

Chapter 12

Genetics of shellfish on a human-dominated planet

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Introduction

Consideration of the genetics of shellfish in the context of aquaculture and the environment requires one to pull focus back from the current scene on the ecological stage, in which humans have realized global ecological dominance, to the broader coevolutionary play, in which shellfish and humans find themselves. This chapter reviews and synthesizes the science behind three issues pertinent to the genetics of shellfish in aquaculture and the environment: (1) genetic impacts of translocations or introductions of shellfish; (2) the genetic impacts of interbreeding between hatchery stocks and wild populations, such as might arise in either shellfish restoration

programs or commercial aquaculture; and (3) domestication and genetic improvement of shellfish for aquaculture.

Shellfish aquaculture and conservation

A long view of global mollusc aquaculture suggests that we tackle these issues in reverse order, starting with domestication. Over the past three decades, world supply of living marine resources has kept pace with human consumption only because of an exponential growth in aquaculture production (FAO 2009a, 2009b). Shellfish aquaculture is a substantial part of this global growth, though it

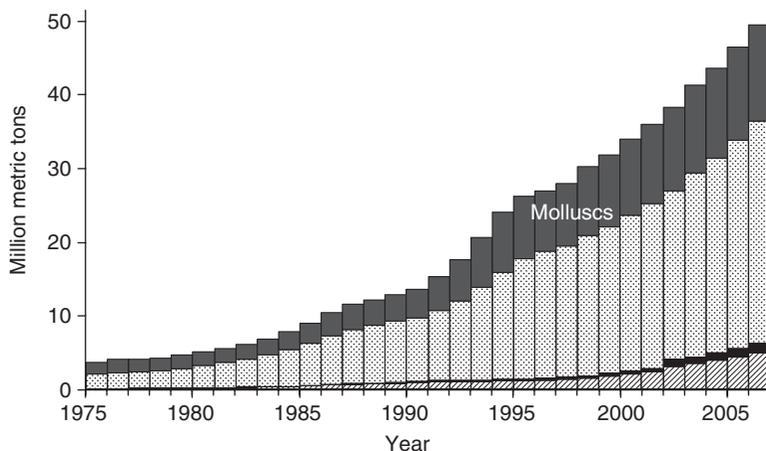


Figure 12.1 World aquaculture production by FAO categories of aquatic animals (FAO 2009b). At top and labeled are molluscs exclusive of cephalopods, followed in order beneath by freshwater and diadromous fish, demersal marine fish, and crustaceans. All other categories (miscellaneous aquatic animals, pelagic marine fish, marine fish, and cephalopods) have contributions too small to be seen. Molluscs, primarily bivalves, comprise a substantial though decreasing share of global production.

has been slowly losing ground to finfish production (Fig. 12.1). In 1975, mollusc production was 1.5 million metric tons (Mt), 42% of total aquatic animal production of 3.6 Mt. Mollusc production had double-digit annual growth during the early 1990s, peaking at 25% in 1993, but has average a more modest annual growth rate of 4.9% over the past decade. Finfish production over the past decade, on the other hand, has expanded at an average annual rate of 6.0%, with the result that mollusc production comprised only 26% of aquatic animal production in 2007, the latest year for which statistics are available (FAO 2009b). Unless mollusc aquaculture moves offshore into the open ocean (Buck 2007; Stevens et al. 2008), future expansion will be constrained by competition for alternative uses of coastal regions, where most humans live, and by the degradation of coastal and estuarine habitats. Thus, shellfish aquaculture will likely need to become more efficient, producing more from less area. Genetic improvement and domestication are proven routes to increasing the efficiency of agricultural production.

At the same time and for many of the same reasons, there is increasing concern about conservation of natural shellfish diversity and the preservation of the ecological services that shellfish provide. The issues of human-mediated translocations and introductions and of interbreeding of hatchery and wild populations will be treated together, as impacts on shellfish conservation.

Genetics of wild and cultivated shellfish

The genetics of bivalve mollusc populations has a long history, pertinent pieces of which have been reviewed (Gaffney 1996; Gosling 2003). Early work on molluscan genetics focused on the geographic structure of natural populations (e.g., Koehn et al. 1976; Buroker 1983; Karl and Avise 1992; Cunningham and Collins 1994; McDonald et al. 1996), on positive correlations of fitness-related traits, such as growth and survival, with allozyme heterozygosity (“allozyme-associated heterosis,” e.g., Zouros et al. 1980; Fujio 1982; Gaffney 1994; Zouros

and Pogson 1994; David 1998; Launey and Hedgecock 2001), on quantitative genetics of complex traits, such as growth, survival, and disease resistance (Lannan 1980; Newkirk 1980; Sheridan 1997; Langdon et al. 2003; Dégremont et al. 2007; Hedgecock and Davis 2007), and most recently on the development and application of genomic tools and resources (Hedgecock et al. 2005, 2007a; Cunningham et al. 2006; Saavedra and Bachere 2006; Gaffney 2008; Tanguy et al. 2008). Genetic evidence for population subdivision has a bearing on the issues of translocations, introductions, and interactions of hatchery and wild populations to be discussed. The causes of allozyme-associated heterosis, the heritability of production characteristics, and the development of genomic approaches to understanding complex traits and physiological ecology are relevant to discussion of domestication and genetic improvement. More recent genetics research has focused on temporal genetic variation in natural shellfish populations (e.g., Hedgecock 1994; Li and Hedgecock 1998; Hedgecock et al. 2007b), as a consequence, potentially, of sweepstakes reproductive success (SRS) (Hedgecock 1994; Hedrick 2005; Sargsyan and Wakeley 2008). Large variance in reproductive success both in natural and hatchery-propagated populations must be considered in evaluating the potential genetic interaction between the two (Gaffney et al. 1993; Boudry et al. 2002).

Domestication of shellfish

Domestication of terrestrial plants and animals was based on a remarkably small number of species (Diamond 1997). Wheat, corn, and rice, for example, provide more than 60% of edible dry matter and 50% of protein consumed by humans (Harlan 1995). Compared with the rather restricted diversity of terrestrial animal domesticates (i.e., birds and mammals), the phylogenetic diversity of cultivated aquatic animals encompasses a full range of bilaterally

symmetric animal life, from the Lophotrochozoa (gastropods and molluscs) and the Ecdysozoa (crustaceans) to the Deuterostomia (sea urchins, sea cucumbers, and teleost fish). In part, this diversity simply reflects the greater phyletic diversity of aquatic life, but it also arises because domestication of shellfish, despite cultivation dating back to Roman times (Günther 1897), is in its infancy.

Shellfish are protodomesticates

Only a handful of aquatic species—all finfish (goldfish, common carp, and perhaps the aquarium fishes, guppies, and neon tetras; Balon 1995, 2004)—have been domesticated, in the sense that they are profoundly changed from wild progenitors. Under this evolutionary definition of domestication, no shellfish species can be considered domesticated. Recently, Duarte et al. (2007) claimed that aquatic species, including over 60 species of bivalve molluscs, were being rapidly domesticated, but their definition of domestication—“breeding, care, and feeding of organisms are controlled by humans”—falls far short of the profound-change criterion that most students of domestication set. Shellfish aquaculture is clearly in the protodomestication phase (Harris and Hilman 1989), in which diverse species of shellfish are no more than exploited captives (Clutton-Brock 1981). The trend documented by Duarte et al. (2007) merely reflects the growth of aquaculture globally and of interest in culturing diverse bivalves. Although the history of shellfish domestication will likely take another century to write, the history of terrestrial domestication suggests that the number of shellfish domesticates will be much smaller than the 60 species of bivalves identified by Duarte et al. (2007). Obvious species on which domestication efforts might be focused are the seven bivalve molluscs that are among the top 40 aquaculture species in the world (Fig. 12.2).

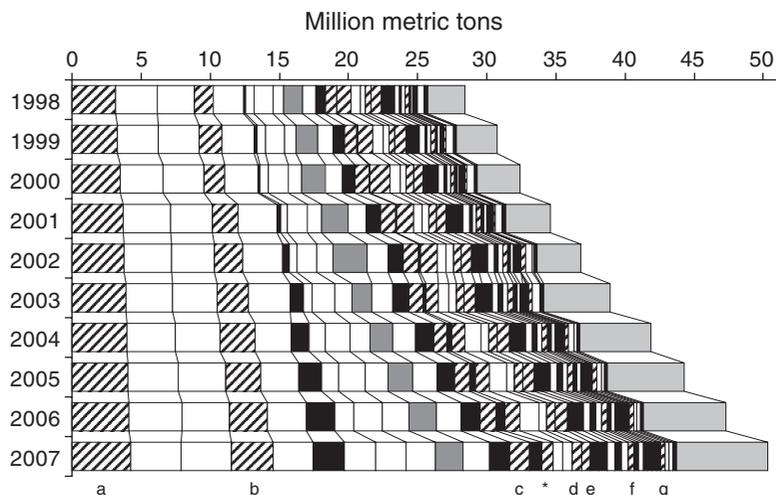


Figure 12.2 Aquaculture production by species (data from FAO 2009b). Segments in each annual bar are coded as follows: cross-hatched segments, filter-feeding bivalve molluscs (a = *Crassostrea gigas*; b = *Ruditapes philippinarum*; c = *Patinopecten yessoensis*; d = *Sinonovacula constricta*; e = Mytilidae; f = *Anadara granosa*; g = *Perna viridis*; * = miscellaneous marine molluscs); unfilled segments, herbivorous or omnivorous freshwater fishes; filled segments, carnivorous fish or crustaceans; dark gray, miscellaneous freshwater fishes; light gray, other species. Species, by rank: (1) Pacific cupped oyster; (2) silver carp; (3) grass carp (white amur); (4) Japanese carpet shell (Manila clam); (5) common carp; (6) whiteleg shrimp; (7) catla; (8) bighead carp; (9) Nile tilapia; (10) freshwater fishes; (11) crucian carp; (12) Atlantic salmon; (13) yesso scallop; (14) *Pangasius* catfishes; (15) marine molluscs; (16) roho labeo; (17) milkfish; (18) constricted tagelus (razor clam); (19) sea mussels; (20) rainbow trout; (21) giant tiger prawn; (22) white amur bream; (23) Chinese mitten crab; (24) channel catfish; (25) blood cockle; (26) black carp; (27) tilapias; (28) marine fishes; (29) amur catfish; (30) red swamp crawfish; (31) snakehead; (32) green mussel; (33) mrigal carp; (34) cyprinids; (35) Japanese eel; (36) flathead gray mullet; (37) Japanese sea bass; (38) sea snails; (39) giant river prawn; (40) Mandarin fish; (41) other species.

At present, the literature on bivalve genetics, breeding, and genomics for the top seven species (*Crassostrea gigas*, *Ruditapes philippinarum*, *Patinopecten yessoensis*, *Sinonovacula constricta*, Mytilidae, *Anadara granosa*, and *Perna viridis*) is extremely thin compared with knowledge of terrestrial domesticates (Table 12.1). With the exception of the Pacific oyster, for which 72 papers were found and for which genomic resources are accumulating at a rapid pace (Hedgecock et al. 2005; Gaffney 2008), little attention is being paid to the other top bivalve species (Saavedra and Bachere 2006; Tanguy et al. 2008). Mussels are a distant second, with 29 papers, but the greater proportion of papers in the genomic resources category than in the quantitative genetics and marker development cat-

egories reflects, in part, greater interest in the ecology rather than culture of these bivalves. Thus, the knowledge base for domesticating top-producing shellfish species is quite narrow.

Advantages of domestication

Why domesticate shellfish? Improvements in the characteristics and yields of domesticated species are well known and widely appreciated. Physiological changes in shellfish species will likely be just as profound as those in terrestrial domesticates and perhaps more dramatic for their rapidity since the principles of breeding are now well developed, widespread, and aided by modern molecular and genomic approaches. More importantly, substantial

Table 12.1 Number of papers retrieved from the Institute for Scientific Information (ISI) Web of Science pertaining to breeding, genetics, and genomics for the top seven globally cultured bivalve species (FAO 2009b).

| Species ¹ | Quantitative genetics and breeding ² | Molecular markers ³ | Genomic resources ⁴ | Union |
|--------------------------------|---|--------------------------------|--------------------------------|-------|
| <i>Crassostrea gigas</i> | 32 | 43 | 26 | 72 |
| <i>Ruditapes philippinarum</i> | 0 | 1 | 1 | 2 |
| <i>Patinopecten yessoensis</i> | 2 | 7 | 1 | 7 |
| <i>Sinonovacula constricta</i> | 0 | 1 | 0 | 1 |
| Mytilidae | 7 | 8 | 17 | 29 |
| <i>Anadara granosa</i> | 0 | 0 | 0 | 0 |
| <i>Perna viridis</i> | 0 | 2 | 0 | 2 |

¹Searches done with an OR statement of common and scientific names (e.g., "*Ruditapes philippinarum*" OR "Manila clam" OR "Japanese carpet shell").

²Separate searches with topics "heritability," "selective breeding," and "genetic improvement" combined.

³Separate searches with topics "microsatellite*" and "SNPs" combined.

⁴Separate searches with topics "linkage map*," "QTL," "Marker assisted selection," "ESTs," "Microarray*," "BAC," "Transcriptomic*," "Genomic Resources" combined.

levels of genetic diversity still exist in extant natural populations, from which most aquaculture species continue to be derived. Improvements in yield of ~10–15% per generation have been obtained for fish (Bentsen et al. 1998; Gjedrem 2000) and for the Pacific oyster (Langdon et al. 2003). Insofar as domestication and genetic improvement of aquatic species will increase the efficiency and sustainability of aquaculture, long-term research toward these ends must be encouraged.

Much of the initial improvement in production characteristics of aquatic species, such as the Atlantic salmon and the Pacific oyster, has been based on additive genetic variance and realized by standard methods of individual or family selection. In this form of breeding, animals with desired characters are bred, and they pass on to their progeny alleles that contribute positively to the desired traits; such selection steadily enriches the population with beneficial alleles, increasing the proportion of animals with desired traits and the population mean. Family selection has nearly doubled the yield of Pacific oysters over that of wild stocks in large-scale field trials (C. Langdon, pers. comm.; Fig. 12.3). There is considerable evidence that selection is effective in improving

shellfish, but there is also evidence that inbreeding and inbreeding depression can rather easily wipe out gains from selection.

Nonadditive variance is also likely to be important in shellfish species. Nonadditive variance causes offspring to deviate from the average of the parental trait values. It is relatively more important in the breeding of common carp, perhaps because additive genetic variance has already been fixed during the longer history of carp domestication (Wohlfarth 1993). Nonadditive variance appears to be relatively more important in highly fecund marine shellfish. The Pacific oyster, for example, shows hybrid vigor (heterosis) for yield (Fig. 12.4) that is as dramatic as that in maize (Shull 1908; Crow 1998), particularly since it emerges, not from crosses among major land races, but from crosses among partially inbred lines derived from a single wild population (Hedgecock and Davis 2007). Dramatic heterosis for yield in oysters is associated with equally dramatic levels of inbreeding depression (Evans et al. 2004), which results from a large load of deleterious recessive mutations (Bierne et al. 1998; Launey and Hedgecock 2001). A large mutational load in shellfish is consistent with the

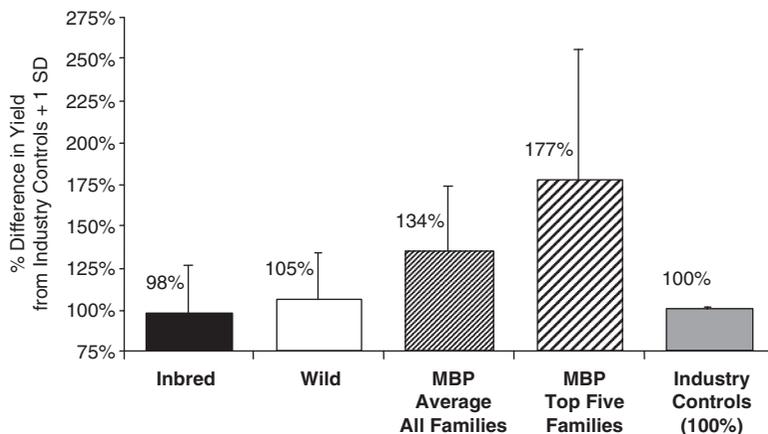


Figure 12.3 Yield of families after two generations of selection by the Molluscan Broodstock Program (MBP) (Langdon et al. 2003; C. Langdon, pers. comm.), compared with yields from inbred families, wild families, and industry controls, to which all values are scaled. The average of all MBP selected families and of the top five families suggest that substantial improvements in yield can be achieved with family selection.

hypothesis that heterosis results from dominance of wild type over deleterious recessive alleles in hybrids (Crow 1998). A large mutational load was also predicted by G. C. Williams' (1975) elm-oyster model for the advantages of sexual reproduction in species with high fecundity and high early mortality. Since high fecundity and high early mortality are the dominant life history features among marine fish (Winemiller and Rose 1992) and invertebrates (Thorson 1950), we might expect considerable scope for genetic improvement of shellfish to come from crossbreeding of inbred lines derived from natural populations of such species.

Commercial crossbreeding for higher shellfish yield

A detailed analysis of four factorial crosses of inbred lines of the Pacific oyster showed that both additive and nonadditive components of variance are important contributors to oyster yield (Hedgecock and Davis 2007). Yield generally increased with the general combining ability (GCA) of inbred lines, a measure of

additive genetic variance, as expected from the response to family selection reported by Langdon et al. (2003). However, high-yielding hybrids with high, positive special combining ability (SCA), a measure of nonadditive genetic variance, and little GCA were also observed. The nonadditive genetic component was often the largest component of variance in yield, accounting for a remarkable 88–96% of yield variance at the seed stage in one cross. Even more remarkable in this study was the finding of large differences in yield between reciprocal hybrids ($AB \neq BA$), which accounted for 21–51% of yield variance and comprised maternal as well as nonmaternal components of variance. The bases of these significant reciprocal effects merit further study, but their existence clearly suggests that the direction of crossbreeding makes a substantial difference in yield.

Shellfish breeding should clearly take advantage of both additive genetic variance, through selection among inbred lines, and nonadditive genetic variance, by identification of elite inbred lines for crossbreeding (Hedgecock and Davis 2007). A breeding scheme to accomplish these dual goals can be conceptualized in the

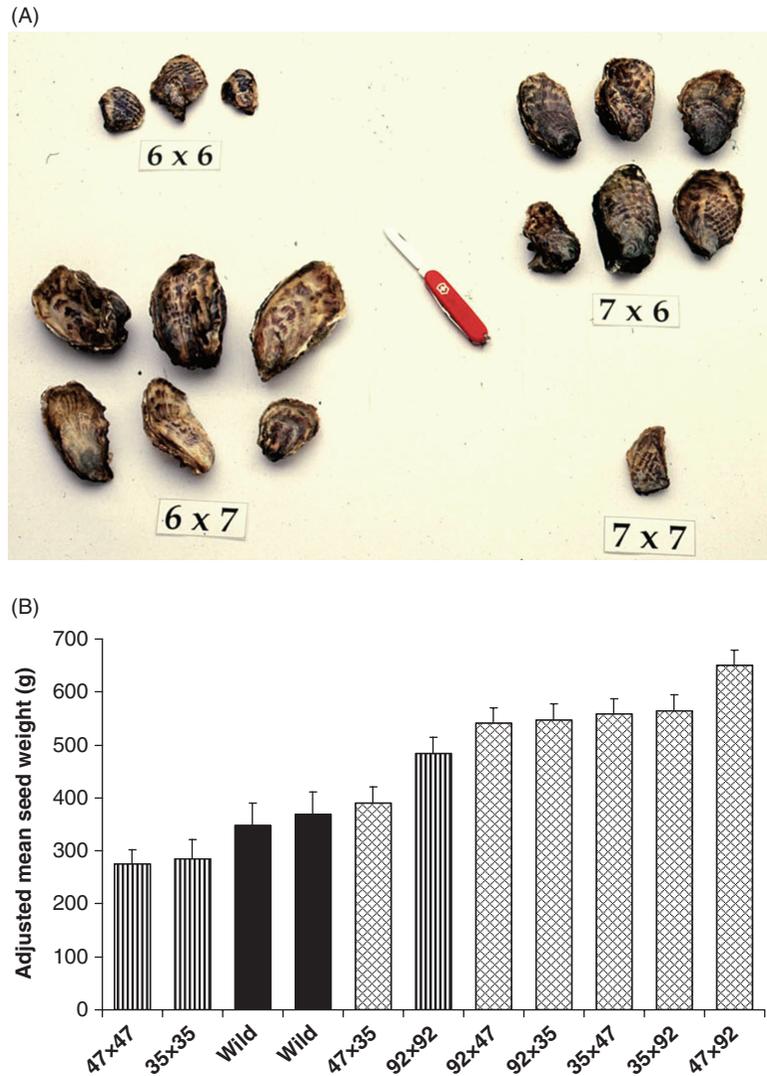


Figure 12.4 Pacific oysters show dramatic growth heterosis (hybrid vigor): (A) Inbred and hybrid Pacific oysters produced by a factorial cross of two partially inbred lines, 6 and 7; (B) comparison of bulk seed weight (adjusted for numbers, ~100, 90-day-old seed, and for initial weight) for top hybrids (cross-hatched bars), their elite inbred parents (vertically striped bars), and the wild stock typically cultured on the U.S. West Coast (solid bars). Five of six hybrids yield significantly more than the current stock cultured, with the best hybrid (47 × 92) yielding nearly twice what the current stock yields.

form of a biomass pyramid, at the top of which is an intense breeding program to develop and select among new inbred lines, to measure the crossbreeding potential of these lines, in order to identify elite lines for further commercial testing (Fig. 12.5). All these activi-

ties require minimal biomass but maximal value per individual. A broodstock oyster, which has resulted from at least two generations of breeding, with a pedigree verified by typing of molecular markers (see below), and which has been conditioned for spawning in a

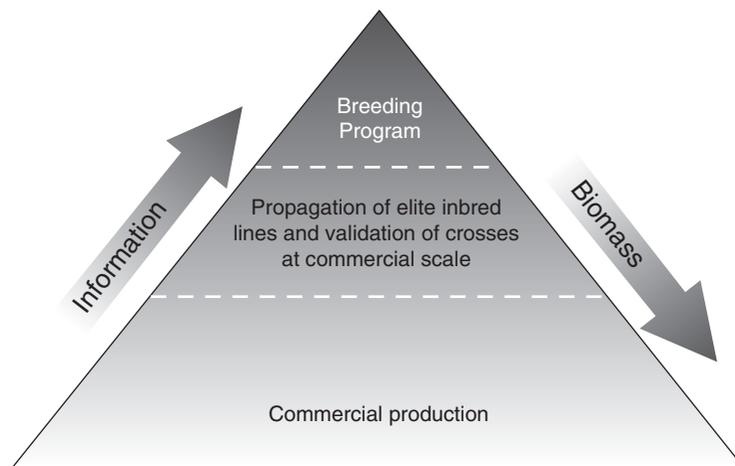


Figure 12.5 A conceptual breeding program for shellfish that captures both additive and nonadditive genetic variance for yield. At the top of the biomass pyramid, breeders develop inbred lines, select among them, cross them and test their hybrids, and make chemical triploids of elite hybrids. The middle tier of the program tests elite crosses on farms, collects yield data, propagates elite inbred lines, and makes and distributes high-yielding seed to production farms. Information flows up the pyramid; biomass increases going down the pyramid.

hatchery, may be worth hundreds to thousands of dollars. At the farm level, on the other hand, each oyster may be worth \$0.25. To get from the research level to the production level requires an intermediate step for amplifying the numbers of inbred broodstock to that required for commercial spawns. Also, at this level, hybrid seed would be produced for large-scale field trials and tracked and evaluated through the production system. A uniform nomenclature for lines and crosses is required to track hybrid stocks through the production system. Information but not broodstock would flow from the middle tier to the top tier of the biomass pyramid, affecting selection among inbred lines. A bioeconomic framework is essential but does not yet exist for determining the value of a commercial shellfish breeding program.

Also required for any shellfish breeding program are genotyping to confirm pedigrees. Hedgecock and Davis (2007) reported that 10.5% of prospective parents for their crosses had multilocus, microsatellite marker genotypes that were incompatible with their parents; another 13.8% were excluded because

of missing information or uncertainty in the genotyping. Curole and Hedgecock (2007) report slightly lower rates of contamination (8.1%) and of uncertain or incomplete genotyping (6.6%) over a larger sampling period. These figures show the necessity of validating parent pedigrees for controlled crosses. Contamination of experimental bivalve populations has been reported previously (e.g., Mallet et al. 1985; Foltz 1986; Zouros et al. 1992; Li and Guo 2004) and must be confronted in all shellfish breeding and experimental research.

As the oyster industry has shifted heavily in the past decade toward production of triploid oysters (oysters carrying three rather than the normal two sets of chromosomes; Nell 2002), a commercial crossbreeding program must also focus on production of triploid as well as diploid hybrid seed (Hedgecock and Davis 2007). Increasing production of triploids, which are effectively sterile (Allen and Downing 1986), is a welcome trend toward sustainability, as discussed in the next section, since it isolates genetically improved farmed stock from natural shellfish populations.

Triploid seed is currently produced by fertilizing diploid eggs with sperm from tetraploid males (Guo et al. 1996). Existing tetraploid stocks of the Pacific oyster were derived haphazardly from a rather narrow genetic base of wild diploid oysters. To take full advantage of nonadditive genetic variance for yield, commercial shellfish breeding programs will need to build new tetraploid lines that incorporate general and specific combining abilities. This can be done by chemically inducing triploidy in eggs from diploid hybrid females fertilized with sperm from an unrelated inbred line and using the resulting three-line triploids and an additional unrelated inbred line to found four-line tetraploid stocks. The suggestion from the maize literature is that heterosis compounds with higher ploidy levels: just as the hybrid AB is better than inbred AA at the diploid level, ABC > AAB at the triploid level, and ABCD > AABC at the tetraploid level (Birchler et al. 2003).

While methods for husbandry and breeding of bivalve molluscs have been steadily improving, genomic resources have also developed at a rapid rate. Development of genomic resources promises to accelerate discovery of phenotypic-genotypic associations, the genes underlying economically important traits, and methods for determining the breeding or crossbreeding values of broodstock at early larval stages (Pace et al. 2006; Hedgecock et al. 2007a).

Conservation

The challenges in conserving while utilizing sustainably the planet's imperiled aquatic biodiversity are enormous and global in nature (Jackson et al. 2001; Dulvy et al. 2003). Conservation of shellfish biodiversity is particularly challenging since diversity is highest in coastal and estuarine habitats, which are heavily impacted by human populations. To counteract or reverse the impacts of overfishing and habitat degradation, resource manag-

ers in the past have used introductions (see Chapter 14, in this book) or translocations as a tool for shellfish remediation. The ecological and genetic risks of these management options are well described but poorly quantified (NRC 2004). Today, those concerned with decimated shellfish populations are turning increasingly to restoration programs for native species, which often involve supplementing natural stocks of shellfish with hatchery-propagated seed. The genetic implications of allowing interaction of wild and hatchery-propagated shellfish populations have been considered (Allen and Hilbish 2000; Gaffney 2006) and are reviewed below.

Introductions of shellfish

Coastal ecosystems are characterized as heavily invaded by nonindigenous species (Grosholz 2002). Most biological invasions are attributable to shipping, via the transport and exchange of ballast water, though purposeful, accidental, or inadvertent introductions of nonnative species, owing to aquaculture (Chapter 14) or the aquarium trade, have also caused profound changes in coastal ecosystems (Ruiz et al. 2000). Although introductions of nonnative shellfish into North America for aquaculture have been portrayed as a current threat (e.g., Naylor et al. 2001), such introductions are largely, if not entirely, historical events. For example, the eastern oyster was introduced into the San Francisco Bay from 1869 until 1940, when that particular "gateway for exotic species" closed forever (Miller et al. 2007).

The difficulty of predicting the ecological consequences of shellfish introductions is well illustrated by the diverse effects that introductions of the Pacific oyster *Crassostrea gigas* have had on different continents and regions (Mann 1979; NRC 2004). MSX (multinucleated sphere unknown), a disease that caused epizootic mortalities of eastern oysters in the Chesapeake

and Delaware Bays in the late 1950s and early 1960s and has now spread along the entire U.S. East Coast (NRC 2004), was introduced from Asia, where it infects Pacific oysters (Burreson et al. 2000); however, the means of MSX introduction—whether from illegal introduction of Pacific oysters for aquaculture, fouling of oysters on ship bottoms, or from ballast water—is unknown (NRC 2004).

Purposeful introductions can be curtailed, in principle, with regulations and enforcement, but often these are lacking or insufficient to prevent some intentional or accidental introductions, as amply illustrated by the controversy over the proposed introduction of the Asian oyster *Crassostrea ariakensis* into the Chesapeake Bay (NRC 2004). The International Council for the Exploration of the Sea (ICES 2005) has developed a code of practice governing introductions and transfers of marine organisms, which is supposed to be enforced by its 20 member countries and to guide policy in nonmember countries. Currently, introductions of exotic shellfish and of interstate transfers of native and naturalized shellfish species are strictly regulated by state agencies along the U.S. West Coast. For example, a recent reintroduction of the Kumamoto oyster *Crassostrea sikamea*, a species that has been cultivated on the U.S. West Coast for six decades, required rearing in closed quarantine, with extensive disease testing, of both the adult oysters imported from Japan and their first-generation offspring (Camara et al. 2008), one generation beyond ICES codes of practice. While introduction of nonnative species raise large ecological concerns, their impact on genetic diversity of local species or of locally established conspecific populations is less well understood but is likely to be less important than their direct impact on ecosystems.

Translocations of shellfish

Translocations of conspecific stocks from one area to another are problematic for disease

transmission and other ecological reasons (see Chapter 13 in this book; Hegaret et al. 2008; NRC 2004). Direct genetic implications of translocations depend on the amount of genetic divergence between local and translocated populations. Like other marine animals with planktonically dispersing larvae, marine bivalves tend to show minimal genetic divergence or high connectivity over oceanic basin scales (Hedgecock et al. 2007c), which makes it difficult to detect any translocation events.

The eastern oyster is a notable exception, having a major genetic divergence between Gulf of Mexico and Atlantic populations (Buroker 1983; Reeb and Avise 1990; Karl and Avise 1992; Cunningham and Collins 1994; McDonald et al. 1996). Regional subpopulations in the northern and southern Atlantic areas, which have also been identified with molecular markers (Hoover and Gaffney 2005; Gaffney 2006), may correspond with physiological races identified earlier on the basis of a latitudinal gradient in spawning season (Loosanoff and Nomejko 1951; Barber et al. 1991). Still, genetic impacts from historical translocations of eastern oysters have yet to be documented. Milbury et al.'s (2004) study of recruitment from 4 million Gulf of Mexico eastern oysters intentionally planted in the Choptank River for restoration purposes detected only three of 3545 spat of Gulf origin. This number was smaller than, but not inconsistent with, the potential reproductive contributions of the planted oysters based on their survival and sex ratio and the size of the natural population. Alternatively, these three spat with a Gulf of Mexico genetic marker could have resulted from previous translocations, a hypothesis supported by detection of a South Atlantic genetic marker in 5% of the spat screened. Despite the difficulty of predicting impacts on local adaptation, any proposed shellfish translocation ought to be preceded, at least, by a determination of the population genetic structure of the target species (Bell et al. 2005; Ward 2006).

Interest in shellfish restoration has grown substantially over the last decade or more, engendering, for example, the establishment by the Nature Conservancy of the Shellfish Restoration Network and the International Conference on Shellfish Restoration (ICSR) that has convened 11 times. Insofar as restoration projects commonly utilize translocation or hatchery enhancement as tools, they have genetic implications for the natural populations being restored. The genetic impacts of translocations have already been covered, so we turn attention, next, to the potential impacts of hatchery propagation on shellfish genetic diversity and fitness. We need, first, to review the reproductive biology of natural bivalve populations, in particular variance in reproductive success and its genetic consequences.

SRS of shellfish

The majority of marine shellfish (and marine fish) share a suite of life history traits—relatively late maturation, high fecundity, small eggs, long-lasting and widely dispersing plankton-feeding larvae, and broad geographic ranges (Thorson 1950; Winemiller and Rose 1992; Palumbi and Hedgecock 2005)—that renders them more vulnerable to loss of variation and extinction than might be expected from their great abundance. These life history traits appear to be adaptations to a biphasic life cycle, in which larvae disperse to planktonic habitats, far from potentially cannibalistic adults, but face tremendously high, though variable, early mortality. Conservation of such species depends, therefore, not only on protection of adult forms but also on the preservation of a vast, poorly delimited and understood planktonic environment.

High fecundity, on the order of a million or more eggs per female per spawning event, and early mortality, typically in the range of 10–20% per day, make possible a high variance among individuals in reproductive success, the number of offspring contributed to the next

generation. Successful reproduction for most marine shellfish requires success at each step in a complex chain of events, from reproductive maturation and spawning of adults to external fertilization of gametes, development, growth and survival of larval forms, metamorphosis and recruitment into the adult habitat, and survival and growth of juveniles to maturity. Reproductive activity, though tuned to the annual seasonal cycle, must still match highly variable local conditions for this chain of events to be complete. The chances of successful reproduction may thus vary dramatically for individuals, perhaps even among individuals adjacent to one another in space but spawning at slightly different times. Consequently, reproductive success in marine organisms might, at times, resemble a sweepstakes lottery, in which there are a few big winners and many losers (Hedgecock 1994).

The hypothesis of SRS, which makes testable predictions about temporal genetic change in populations and variance in the genetic composition of new recruits, has received support from both empirical (e.g., Li and Hedgecock 1998; Hauser et al. 2002; Turner et al. 2002; Lee and Boulding 2007, 2009; Hedgecock et al. 2007b) and theoretical studies (Waples 2002; Hedrick 2005; Eldon and Wakeley 2006; Sargsyan and Wakeley 2008). The implication of the SRS hypothesis for conservation is that these seemingly inexhaustible living marine resources may have effective population sizes that are orders of magnitude smaller than census sizes, and thus rates of genetic drift and inbreeding that can erode biodiversity on ecological timescales.

Interaction of hatchery-propagated and natural shellfish populations

Adverse interactions of wild and hatchery-propagated stocks are likely growing with the global expansion of aquaculture (McGinnity et al. 2003; Hindar et al. 2006) and stock enhancement programs, including shellfish

restoration efforts (Born et al. 2004; Gaffney 2006). High fecundity creates the risk that reproduction and spread of hatchery-propagated shellfish stocks will dilute the genetic diversity of wild populations. Ryman and Laikre (1991) provided a model for this problem, in which the effective size of a population comprising both naturally (wild) and artificially (hatchery or captive) propagated components is given by

$$\frac{1}{N_e} = \frac{x^2}{N_c} + \frac{(1-x)^2}{N_w}, \quad (12.1)$$

where N_e is the effective size of the mixed population, N_c is the effective size of the captive or hatchery population, N_w is the effective size of the wild component, and x is the proportion of the spawning population of hatchery origin.

Hatchery effects on genetic diversity and adaptedness

For highly fecund shellfish, the risk that an enhancement program might dilute genetic diversity and reduce the effective size of a wild population is potentially great, but there are few cases in which a genetic impact has been rigorously quantified (Gaffney 2006). Initial enthusiasm for the intuitive simplicity of the Ryman–Laikre model can give way to skepticism about its application, when difficulties or uncertainties in measuring its parameters are fully appreciated. N_c is knowable, certainly, given current capabilities for high-throughput analysis of genetic markers and statistical tools for estimating the effective number of hatchery parents; this parameter can and should be determined. The other two parameters, x and N_w , are much more difficult to quantify, however, since they depend on information about the natural population and on proper spatial scales of measurement. One temptation is to estimate x , for example, by estimating the proportion of seed that are of hatchery origin;

such an estimate is limited by uncertainty in estimating wild seed production at an appropriate spatial scale. Moreover, since x should be determined at spawning, the relative rates of wild and hatchery seed survival to reproduction need to be known.

Another genetic risk of hatchery-based shellfish restoration programs is the effect on fitness or adaptedness of natural populations. A hatchery stock is almost inevitably subject to intentional or unintentional artificial selection (“domestication” selection) in the hatchery environment. For example, fine-mesh screens are used universally in shellfish hatcheries to cull small individuals from larval cultures. This practice could select for size-at-age and therefore rapid larval development. If this trait were maladaptive in natural environments and if, through a restoration program, a hatchery stock were to swamp a local population, then the reproductive success of the “enhanced” population could, in principle, be reduced. Unfortunately, there are almost no data on the genetic impacts of such hatchery practices; indeed, it would be challenging to measure genotype-by-environment interaction for larval traits across both hatchery and natural habitats. Nevertheless, the risk of domestication selection can be mitigated to a large extent by continual replacement of hatchery broodstock with wild adults and exclusion of hatchery-bred adults from the hatchery broodstock, which would prevent cumulative effects of domestication selection across generations. Risks from hatchery enhancements on genetic diversity or adaptation are manageable with appropriate designs and monitoring (Hedgecock and Coykendall 2007).

Restoration of disease-ravaged shellfish populations

Heavy disease pressure, such as experienced in the Chesapeake and Delaware Bays, poses further challenges for a traditional

hatchery-based approach to shellfish restoration (Mann and Powell 2007). Planting disease-susceptible hatchery seed is unlikely to increase the adult population of oysters, except in low-salinity refuges where disease pressure is weak or absent. Still, hatcheries could conceivably play a role in restoration of natural populations by seeding stock selected for disease resistance or disease tolerance, a concept termed *genetic rehabilitation* (Allen and Hilbish 2000). The goal of genetic rehabilitation is not to enhance populations directly but to increase the frequencies of disease-resistant or disease-tolerant genotypes to the point at which natural selection can amplify and spread them further. At the same time, however, the effective size of the selected stock needs to be large enough to minimize inbreeding depression, a decline in fitness that might offset any advantage of disease tolerance. Unfortunately, closed shellfish populations, including some selected for disease resistance, tend to have small effective sizes and inbreeding depression (Vrijenhoek et al. 1990; Hedgecock et al. 1992; Carlsson et al. 2006). Genetic analyses of juvenile oysters in the Great Wicomico River, Virginia, showed that less than 10% came from a disease-resistant line that had been planted there (Hare et al. 2006); in a second genetic study of hatchery-supplemented Virginia populations, no significant contribution of selectively bred oysters was observed over a 4-year period (Carlsson et al. 2008). Thus, the fitness of selectively bred oysters in the natural environment must be taken into account in assessing the viability of a genetic rehabilitation strategy.

Geoduck aquaculture: a case history

The risk of interaction between wild and hatchery-propagated farmed stocks has been raised as an issue in the culture of geoduck clams (*Panopea generosa*, formerly *Panopea abrupta*; Vadopalas et al. 2010) in the U.S.

Pacific Northwest. Washington State House Bill (HB) 2220, passed in April 2007, directed the Washington Sea Grant College Program to measure and assess, among other things, “[g]enetic interactions between cultured and wild geoduck, including measurements of differences between cultured geoducks and wild geoducks in terms of genetics and reproductive status.”

Lack of demonstrable population genetic structure for geoduck (Vadopalas et al. 2004; Miller et al. 2006), as in most marine bivalves, suggests that mixing of divergent gene pools through translocation of farmed stock is unlikely to be a great risk. Dilution of genetic diversity or effects of intentional or inadvertent selection on cultured clams are potential issues, but several factors appear to diminish these risks. Current hatchery practices—to collect local broodstock, to rotate broodstock within spawning seasons and between years, and to avoid using cultured geoduck as broodstock—eliminate the possibility of cumulative genetic change from domestication selection and reduce the risk of random genetic changes from use or reuse of too few broodstock.

What part of geoduck reproduction in the Puget Sound might be attributable to spawning of hatchery-propagated stocks (x in the Ryman–Laikre model) is unknown but likely small for the foreseeable future. Presently, geoduck aquaculture occupies a little more than 140 ha of intertidal area in Washington State and could grow to as much as 245 ha, which would be 1.4% of the nearly 18,000 ha of subtidal geoduck beds available for commercial harvest in Washington State. Relative to the reproductive output of an exploitable geoduck biomass of 73,848 t in 1999 (Sizemore and Ulrich 1999), spawning by a few hundred metric tons of farmed geoduck (annual farm harvest is ~400 t in Washington State) is unlikely to be a significant contribution. Moreover, farmed geoduck clams are harvested at 5–7 years of age, when they are

just reaching reproductive maturity and are mostly male (Straus et al. 2008). The age and size of farmed geoducks at harvest, about equal to the minimum size of clams in the exploitable biomass, is well below the size or age at which the clams reach maximum fecundity. While it seems improbable that geoduck aquaculture could have a measurable impact on natural diversity, precautionary research on the interaction of wild and farmed geoduck clams is underway with funding from HB 2220.

Sterilizing farmed shellfish stocks through triploidy

One way to eliminate much of the risk of interaction between wild and hatchery stocks is to render farmed stocks sterile. Triploidy is commonly induced in shellfish to reduce reproductive effort, divert energy to growth, and improve meat quality during the normal spawning season (Allen and Downing 1986; Nell 2002; Vadopalas and Davis 2004). Because triploids are effectively sterile, their use in shellfish aquaculture dramatically reduces the risk of spawning and mixing with local native or naturalized stocks. At the same time, triploidy offers only a temporary reduction in the risk of an introduction, if the farmed species is a nonnative (NRC 2004). Gene knockout technology offers another route to sterilization (Grewe et al. 2007; Wong and Van Eenennaam 2008), although widespread public resistance to genetically modified organisms is likely to make this a nonviable strategy. The ability to culture sterile stocks frees aquaculture to pursue the benefits of domestication while minimizing or preventing the interactions of farmed and wild stocks. Biosecurity of reproductively competent tetraploid stocks in the environment is just beginning to be addressed (Piferrer et al. 2009); early experience with tetraploid Pacific oysters suggests that they are not robust enough, at present, to outcompete diploid stocks.

Conclusions

Long-term research on developing and improving domesticated shellfish stocks is needed to make shellfish farming more efficient and to relieve fishing pressure on natural populations. Such research should be coupled with research on reducing or eliminating interactions between wild and farmed populations (e.g., by inducing triploidy in hatchery-propagated stocks).

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