic pathways to sustain calcification in a low pH environment. Additionally, larvae use different strategies for coping with different magnitudes of pH stress, initiating a robust transcriptional response to present-day pH regimes, but only a muted response to near-future conditions. The authors concluded that an enhanced capacity to cope with present-day pH variation may not translate into success in future oceans.

**Genetics as the basis for hatchery-based fishery supplementation**

*Genetic principles*. – Faced with a fishery that has declined, managers often choose to boost it demographically by stocking fish or shellfish propagated in hatcheries. Several different contexts have been distinguished in the genetics literature (Miller and Kapuscinski, 2003). Supplementation is the use of artificial propagation, while conserving genetic resources, for the goal of augmenting or restoring targeted populations. Augmentation is the rehabilitation of a demographically depressed population by adding hatchery-produced fish to a naturally reproducing population. Restoration is the reestablishment of a self-sustaining population in an ecosystem no longer occupied by that species.

Technical advances in fish tagging and mariculture have been such that purposeful marine stock enhancement can be approached with greater purposefulness than previously. To increase the likelihood of success and avoid repetition of mistakes made in historic marine stock enhancement programs, Blankenship and Leber (1995) described a responsible approach to developing, evaluating, and managing marine stock enhancement programs. The components of the approach include: (1) prioritizing and selecting target species for enhancement, (2) developing a species management plan that identifies harvest opportunity, stock rebuilding goals and genetic objectives, (3) defining quantitative measures of success, (4) using genetic resource management to avoid deleterious genetic effects, (5) employing disease and health management practices, (6) considering ecological, biological, and life-history patterns when forming enhancement objectives and tactics, (7) identifying released hatchery fish and assessing stocking effects, (8) using an empirical process for defining optimum release strategies, (9) identifying economic and policy guidelines, and (10) using adaptive management to learn and adjust practices as the program goes forward. The authors presented case studies on Atlantic cod *Gadus morhua*, red drum *Sciaenops ocellatus*, striped mullet *Mugil cephalus*, and white seabass *Atractoscion nobilus* to illustrate application of their approach to stock enhancement.

Achieving conservation of genetic resources while supplementing wild populations requires awareness of four types of genetic hazards related to hatchery-based propagation and rearing, as well as implementation of programmatic activities to minimize the associated risks (Busack and Currens, 1995). Hazard is defined as an adverse genetic consequence of hatchery activities on a population, and the associated risk is the probability that the hazard will be realized. Type 1 hazard is extinction, the complete loss of a population; in the context of hatchery-based propagation of a focal population,

extinction could result from a catastrophe due to disease, power failure, or loss of water. Extinction also could result from removal of all wild spawners for captive propagation, followed by failure of hatchery-propagated fish to survive or reproduce after release. Type 2 hazard, loss of within-population genetic variation, may result from random genetic drift or inbreeding. This process could be realized within the hatchery due to inadequate attention to crosses made and family representation in the progeny or in the receiving environment if the offspring of a limited number of spawners overwhelm the genetic variability of the supplemented population (Ryman and Laikre, 1991). Type 3 hazard, loss of between-population genetic variation, can result from gene flow at excessive levels among source and recipient populations. Type 3 hazard can result in loss of genetic uniqueness of populations and possible reduction in the fitness of the fish due to outbreeding depression. Type 4 hazard, domestication selection, results from adaptation of a cultured fish stock to the aquaculture environment, which may decrease the fitness of hatchery fish and their descendants in the wild.

Against this background, several groups of authors have developed and presented guidelines for hatchery-based fishery supplementation that are convergent regarding the underlying genetic principles, but that differ in terms of application to fished or unfished taxa in marine or freshwater contexts. The U.S. Fish and Wildlife Service and National Marine Fisheries Service (2000) adopted a joint policy to guide controlled propagation of species listed under the Endangered Species Act. While drawing most heavily on the literature for salmonids, Miller and Kapuscinski (2003) presented general guidelines for hatchery supplementation. Jones *et al.* (2006) presented genetic management guidelines for conservation and captive propagation of freshwater mussels, and George *et al.* (2009) for propagation and translocation for freshwater fishes for purposes of conservation. While the respective guidelines vary to reflect the life histories of the species groups and the intent of the programs at issue (augmentation of imperiled taxa or of commercially fished populations), they tend to share certain key recommendations. Broodstock should be selected to show the greatest similarity to the population targeted for management in terms of genetic lineage, life history, and ecology of the originating environment. Sufficient numbers of broodstock should be chosen to minimize differences among the hatchery and wild spawning populations and to avoid loss of genetic variation due to random genetic drift. Hatchery managers generally should avoid keeping captive stocks for more than one generation in order to avoid domestication effects. Mating schemes, including factorial mating designs, should be adopted in order to maximize effective population size $N_e$ and to minimize risk of inbreeding. Rearing practices should be aimed at producing fish that are qualitatively similar to those of the targeted population in terms of size, behavior, physiological status, heath, and other key attributes, and should be released at locations where the target populations' naturally produced fish occur at that life stage. For elaboration, readers should consult the guideline most pertinent to their interest.

Artificial propagation and stocking programs can be large, expensive undertakings, and hence it is important to know whether

they succeed in achieving their objectives within changing ecological and economic contexts. Hence, it is appropriate that program managers conceive and operate such programs within a context of adaptive management. Adaptive management (Holling, 1978) is an approach aimed at gaining experience about complex system behavior and actively learning as a system is managed. Widely applied in many engineering contexts, adaptive management also has been successfully applied in management of Pacific salmonids and timberlands in western North America. Adaptive management has four steps: (1) setting management goals, (2) problem analysis and policy design, (3) implementation of management actions, and (4) monitoring the system to determine whether and to what degree management goals were achieved. Critically, adaptive management is not aimed at passing through these steps just the one time, but rather to go through them repeatedly, each time learning and adjusting goals, technical approaches, and implementation as appropriate to as successfully as possible manage the behavior of a complex and changing system. Whether or not conceived as an exercise in adaptive management, as we shall see below, some fisheries augmentation programs have included monitoring and adaptation as the program moves forward, including (demographic and genetic) monitoring of augmented populations.

The principles laid out under Blankenship and Leber's (1995) responsible approach to marine stock enhancement have gained widespread acceptance, and have been used to guide hatchery-based stock enhancement in many countries. Evaluating implementation 15 years after the publication of the responsible approach framework, Lorenzen *et al*. (2010) found, however, that most enhancement programs remain weak in four particular areas: (1) while fishery stock assessments and modelling are integral to exploring the potential contribution of stocking to achieving fisheries management goals, they are lacking in most stock enhancement efforts in coastal systems; (2) establishing a governance framework for enhancements is largely ignored in stocking programs, thereby diminishing opportunities for integrating enhancement into fishery management; (3) involvement of stakeholders in planning and execution of stocking programs from the start is key, but is only rarely an integral part of program development; and (4) adaptive management of stocking is critical to achieving goals, improving efficiencies, and understanding and controlling the effects of stocking on fisheries and wild stocks, but is not well integrated into most enhancement plans.

*Application of genetic principles*. – Many fisheries that have suffered declines have been targeted for demographic boost using cultured or translocated individuals, yielding a rich literature of experience. Here, I highlight a few chosen case studies in order to illustrate key points.

A first set of cases aims at demographically boosting fisheries that are not exploited, but that are the focus of conservation concern. The southeastern United States exhibits high levels of endemic aquatic biodiversity. The upper Tennessee River watershed is an aquatic biodiversity hotspot; the Clinch River alone has 118 fish and 45 mussel species. However, this biodiversity is imperiled by threats ranging from siltation to dams to toxic spills. At the

same time, some aquatic ecosystems have recovered to the point that extirpated populations can be restored. Against this background, "conservation aquaculture" is going forward at federal and state hatcheries, universities, and non-governmental organizations. Conforming to George *et al.*'s (2009) guidelines for propagation and translocation for freshwater conservation, ongoing conservation aquaculture activities aim at restoring non-game fishes and freshwater mussels of the upper Tennessee basin. Among notable successes, Abrams Creek in Great Smoky Mountains National Park formerly held 67 of the Park's 79 known fish species. However, the stream was treated with rotenone in 1957 to improve fishing for non-native rainbow trout, causing the loss of many native fishes; only 35 species persisted or returned from tributaries. A multi-agency restoration project was initiated in 1986, focusing on federally endangered smoky madtom *Noturus baileyi*, yellowfin madtom *Noturus flavipinnus*, Citico darter *Etheostoma sitikuense*, and spotfin chub *Erimonax monachus*. Spotfin chub was the only species with a source population large enough to support translocations; the madtoms and darter would require propagation to produce numbers sufficient for reintroductions. Over 20 years, 3,000 smoky madtoms, 1,600 yellowfin madtoms, and 3,500 Citico darters were released into Abrams Creek. Ongoing monitoring has shown that the species are reproducing, recruiting, and dispersing into suitable habitat (Schute *et al.*, 2005), although it took 10 years before in-stream recruitment was observed. Several attempts to establish threatened spotfin chub have proven unsuccessful.

In another restoration-oriented project, Virginia Tech University and the U.S. Fish and Wildlife Service have partnered since 1997 to develop methods for the culture of imperiled freshwater mussels. Over the ensuing years, the annual production of juveniles has gradually increased. The project typically produces 10,000 or more juveniles of 6-10 species each year for release to natal rivers in the upper Tennessee basin to restore populations destroyed by toxic spills. Since the partnership began, over 1.5 million juveniles of 39 species have been produced. All aspects of broodstock sourcing and collection, propagation, and release of progeny conform to genetic management guidelines for captive propagation of freshwater mussels (Jones *et al.*, 2006). Persistence and growth of planted mussels have been observed for several years, and more recently, signs of their spawning and recruitment (Carey *et al.*, in review).

A second set of cases aims at boosting populations that are the object of commercial fisheries. Some of these commercial fisheries augmentation programs are quite large. Examples include Japanese chum salmon *Oncorhynchus keta*, Japanese red sea bream *Pagrus major*, Alaskan pink *Oncorhychus gorbuscha* and other salmon, and Gulf of Mexico red drum. These programs are of such scale that multiple hatcheries produce juveniles for stocking, sometimes under central coordination and sometimes not. In some cases, managers and culturists simply stock cultured fish and subsequently seek evidence of heightened catch. Critically to the point of this review, sometimes the results of the augmentation are subject to genetic monitoring supporting the evaluation and purposeful implementation of augmentation. As we shall see, genetic monitoring has shown both failures and successes for augmentation of fished species.

Red abalone *Haliotis rufescens* is the largest, most common abalone along the northern coast of California, where following a decline, it is harvested on a restricted basis. Numerous out-plantings of hatchery-derived red abalone seed exhibited poor survival. In 1979, the California Department of Fish and Game out-planted 42,000 3-cm abalone at San Miguel Island, California. Allozyme frequencies (Gaffney *et al*., 1996) showed that while out-planted abalone dominated the catch for years afterwards, the $N_e$ for the targeted population (< 10) actually was reduced by out-planting. The finding called into question the efficacy of the stocking program as then practiced and led to changes in its design and implementation.

Atlantic salmon *Salmo salar* has declined through much of its range, leading to establishment of multiple hatchery-based restoration efforts, some accompanied by genetic monitoring for efficacy. One such effort focused on rebuilding the population in the Connecticut River in the northeastern United States, which lost its Atlantic salmon population as a result of human activity 200 years ago. Cultured stocks, derived mainly from the Penobscot River in Maine, were employed to restore the population. An annual run of salmon has been re-established (Letcher and King, 2001), although the population is not yet self-sustaining. Within the monitoring portion of the program, an approach termed family-printing (Letcher and King, 1999) was applied, in which multilocus microsatellite genotypes are used to identify offspring from a specific targeted stock of randomly-mated parents. Whole families were stocked in given tributaries and family-printing was used to see which tributaries supported high recruitment. Changes in allozyme and microsatellite alleles frequencies and age-at-maturity have been reported for the stock adapted to the Connecticut River as compared to the donor Penobscot River population (Martinez *et al*., 2001; Spidel *et al*., 2004). Ayllon *et al*. (2006) examined variation at microsatellite loci in historical scale and modern tissue samples to evaluate the degree and direction of any genetic changes that occurred in the introduced population. These authors found the current genetic composition of the Connecticut River population very similar to that of its Penobscot River donor population, with no differences in heterozygosity, mean number of alleles per locus, number of migrants, or $F_{ST}$ values between the two populations, suggesting that no genetic bottlenecks had occurred during the restoration program. The estimated effective number of breeders per generation for both the Penobscot and Connecticut Rivers, more than 200 in each river, was large enough to preserve genetic variability without expecting short-term gene drift. Large effective population sizes were estimated to have been used for the restoration program in the Connecticut River during the 1990s (Spidel *et al*., 2004). Ayllon *et al*. (2006) extended this inference to the 1970s and 1980s, suggesting that managers carefully followed the principle of using sufficient numbers of spawners to preserve genetic variability.

Genetic markers have been applied to assess the success of Southeast Asian fisheries augmentation programs. For example, the findings from Na-Nakorn *et al*. (2006) posed important implications for the design and implementation of the artificial breeding program supporting augmentation of Mekong giant catfish populations. Analysis of 127

hatchery-bred individuals showed that common haplotypes seemed to dominate the cultured stock and did not show the level of genetic variation observed in the wild. Thus, the broodstock management plan should be modified so that broodstock include all available haplotypes in order to maximize effective population size and maintain the genetic integrity of *Pangasianodon gigas* held in captivity and of augmented populations.

In a third set of cases, genetics can be applied for genetic marker-assisted fishery restoration. In some cases, managers have chosen to demographically boost a particular stock within a mixed-stock fishery. In such a case, broodstock selected for propagation must represent the target stock. Identification of suitable broodstock candidates must be carried out on the basis of genetic markers characterizing that stock. I know of two cases where maker-assisted restoration of a target stock has been implemented.

Olson *et al*. (2000) evaluated use of genetic markers for estimating population identity of pink salmon *Oncorhynchus gorbuscha* in a supportive breeding program on the Dungeness River in Washington state in the northwestern United States. Fall-run pink salmon of the Dungeness River were the target of restoration, but they presumably overlap in terms of timing with an earlier summer run. Microsatellite markers provided an accurate estimate of population identity; when applying a log-likelihood ratio criterion of greater than 1.3, 74.8% of individuals were correctly assigned to population. The results suggested that genetic markers with many alleles are preferred when populations exhibit little genetic differentiation. The key point in our context is that use of microsatellites to select fall-run pink salmon for supportive breeding confirmed the run-timing overlap and prevented unintentional crosses between the two populations.

There are historic reports of a thriving walleye *Zander vitreus* population with many large (5-8 kg) individuals in the New River of Virginia in the eastern United States. However, construction of Claytor Dam in 1939 and other dams on New River led to a crash of the walleye population and to subsequent stocking of non-native walleye stocks sourced from elsewhere in the United States. A number of high-water years led to catches of large fish in the 1990s, leading to impetus for study of the fishery. Studies of fish movement from 1997-1999 showed three spawning locations in the river and of genetics showed stock differentiation on the spawning grounds. Genetic markers not seen in other walleye populations included a unique mitochondrial haplotype and high frequencies of particular alleles at two microsatellite loci. The latter provided the basis for genetic marker-based restoration. Broodstock candidates are collected from spawning locations known to have high frequencies of the native stock. Fisheries managers tag and hold broodstock candidates while geneticists screen microsatellite markers for alleles indicative of the native stock. Culturists then spawn selected individuals bearing native alleles and rear the young in hatcheries. Through 2009, 600,000 fry and 900,000 fingerlings had been stocked in the New River in Virginia and West Virginia. Notably in our context, Palmer *et al*. (2007) evaluated the success of the restoration program. Catch of walleye in spring electrofishing and fall gillnet surveys increased. Genetic screening of samples indicated

increased frequencies of alleles diagnostic for the native walleye population. Further, angler catches indicated increased fishing effort for walleye and increased catch and harvest.

The upshot of these case studies is that with careful attention to principles of population genetics in the design and implementation of hatchery-based supplementation programs, declining fish populations can be rebuilt while conserving genetic resources.

## CONCLUSION

Just as the problems of overexploitation and declining fisheries arise from issues of economics, sociology, and politics, an effective approach to their solution must itself be interdisciplinary (McGoodwin, 1990). Against this background, I have aimed to make the case that application of genetic principles provides a valuable addition to a holistic fisheries management program. Such applications include use of genetic baseline data to define biologically meaningful management units, monitoring the impacts of fisheries and fishery management actions, and guiding and evaluating restoration activities. Newer applications promise advances in the basic science underlying fisheries management – e.g., applications of functional genetics to infer the causes of fishery declines.

Increasingly, progressive fisheries management agencies have in-house capabilities for genetic assessment and monitoring functions. Against this background, developing countries might seek to build their capacity for applied population genetics, either within fisheries management agencies or via scientific collaboration with research-oriented universities. A key aspect of such capacity building is the training of nationals to do applied genetics work, and some Southeast Asian countries have made notable gains in this regard. It may be necessary to partner with institutions outside the country for such training. While much progress has been achieved, the task of applying genetics to the effective management of declining fisheries is large and mostly before us.

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