

The Chesapeake Bay Blue Crab (*Callinectes sapidus*): A Multidisciplinary Approach to Responsible Stock Replenishment

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The Chesapeake Bay has traditionally been one of North America's most productive fishing grounds, supporting the world's largest blue crab fishery. During the last several decades, fishing mortality and environmental degradation led to ~70% drop in the bay's blue crab abundance, 84% decline in its spawning stock, and historically low levels of juvenile recruitment as well as nursery habitats being below carrying capacity. This situation makes the Chesapeake Bay blue crab an appropriate candidate for responsible stock enhancement. A multidisciplinary, multi-institutional program was developed to study the basic biology and life cycle of the blue crab, develop hatchery and nursery technologies for mass production of blue crab juveniles, and assess the potential of using cultured juveniles to enhance blue crab breeding stocks and, in turn, bay-wide abundance and harvests. Basic biology and culture studies enabled closing the life cycle of the blue crab in captivity. Juvenile crabs have been produced year round, with excellent survival. During 2002–2006, over 290,000 cultured crabs were tagged and experimentally released into the bay's nursery habitats. Cultured crabs survived as well as their wild counterparts, increased local populations at release sites by 50–250%, grew quickly to sexual maturity, mated, and migrated from the release sites to spawning grounds, contributing to the breeding stock as soon as 5 to 6 months post-release. Findings reported in this text and other articles in this volume are indicative of the feasibility of our approach of using hatchery juveniles to replenish the blue crab breeding stocks in the Chesapeake Bay.

Keywords breeding stock restoration, hatchery, tag and recapture, release strategies, stock enhancement

INTRODUCTION

Chesapeake Bay has traditionally been one of North America's most productive fishing grounds, supporting substantial bivalve, crustacean, and finfish harvests. In recent decades, com-

Authors' Note: Since the submission of this manuscript, the total number of released hatchery crabs has increased significantly. A total of 215,000 hatchery-raised crabs were released into Chesapeake tributaries in 2007, bringing the 2001–2007 cumulative release total to 515,000 juvenile hatchery crabs.

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mercial landings declined severely. Sustained fishing mortality and environmental deterioration led to an ~70% decrease in blue crab abundance in Chesapeake Bay during the last 15 years, from an estimated 900 million crabs down to ~300 million, with 45–55% of those crabs harvested annually (Miller et al., 2005; Bi-State Blue Crab Technical Advisory Committee [BBC-TAC], 2006; Lipcius et al., 2005a; Bunnell and Miller, 2005). Consequently, the blue crab fishery, which in the early 1990s was a 52,000-ton, \$72-million industry, declined to a 28,000-ton, \$61-million harvest in 2004. However, more alarming than the drop in abundance and harvest of the blue crab is the concurrent decline in the blue crab spawning stock and, in turn,

decreased larval abundance and recruitment (Lipcius and Stockhausen, 2002). Detailed analyses conducted over 17 successive years (1988–2004) showed that spawning stock abundance and biomass in Chesapeake Bay declined by 81% and 84%, respectively (Lipcius and Stockhausen, 2002; Miller et al., 2005). These studies, along with similar research conducted south of Chesapeake Bay (Eggleston et al., 2004) also provided strong evidence for a direct relationship between abundance of spawning stock and larval abundance and recruitment, which has caused both larval parameters to drop by an order of magnitude over the same time span. This drastic depletion of blue crab “seed stock” resulted in a continuous decline of juvenile abundance in nursery habitats along tributaries of Chesapeake Bay (Hines, 2003; Lipcius et al., 2005a), which are now considered to be largely below their carrying capacities for this species (Hines et al., 2008; Seitz et al., 2008). This situation makes the blue crab in Chesapeake Bay an excellent candidate for restocking efforts, focusing on recovering its spawning stock biomass and, in turn, larval abundance and recruitment, ultimately leading to restoring blue crab populations and harvests.

Consequently, in 2001 we established the Blue Crab Advanced Research Consortium (BCARC), a multidisciplinary scientific team that consists of aquaculture and hatchery experts, crustacean endocrinologists and pathobiologists, geneticists, and benthic and fishery ecologists. The BCARC also includes industry partners (Maryland Watermen’s Association) and interacts closely with regulatory authorities that are in charge of managing the blue crab fishery in Chesapeake Bay. The objectives of the BCARC are the following:

1. Advance our understanding of the basic biology of the blue crab (reproduction, development, nutrition, genetics, immune function).
2. Develop hatchery and nursery technologies for mass production of blue crab juveniles.
3. Assess the feasibility of using hatchery-produced juvenile blue crabs to enhance blue crab breeding stocks and, in turn, their overall abundance in Chesapeake Bay.
4. If successful, scale-up hatchery/nursery operations and transfer the technology to the fishery industry.

This volume includes eight reports from BCARC partners, demonstrating significant progress towards all the above objectives. This article summarizes the major consortium findings and the status of the program in the context of the long-term objectives of developing a responsible approach to reversing the trend and enhancing the dwindling abundance of the economically and ecologically important blue crab in Chesapeake Bay.

MANAGING THE BLUE CRAB FISHERY

As indicated by several key reviews of the field (Blankenship and Leber, 1995; Leber, 1999, 2002, 2004; Munro and Bell, 1997; Hilborn 1999, 2004; Bell et al., 2006; Kitada and Kishino,

2006), restocking is but one of the diverse management tools and options needed to restore severely depleted marine fisheries. Obviously, blue crab stock replenishment would not be effective without a strict management plan for the blue crab fishery, as well as adequate measures to protect breeding stocks from harvest. Responding to the recent trends of severe decline in blue crab abundance and harvests, the regulatory authorities that oversee fisheries in Chesapeake Bay have conducted multiple assessments to monitor and predict the state of the crab fishery, and have implemented management plans to reduce fishing mortality. Fishing efforts were limited by season, days, and hours of harvest allowed; type of gear; minimum legal size; and location of fishing activities. Measures to protect female broodstock migrating to their spawning grounds were also partially implemented. Additionally, 2400 km² in the lower bay, covering approximately 75% of the blue crab spawning grounds, has been designated as a spawning sanctuary and is closed to harvests during the spawning season (June 1–September 15; Lipcius et al. 2003, 2005b; Lambert et al., 2006). Nevertheless, these stringent management strategies have had a limited impact on the population, as trends in the spawning stock and harvestable crabs have not shown any significant increases in the recent past (Miller et al., 2005; BBCTAC, 2006; Chesapeake Bay Stock Assessment Committee, 2005). These measures did, however, halt declines and stabilized blue crab populations at lower levels of abundance.

BLUE CRAB LIFE HISTORY

As also advocated in recent stock replenishment and marine conservation biology reviews (Svåsand et al., 2000; Lipcius et al., 2003, 2005b; Taylor et al., 2005; Bell et al., 2006; Obata et al., 2006), managing the blue crab fishery and establishing an effective stock restoration program must take into account both the blue crab complex life history and its migratory patterns in Chesapeake Bay. The life history of the blue crab consists of multiple phases in diverse and varied environmental niches throughout the entire bay. Chesapeake Bay is North America’s largest estuary, with a surface area of over 11,600 km² (165,000 km² in total watershed area). It is 320 km long (north to south) and 5–56 km wide (east to west), draining 15 major rivers and opening to the Atlantic Ocean through a narrow mouth at its southern end. It has a wide salinity gradient, from full-strength seawater in the south to brackish and almost fresh water in the north. It is relatively shallow (average depth of 6.5 m) and is thus subject to major seasonal fluctuations in temperature and salinity.

The blue crab life cycle involves very specific seasonal and geographical breeding and spawning patterns (Hines, 2003; Miller et al., 2005; Hines, 2007; Jivoff et al., 2007). Most blue crabs mate in the upper (northern) parts of Chesapeake Bay during summer and early fall. During mating, the male transfers a spermatophore or “sperm package” to the female, which has

just undergone her final (or pubertal) molt. The female stores the spermatophore until she is ready to ovulate months later. The inseminated females start to migrate toward the spawning ground in the lower (southern) higher-salinity regions of the estuary, reaching these waters before the onset of the winter (Aguilar et al., 2005). They then bury in the sand where they hibernate and over-winter until temperatures begin to rise during early spring. Females emerge from hibernation and ovulate, and the ovulated eggs travel through the sperm stored by the female and are fertilized. They are then deposited on pleopods as an egg mass ("brood"), and the embryos develop for 2–3 weeks before hatching, which occurs throughout the summer. A typical brood will produce 3–5 million pelagic larvae called zoeae. The hatched zoeae are swept by water currents out of the mouth of Chesapeake Bay and into Atlantic waters. They develop through 8 zoeal stages and recruit back to the bay as megalopae (9th larval stage) and settle primarily in seagrass nurseries in the lower part of the bay. Megalopae metamorphose into juvenile crabs, which disperse into a wide range of nursery habitats in subestuaries of the lower and upper bay.

ADOPTING THE RESPONSIBLE APPROACH

The main objective of our program is to supplement fishery management with a complementary approach to help reverse the declines of the blue crab in Chesapeake Bay. Our approach is to explore the feasibility of enhancing the severely depleted breeding stock and, in turn, the abundance and harvest of crabs. A similar program occurred in Japan for a closely related portunid crab, the swimming crab (*Portunus trituberculatus*). Because of a severe depletion of that over-fished species in various marine systems such as Osaka Bay and the Seto Inland Sea, an aggressive hatchery-based stock replenishment program started in the 1970s. During the last decade, multiple regional and national Japanese hatcheries annually released 35–45 million swimming crab juveniles into the depleted waters (Ariyama 2000, 2001; Ariyama et al., 2001; Secor et al., 2002). While an overall increase in harvests of the swimming crab has been documented since the start of the program, there is no direct evidence that the increase resulted from the releases of cultured juveniles, rather than from other management initiatives.

Although the Japanese swimming crab replenishment program provided inspiration to our research in Chesapeake Bay, we sought a thorough science-based approach to provide the foundation and proof-of-concept for a sound, long-term restoration program. Thus, we set out to (1) engage in a blue crab restoration program that meets the standards of responsible stock replenishment, and (2) conduct a thorough, multidisciplinary research program to test the feasibility of our stock replenishment approach before recommending large-scale implementation.

The responsible approach to stock replenishment, as defined by several reviews of the field published in the last decade, highlights several key components that must be included in the program (Blankenship and Leber, 1995; McKinnell and Lundqvist,

2000; Secor et al., 2002; Leber, 2004; Taylor et al., 2005; Bartley et al., 2006). This article summarizes the progress made by the blue crab restoration program in addressing several of the most critical components, with more details provided by the companion articles in this volume.

CLOSING THE LIFE CYCLE AND DEVELOPING HATCHERY AND NURSERY TECHNOLOGIES

Despite its complex life cycle, we have established detailed protocols for successful mass production of blue crab larvae and juveniles (Zmora et al., 2005). To determine the optimal timing of juvenile releases in Chesapeake Bay, and to consistently provide larvae and juvenile crabs for both laboratory and field experiments, we aimed to produce larvae and juveniles on a year-round basis. In the wild, adult blue crabs mate during the summer and ovulate, produce ovigerous masses, and hatch during the next spring and summer (Van Engel, 1958; Millikin and Williams, 1984; Jones et al., 1990; Hines, 2007; Jivoff et al., 2007). Mated, inseminated blue crab females were collected every fall and carefully transferred to our broodstock tanks (volume = 0.5–4 m³), in which all environmental conditions, including water temperature, salinity, and photoperiod, were fully computer controlled. Although we could allow mating of hatchery-produced crabs (F1 progeny), collecting a large number (80–120) of inseminated females from the wild is part of an overall plan to address genetic resource management and prevent inbreeding (see below).

In the hatchery, mated females were exposed to phase-shifted environmental conditions to obtain on-demand and year-round spawning and juvenile production. To keep a stock of mated females ready to be induced to ovulate and produce broods, they were exposed to winter conditions, i.e., low temperatures (15°C) and short days (8 hours of light). To induce ovulation and brood production, the external conditions were gradually shifted to simulate those that females experience on their spawning grounds, i.e., warm temperatures (up to 22°C), long days (16 hours of light), and high salinity (30 ppt). Using this approach, we have been able to obtain ovulated, ovigerous females (Figure 1) and produce blue crab larvae and juveniles all year.

We continued to optimize the published protocols (Zmora et al., 2005) for larval rearing and juvenile production and consistently increased our production (Figure 2). Intensive larval rearing (60–140 larvae/liter) using three microalgae species and omega-3 enriched rotifers and *Artemia* nauplii resulted in 30–80% survival from hatch to megalopae in 3–4 weeks. Megalopae were reared to 20-mm juveniles (mean carapace width) at lower densities (5–20/l) in 4 weeks at survival rates ranging from 10–30%, depending on rearing density. From 2002 through 2006, a total of 570,000 blue crab juveniles were produced in our hatchery/nursery operations; over 290,000 of these were individually tagged and experimentally released into Chesapeake Bay (many thousands were used for other experiments). During 2006, we

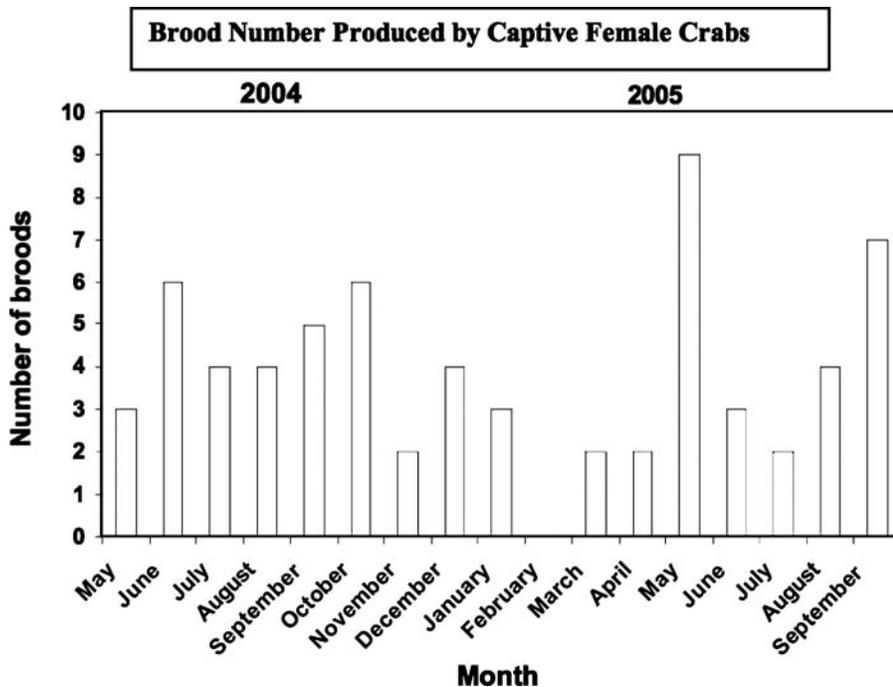


Figure 1 The number of broods (egg masses) produced by captive blue crab females during May 2004 to September 2005. Natural “brooding” period is May through August. Arrows indicate “out of season” brood production.

produced 288,000 juveniles, of which 123,000 tagged individuals were released into the bay.

The strategy of our blue crab replenishment program is to conduct initial experiments in small (1–100 ha.) nursery habitats in both the upper and lower parts of the estuary, and then expand to a larger, bay-wide approach (Hines et al., 2008). Because Chesapeake Bay is a very large system (see above), our strategy requires an incremental increase in juvenile production for larger release experiments. To achieve that goal, we have recently started to scale-up juvenile production. Working with Chesapeake Bay stakeholders, including the Maryland Department

of Natural Resources and the Maryland Watermen’s Association, we have begun to produce juvenile crabs in a large-scale state-owned nursery. Further expanding this facility, we plan to gradually increase production to 0.5–1 million juveniles per year during the next 3–5 years.

BASIC STUDIES OF BLUE CRAB BIOLOGY

Our program strongly emphasizes the role of basic biological understanding in multiple aspects of the effort, including aquaculture (hatchery, nursery), optimal release strategies, and adaptive management of the fishery. Several ecological and life history patterns and their relevance to our program are addressed in more detail elsewhere in this volume (Aguilar et al., 2008; Eggleston et al., 2008; Hines et al., 2008; Johnson et al., 2008; Lipcius et al., 2008; Seitz et al., 2008; Young et al., 2008) and other peer-reviewed literature (Place et al., 2005; Steven et al., 2003, 2005). With respect to the economic feasibility of the program, it is very clear that understanding blue crab biology is essential to reducing the production cost of cultured juveniles.

To scale-up juvenile crab production, it is necessary to control and synchronize ovulation, brood production, and hatching. As has been the case with many cultured finfish (Zohar and Mylonas, 2001), obtaining such control requires a full understanding of blue crab reproduction at both the gene and hormonal level. Additionally, reducing cost requires better control of early development and growth processes. Specifically, being able to synchronize early molting in the nursery is expected to solve one of the main obstacles in the efficient and cost-effective

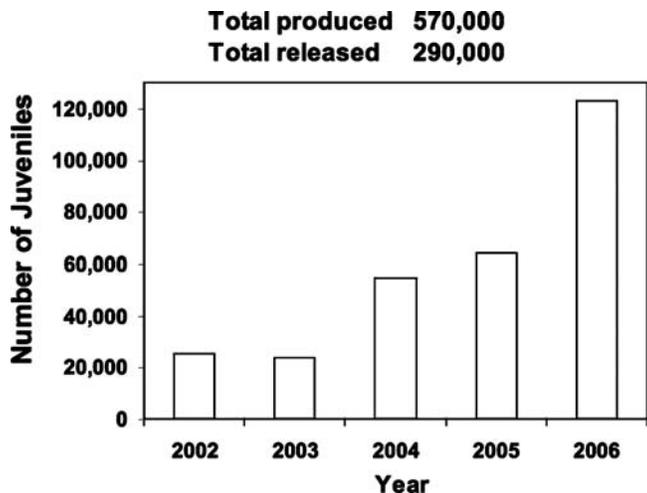


Figure 2 The number of hatchery crabs tagged and released into studied nursery areas around Chesapeake Bay during 2002–2006.

production of juvenile blue crabs—high mortality due to cannibalism. Our program has engaged in comprehensive studies at the molecular through functional levels, aimed at understanding multiple aspects of the physiology and endocrinology of blue crab development, growth, and reproduction (Chung et al., 2006; Zmora et al., 2007). The ultimate goal is to devise strategies to increase efficiency and reduce the cost of producing juveniles.

ARE HATCHERY-PRODUCED JUVENILES COMPETENT?

The basic assumption of any restocking program is that hatchery-produced juveniles are similar to wild-born ones and, thus, after release into the natural environment, can survive equally well as wild individuals. However, several studies, conducted mainly with finfishes, demonstrated that hatchery-produced individuals were different from wild counterparts in both behavioral and morphological aspects (Swain and Riddell, 1990; Furata, 1998; Ellis et al., 1997; Hard et al., 2000), and thus did not survive as well (Kellison et al., 2000; Stunz and Minello, 2001). Exposing the cultured animals to conditions that mimic those they will experience in the nature environment is an effective way to significantly increase survival (Olla and Davis, 1989; Brown and Smith, 1998; Hossain et al., 2002; Kellison et al., 2003).

To test the competence of our hatchery-produced blue crabs, we compared behavioral, morphological, and performance (growth, survival) parameters between cultured and wild juveniles (Davis et al., 2004a, 2005a; Young et al., 2008). Those studies showed that crabs from the hatchery were morphologically different from wild-born ones in having significantly shorter lateral spines, which may lead to higher vulnerability of the cultured crabs to predators. However, after 10 days post-release, the lateral spines of hatchery-raised crabs were no different from those of their wild counterparts. Laboratory and field experiments demonstrated that cultured crabs did not differ from wild ones in their use of habitat and refuge, movement and dispersal patterns in the field, or feeding rates on natural prey. However, cultured crabs, which are grown without sediment in the tanks, buried less often than wild crabs, although this behavior did not translate to higher mortality of cultured crabs in the field. Conditioning the hatchery-raised juveniles for 48 hours by exposing them to sediment eliminated the difference in their burial frequency.

Cultured crabs grew as well as wild-born crabs—they molted at similar intervals and grew at a similar rate between molts (Young et al., 2008). However, the most promising finding of the study, which used both tethered crabs and simultaneous releases of individually tagged cultured and wild crabs, was that in the field crabs produced in the hatchery had similar survival rates to their wild counterparts. This finding, combined with the relatively high long-term survival rates of our cultured crabs in Chesapeake Bay (see below), is a strong indication that hatchery-produced juveniles released under optimal conditions can perform and survive well to sexual maturity.

IDENTIFYING RELEASED CULTURED ANIMALS AND ASSESSING STOCKING EFFECTS

We tested two types of internal physical tags for blue crab juveniles, coded microwire tags and fluorescent elastomer tags (Davis et al., 2004b). Based on higher tag retention and faster rate of tagging, microwire tags were selected as the best mass-tagging method for juvenile blue crabs. However, elastomer tags were found to be efficient also, and, in several of our experiments requiring double-tagging, crabs were marked with both types of tags. To date, all of the 290,000 juvenile blue crabs released experimentally into Chesapeake Bay have been individually tagged before release, the vast majority with coded microwire tags. This makes our effort the world's largest tag and release experiment for any species of crab. Approximately 175,000 cultured crabs were released in tributaries of the upper Chesapeake Bay (Hines et al., 2008; Johnson et al., 2008), in typical nursery habitats. The remaining 115,000 were released in the lower bay (Hines et al., 2008; Lipcius et al., 2008; Seitz et al., 2008) in nursery habitats that are much closer to the blue crab spawning grounds.

The tagged juvenile crabs were released in cohorts of 1,000–20,000 individuals into several relatively small (1–100 hectares) coves and embayments, for which we have extensive background information on population structure and ecology. We released crabs at increasingly higher densities in an effort to establish the carrying capacity of the tested nursery habitats. Stocking densities ranged from 0.18–0.7 crabs per m². The release and control sites were then thoroughly sampled, using multiple sampling methods, including seines, tows, and scrapes, in an effort to capture and assess the presence of wild crabs and hatchery-released crabs. Sampling continued for up to 16 months after the releases, at intervals ranging from 1 week during the initial months post-release to 2–4 weeks in the latter months. Survival and growth rates of the cultured juveniles, their contribution to the wild crab population at release sites, wild crab displacement by cultured crabs, enhancement values, and dispersal patterns within and from the release sites were assessed.

The significant volume of data produced is described elsewhere (Davis et al., 2004a, 2004b, 2005a, 2005b; Eggleston et al., 2008; Hines et al., 2008; Johnson et al., 2008; Lipcius et al., 2008; Seitz et al., 2008). The measures of success have so far been very encouraging, although enhancement effect varied considerably as a function of site and season of release, environmental conditions, size of released crabs, density of wild and released crabs, and occurrence of predators in the release sites. Survival of the released crabs to sexual maturity averaged 15% (and ranged from 5–30%). Increasing stocking density did not lead to displacement of wild juveniles and resulted in increased enhancement and production, indicating that the released juveniles did not saturate the carrying capacity at the densities and study sites we used. The cultured crabs enhanced the wild population at the release sites by 50–250% (and thus, in some cases, as much as tripled the abundance of wild crabs). When released in the spring or early summer, with the ensuing exposure to warm bay temperatures, crabs grew extremely fast and reached

sexual maturity as soon as 3 months after release. At this time, they mated and left the release (nursery) sites on their migration towards the spawning grounds in the lower Chesapeake Bay, thus contributing to the breeding stock during the same year of their release (see below for additional discussion).

The results obtained to date strongly indicate that using hatchery releases to enhance the blue crab spawning stock is feasible. However, the ultimate measure of success will be to demonstrate that cultured crabs contribute to the next generations of crab offspring and, in turn, to increased abundance of adult crabs in Chesapeake Bay. To measure and demonstrate such a success we will have to (1) monitor gene flow from cultured crabs to wild-born offspring, and (2) quantify the impact of releases on overall abundance of blue crabs in Chesapeake Bay. Scaling-up of production and bay-wide releases will be required, as well as development of additional genetic and molecular tools (see below).

GENETIC CONSIDERATIONS AND TOOLS

There are numerous ways in which cultured organisms can have a direct genetic impact on recipient stocks (Blankenship and Leber, 1995; Tringali and Bert, 1998; Utter, 1998; Tringali and Leber, 1999; Tringali et al., 2003; Taylor et al., 2005; Tringali, 2006). To adopt responsible broodstock selection and juvenile production strategies, and to avoid the potential adverse impacts of releases of hatchery-raised crabs, the genetic structure of blue crab populations around the replenishment sites along Chesapeake Bay had to be studied.

We also realized that, as we increase the number of juveniles released, the laborious mechanical tagging process will need to be replaced by a genetic tag (e.g., DNA markers). Additionally, assessing gene flow and the contribution of cultured crabs to breeding stocks and future generations can only be accomplished by using genetic markers. Accordingly, we engaged in a comprehensive genetics program for *Callinectes sapidus*.

The complete mitochondrial (mt) genome of the blue crab was determined (Place et al., 2005), and blue crab microsatellite markers were isolated and characterized (Steven et al., 2005). Using both tools, the population structure of the blue crab in Chesapeake Bay was shown to be highly genetically diversified (haplotype diversity >0.7 ; lack of common haplotypes; microsatellite heterozygosity varies from 40–97%, depending on loci; A. Place, unpublished). However, all current analyses indicate that the crab population in the bay is a single panmictic breeding population. Based on mtDNA data, significant yearly variation of genetic composition was observed ($p < 0.005$), despite a low F_{st} value (<0.03), suggesting that the genetic structure of local populations change from year to year. These findings imply that our restocking program must be very careful about conserving blue crab genetic diversity. Consequently, when implementing the bay-wide program, the recommended approach would be to use only wild inseminated females caught on the spawning grounds in the lower bay (the end

migration point for inseminated females from around the bay) and to release their hatchery-produced offspring for spawning stock restoration. However, none of the hatchery-produced offspring should be used for future broodstock (A. Place, personal communication).

Using only mitochondrial DNA sequences, we were able to very reliably distinguish crabs of hatchery origin from wild crabs with greater than 95% accuracy. Adding microsatellite markers, nearly 100% accuracy could be obtained. During the 2003–2006 releases, we used both mechanical tags and mitochondrial DNA markers to monitor the hatchery-released crabs. As we scale up releases, monitoring of juveniles in the bay will gradually shift from using microwire tags to using mitochondrial DNA and microsatellite markers to identify cultured crabs.

OPTIMAL RELEASE STRATEGIES

Survival of released crabs, their growth rate to sexual maturity, and likelihood of successful integration into the spawning stock is a direct function of release strategy and protocols. We conducted detailed experiments to identify optimal sites and timing of releases, as well as optimal size of crabs to be released and density of releases. As these factors are interconnected, we used multi-factorial analysis and modeling to assess and predict optimal protocols of release. Release sites in the upper and lower bay were over 200 km apart and had very different environmental conditions, predator guilds, and proximity to the spawning grounds. As juveniles were released at sites that harbor plants and other organisms, a community analysis approach was used to better understand the role of refuge habitats (seagrass beds, oyster reefs), prey organisms, and predators.

The findings of those studies are detailed in this volume (Hines et al., 2008; Lipcius et al., 2008; Johnson et al., 2008; Seitz et al., 2008) and elsewhere (Lipcius et al., 2005a, 2007; Seitz et al., 2005; Davis et al., 2005b). Of greatest importance are the data related to optimal crab size, location, and timing of release. Determining size at release in any restocking program reflects a “trade off” between survival and cost of producing juveniles. Field experiments for both upper and lower bay habitats consistently showed that survival of cultured crabs increased with their body size in the range of 10–70 mm carapace width (CW). However, the cost of juvenile production increases significantly with size, mainly reflecting increased aggression and mortality due to cannibalism. In Chesapeake Bay, at a size of approximately 20 mm CW, wild juvenile crabs undergo a secondary dispersal phase and recruit into shallow nursery habitats in both the upper and lower bay (Pile et al., 1996; Lipcius et al., 2007, 2008). Based on those studies, we chose to release crabs at the minimal size that matches the habitat requirement of the juveniles in the wild, which is 20 mm CW. Food requirements of the blue crab change as they grow; therefore, all release sites are analyzed for availability of sufficient prey, and release density is adjusted accordingly (Davis et al., 2005b; Seitz et al., 2005,

2008). Other important considerations in selecting release habitats and determining release densities are the occurrence and type of refuge structures (seagrasses, woody debris, etc.), the presence and density of wild crabs, prevalence of disease, and abundance and type of predators.

To maximize the likelihood of released juveniles contributing successfully to the spawning stock, we tested the impact of timing-of-release on survival to sexual maturity. Between April and October (when water temperature is most conducive to survival), we observed significant seasonal differences in survival rates, mainly reflecting the abundance of predators in release habitats (Hines et al., 2008; Johnson et al., 2008). Those predators consist of blue crabs of the same or larger size and some species of fish. However, of more importance to success were seasonal differences in growth rate and acquisition of sexual maturity. Juvenile crabs released during the spring (April–June), as soon as the temperature in the upper bay started to increase and approach summer conditions, reached sexual maturity at ~100 mm CW within as little as 3 months post-release (at 5 months of age). They then mated and left the release areas, starting their southward migration to the spawning grounds in the lower bay, and contributed to the breeding stocks as early as 5–6 months after release (Hines et al., 2008; Johnson et al., 2008). This is the overarching objective of the replenishment program. Although it is most probable that those crabs hibernate before spawning the next spring/summer, they still produce offspring a year ahead of their wild counterparts. Crabs released later in summer or in the fall stopped growing as soon as the water temperature dropped and did not reach maturity. They over-wintered in the release areas and were tracked again the next summer as they continued to grow to sexual maturity. These late-release crabs follow the same pattern to spawning as the wild crabs.

Collectively, these experiments led to a release strategy that significantly expedites the contribution of hatchery-produced crabs to the breeding stocks. Through the exposure of mated females in the hatchery to phase-shifted, photo-thermal regimes, we induce hatching in early March, about 3–4 months before it occurs in the wild (Figure 1). Sixty days later, in early May, as soon as temperatures in the bay enter the optimal range for survival and growth, we release 20-mm CW juveniles, again around 3–4 months before wild juveniles of the same size reach nursery habitats (at least for the upper bay). Therefore, the juveniles released early enjoy an environment with reduced populations of competitors and predators, as well as warm (summer) temperatures. Consequently, they survive well and grow fast, reach sexual maturity in as few as 3 months (as early as August), mate, and migrate to the spawning grounds before the onset of winter. They will over-winter and spawn in their second year of life. Their wild counterparts will not grow enough to reach maturity before the end of summer, and will therefore over-winter as immature animals that will attain sexual maturity only in the following summer and spawn in the next (third) year of their life. Because water temperature in the lower bay is higher for longer periods, compared to the upper bay, and nursery habitats in the lower bay are closer to the spawning grounds, the same result

can be achieved by releasing the crabs throughout early fall. This strategy results in the presence of larger crabs (of hatchery origin) in the nursery habitats when the smaller wild juveniles are recruited to the same sites. Based on the feeding habits of different size classes of crabs, it is unlikely that released crabs preying upon the smaller wild crabs had a significant impact on their abundance. Analyzing gut contents of crabs smaller than 60 mm CW showed only 3% blue crab prey, while a majority of their diet (38%) was composed of bivalves (Tagatz 1968; Laughlin 1982; Hines et al., 1990; Mansour, 1992; Lipcius et al., 2007).

The release strategy described above led to a one-year shortening of the time required for cultured crabs to reach the spawning grounds, compared to the wild juveniles. Therefore, the probability of released crabs quickly contributing to the breeding stocks is dramatically increased. The findings and considerations leading to this release strategy have been modeled and are discussed in more detail later in this volume (Hines et al., 2008; Johnson et al., 2008).

DISEASE AND HEALTH MANAGEMENT

Responsible aquaculture-based blue crab stock replenishment faces a series of challenges with respect to disease. First, in high density aquaculture, there is the potential for pathogens to spread rapidly with devastating effects on juvenile crab production. Second, to avoid introducing pathogens into the environment, the facility must produce juveniles for release that are free of disease-causing organisms. Finally, it would be counter-productive to release “clean” healthy juveniles into habitat harboring endemic crab diseases. BCARC partners are using a three-pronged strategy to address these challenges, consisting of development of improved diagnostic tools, quality assurance in the aquaculture setting, and habitat assessments.

1. *Diagnostic Tools.* Molecular techniques can rapidly detect cryptic infections in incoming broodstock simply by sampling hemolymph and leg muscle tissue. We are employing and developing rapid and sensitive molecular tools for detection of known blue crab pathogens, including quantitative PCR assays for *Hematodinium* sp. (Steven et al., 2003), White Spot Syndrome Virus (WSSV; Powell et al., 2006), and pathogenic *Vibrio* species (Panicker et al., 2004). Examples of additional pathogens for which molecular probes are anticipated are the microsporidian *Ameson michaelis* (cotton crab disease) and Chesapeake Bay Virus (Messick and Sindermann, 1992).
2. *Disease-free Releases.* To date, we have not experienced any disease outbreaks in the hatchery. To produce specific pathogen-free juveniles and remain attentive to the threat of diseases and parasites, we follow quality assurance measures that include quarantine and histological examination of incoming wild broodstock females, and inspection of juveniles before release. We have partnered with experts on

blue crab diseases at the Cooperative Oxford Lab (NOAA Oceans Service) who serve as independent inspectors and advise us on potential disease outbreaks. By careful histological examination, Oxford Lab scientists screen for a long checklist of potential parasites and pathogens, including metazoans (e.g., Nemertean worms), protozoa (*Paramoeba*, microsporidia, *Hematodinium* sp.), bacteria (rickettsias, *Vibrio* spp.), and viruses (Chesapeake Bay Virus, bi-facies virus; Messick and Sindermann, 1992).

3. *Habitat Assessments.* Disease assessments will be included in habitat surveys to ensure that juveniles are not released into areas of Chesapeake Bay with recent disease-related mortality of blue crabs. Of immediate concern are potential hotspots for pathogens such as the parasitic dinoflagellate, *Hematodinium* sp. (Messick and Shields, 2000). Field sampling of blue crabs, blue crab prey, and water and sediment samples will include both microscopic (histological) and molecular assays for available pathogens of concern. Habitat with potential blue crab disease organisms will be avoided as release sites.

ECONOMIC FEASIBILITY

Predicting the cost-effectiveness and economic feasibility of blue crab replenishment efforts is one of the major factors governing success of large-scale implementation of the concept. In addition to its considerable economic value, the blue crab is an icon of traditional and cultural value in the Chesapeake Bay region. Moreover, it is an important organism in the bay's benthic and pelagic communities. Therefore, maintaining robust populations and preserving the traditional fishery has tremendous non-monetary value.

Owing to the drastic decline in number of spawning females in Chesapeake Bay in the last 15 years (Seitz et al., 2001; Lipcius and Stockhausen, 2002; Miller et al., 2005), and the fact that the spawning sanctuary in the lower bay protects only 50% of the spawning females (Lipcius et al., 2003), we estimate that the spawning sanctuary currently supports 3–8 million spawning females during the summer (A. Hines, R. Lipcius, and R. Seitz, personal communications). A reasonable goal would be to enhance that stock by 10% annually, thereby increasing it by 300,000–800,000 females per year. Assuming 10% survival of cultured females until spawning in the sanctuary (which will entail a strict management plan to protect seasonal migration corridors used by mated females to reach their spawning ground), this goal will require production and release of 3–8 million juvenile females or 6–16 million crabs of both sexes per year. The Japanese swimming crab stock enhancement program has been releasing approximately 35–45 million juveniles per year. Roughly 10 million are released from a single hatchery (Tamano Hatchery; Ariyama, 2000, 2001; Ariyama et al., 2001; Secor et al., 2002). Producing and releasing millions of blue crabs annually is certainly feasible. The obvious question is whether this will be cost-effective. The nursery stage is currently the princi-

pal bottleneck because of intensive cannibalism, which in turn dictates relatively low stocking densities, and because of the extensive labor involved in grading (size-sorting) the juveniles and preparing shelter structure in the rearing tanks (Zmora et al., 2005). As soon as we overcome the cannibalism issue (likely via improved biological understanding of animal physiology), we expect the production cost of blue crab juveniles will be no different from that of fingerlings of many farmed marine finfish, i.e., in the range of US\$0.15–0.30/juvenile.

The ultimate economic feasibility of our approach will depend upon data generated once the release strategy is further optimized and more monitoring is done on a bay-wide scale. Further cost-benefit examination of the proposed restocking approach will have to consist of a stringent socioeconomic analysis, including a comparison of alternative (non-hatchery) methods to increase the number of spawning adults. Modeling exercises will be implemented in the future to compare the cost and impact of the restocking approach with other management tools, e.g., fishermen buyouts and harvest size restrictions.

SUMMARY AND LESSONS LEARNED

During the 5 years since inception of the multidisciplinary blue crab restoration program, significant progress has been made in addressing many of the components and tenets of responsible stock enhancement. Being able to close the entire life cycle of the blue crab in captivity, and develop hatchery protocols for mass production of its juveniles, enabled progressively broader experiments to assess the feasibility of the program concept. Increasing numbers of juvenile crabs have been individually tagged and experimentally released, and monitored for a wide range of studies. Cultured crabs were shown to survive in the field as well as their wild counterparts. Release strategies are being studied and optimized in terms of crab size, site suitability, and timing of release. Careful attention is being paid to the health of released juveniles and to preserving their genetic diversity. Optimal releases into nursery habitats that are below carrying capacity resulted in significant enhancement of local populations. Released crabs grew quickly to sexual maturity with excellent survival. Genetic markers to monitor the released crabs are now also being used to study the genetic structure of the Chesapeake Bay population and will be gradually employed to assess the ultimate success of the program in terms of contribution of cultured crabs to breeding stocks and their offspring. At the present stage, we consider our findings to be very encouraging and indicative of the feasibility of our approach of using hatchery-raised juveniles to enhance the blue crab breeding stocks in Chesapeake Bay.

This multidisciplinary program has taught us several valuable lessons. First and foremost is the importance of understanding the basic biology and life cycle of the studied model (i.e., the candidate for restocking) within its ecosystem. Specific restoration approaches must be tailored to the species of interest in the context of the targeted system. Second is the importance of

integrated approaches to stock restoration. A blue crab restoration program must be based on a holistic approach, which integrates understanding of the released species with the system to be restored, such as the ecology of the habitat, seasonality patterns, and biological cycles. Moreover, blue crab restoration cannot be successful without integrating adequate management strategies to protect the wild and released animals until sexual maturity and spawning. All stakeholders in the resource to be restored must join forces and work in concert, including the fishery and seafood industry, policymakers, environmental activists, and scientists. Finally, a successful stock restoration program must be guided by thorough and solid science that addresses the multiple and complex facets reviewed here. This type of research is costly and typically spans several years. However, it is essential to avoid the expensive mistakes of the past, such as precociously implementing major marine stock enhancement programs. Throughout the process, the scientific research must be scrutinized via publication in the top-quality peer-reviewed literature. As global fisheries face increasing threats from human-driven activities, implementing scientifically solid stock enhancement or restoration programs may provide substantial and long-term ecological and financial benefits.

FUTURE DIRECTIONS

Taking our program to the next level will require scaling up our experimental releases to additional and larger ecosystems within Chesapeake Bay. A bay-wide and multi-species approach, testing different habitats and larger cohorts of released crabs, is required to model and predict full-scale impact and success. Based on such a model, a detailed cost-benefit analysis must be conducted to weigh the hatchery-based replenishment strategy against other avenues to restore crab stocks in Chesapeake Bay. Close partnership with policymakers, regulatory agencies, and stakeholders is required to fully integrate our efforts with improved management of the fishery, ongoing efforts to clean the bay, and restoration of its coastal habitats. Ensuring successful contribution of released cultured crabs to spawning stocks will require strong measures to protect females along their migratory route from nursery habitats to spawning grounds. The impact of changes in climate and oceanic conditions as well as alterations in coastal nursery habitats on recruitment of juveniles from either wild or hatchery-produced crabs will have to be further understood. Basic biological studies of blue crab life history and ecology will continue to provide critical information needed to improve and optimize replenishment and management of the fishery.

ACKNOWLEDGEMENTS

The Blue Crab Advanced Research Consortium and its extensive studies would not have been possible without the vision and continuous support of U.S. Senator Barbara Mikulski. The

partnership and cooperation of the Maryland Watermen's Association, especially its president, Larry Simms, and Mick Blackstone, Executive Director of Crab Restoration Around the Bay, is highly appreciated. We are grateful to Steve Phillips, Chief Executive Office, Phillips Foods Inc., for his financial and moral support. We are also thankful to the staff of the Maryland Department of Natural Resources for allowing us to use their facilities. This research was supported by a NOAA Chesapeake Bay Program Grant (NA17FU2841) to the Blue Crab Advanced Research Consortium.

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