



## Morphological conditioning of a hatchery-raised invertebrate, *Callinectes sapidus*, to improve field survivorship after release

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Received 22 June 2004; received in revised form 20 September 2004; accepted 22 September 2004

### Abstract

For recruitment-limited, severely depleted fishery stocks, stock enhancement may become an important technique in the return of population sizes to sustainable levels. Aquaculture-reared individuals, however, may face some disadvantages upon release into the wild due to differences between natural conditions and the hatchery. The goal of this study was to test whether field survivorship of hatchery-raised blue crabs, *Callinectes sapidus*, could be improved by simple conditioning steps, taking advantage of phenotypic plasticity in certain traits. This species is currently the focus of a preliminary stock enhancement program in the Chesapeake Bay. Results indicate that unconditioned hatchery crabs had lower survivorship than wild crabs in the field and differed in carapace color and lateral spine length. Both traits were plastic. Carapace color was changeable within 1–2 days, without a molt, upon the exposure of crabs to new substrates. However, colors within the range produced in this study did not significantly affect survivorship in a field or a laboratory experiment. Change in spine length required exposure to predators for 1–4 weeks. Exposure to fish predators resulted in increased spine length, though exposure to adult blue crabs had no significant effect. Crabs with lengthened spines had significantly higher survivorship in both laboratory and field experiments, suggesting that this feature may be one on which to focus large-scale conditioning efforts. Results of this study suggest a level of phenotypic plasticity that may contribute to the blue crab's ability to take advantage of multiple estuarine habitat types. On a more applied level, results of this study suggest that at least some deficiencies in hatchery-raised organisms can be alleviated and would likely lead to improved success and efficiency of stock enhancement efforts. Similar studies on other hatchery-raised invertebrates and finfishes may also lead to improvements in their enhancement programs.

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**Keywords:** Stock enhancement; Decapod; *Callinectes sapidus*; Lateral spines; Carapace color

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## 1. Introduction

Stock enhancement in which hatchery-raised juveniles of an aquatic species are released into the wild has been used worldwide in a number of finfishes (e.g., Secor and Houde, 1998), mollusks (e.g., Beal et al., 2002), and less often, crustaceans (e.g., Bannister and Addison, 1998). In many cases, the released juveniles survive well enough to bolster population sizes (e.g., McEachron et al., 1998; Bannister, 2000). However, even in most of those cases, it is not known whether the released juveniles survived as well as their wild counterparts or simply well enough to be detected.

The goal of most stock enhancement programs is to produce individuals that survive equally as well as wild individuals once released, rather than better (the genetically overweighted “super individual”) or worse (less product per unit effort). Work comparing such factors as behavior (Swain and Riddell, 1990; Furata, 1998), feeding efficiency (Furata, 1998; Sundstrom and Johnsson, 2001), morphology (Ellis et al., 1997; Hard et al., 2000), and ultimate survivorship (Kellison et al., 2000; Stunz and Minello, 2001; Davis et al., 2004b) indicates that often hatchery-raised individuals are not as well-equipped as their wild counterparts to deal with natural environmental conditions. Sometimes factors related to the hatchery environment, such as lower flow, uniformity in substrate color, and different food, have behavioral or morphologic ramifications that lead to lower survivorship in the field than expected for a similarly sized or aged wild individual (Schiel and Welden, 1987; Stoner and Davis, 1994; Ray et al., 1994; Furata, 1998; Bolker and Hill, 2000; Kellison et al., 2000).

Many of these behavioral and morphologic expressions reflect an organism’s ability to respond phenotypically to its environment, rather than genetic differences between hatchery and wild organisms. It may be possible to use this phenotypic plasticity to the advantage of the stock enhancement program. To improve the success of such programs, it has been proposed that hatchery individuals be conditioned to alleviate some of their deficiencies before their release (Olla and Davis, 1989; Berejikian, 1995; Brown and Smith, 1998; Kellison et al., 2000). Though this process might produce added expense, the extra cost might be offset by improvements in survivorship. For

example, hatchery flounder conditioned with a predator-exposure treatment had higher survivorship in the laboratory than unconditioned hatchery flounder (Hossain et al., 2002). The key is to identify traits of the hatchery organism both that differ enough from the wild population to cause differential survivorship, and that are plastic enough to respond quickly and easily to conditioning.

Though stock enhancement is not often used in crustacean cases, recent investigations have begun into the possibility of using stock enhancement to bolster declining populations of blue crabs, *Callinectes sapidus*, in the Chesapeake Bay. Populations of this species have declined more than 80% in the past decade and are thought to be recruitment-limited (Lipcius and Stockhausen, 2002). Initial studies indicate that hatchery-raised blue crabs can contribute significantly to local populations without displacing wild individuals and do survive to maturity in subestuaries of the Chesapeake Bay (Davis et al., in press). However, one type of field survivorship comparison, relying on tethering techniques, suggested that survivorship of hatchery-raised crabs was not as high as that of wild crabs (Davis et al., 2004b).

The purpose of the present study was to determine whether survivorship of hatchery crabs could be improved by simple methods that could be applied to crabs before they are released. Specific goals were to identify differences between hatchery and wild crabs, determine whether differences led to differential mortality, and then determine whether differences could be eliminated through conditioning. We focused on the morphological factors of carapace color and lateral spine length.

## 2. Methods

Blue crabs were raised at the University of Maryland Biotechnology Institute’s Center of Marine Biotechnology (COMB). Those used in the study were raised in 3 m<sup>3</sup> cylindrical, blue, fiberglass tanks and fed a combination of pellet food, flake food, and squid several times per day (Zmora et al., unpublished data). One cohort (Cohort C) was the offspring of a laboratory-born female and a wild male. All others were produced from wild-caught mature females that had mated in the wild. In total, crabs from four cohorts

(Cohorts A, B, C, and D), which were born in early March, late March, April, and May 2003, respectively, were used.

### 2.1. Differences in survivorship between hatchery and wild crabs

Hatchery crabs produced in 2002 had lower survivorship than wild crabs in tethering experiments (Davis et al., 2004b). Two experiments were designed to test whether hatchery crabs produced in 2003 also had lower survivorship than wild crabs. The first was a repeat of the tethering experiment, in which four groups of 10 crabs were tethered each in Aberdeen Cove (1 ha) in the South River and Boathouse Creek

(5.5 ha) in the Rhode River (Fig. 1). Within each group of 10 crabs, five were hatchery-raised (11–23 mm carapace width (CW), mean  $15.7 \pm 0.06$  mm SE) and five were wild (11–22 mm CW, mean  $15.8 \pm 0.07$  mm SE). Hatchery crabs were members of hatchery cohorts A and B, and wild crabs were from the South and Rhode Rivers on the upper western shore of the bay (Fig. 1) and waters near Deal Island on the eastern shore of the bay ( $38.1^\circ$  N,  $75.9^\circ$  W). Most crabs had all of their limbs, and none were used if missing both claws or both swimming legs. *t*-tests revealed no differences in the number of missing appendages between hatchery and wild crabs. Tethering experiments, which prevent a full range of escape methods, do not reveal actual mortality rates but rather

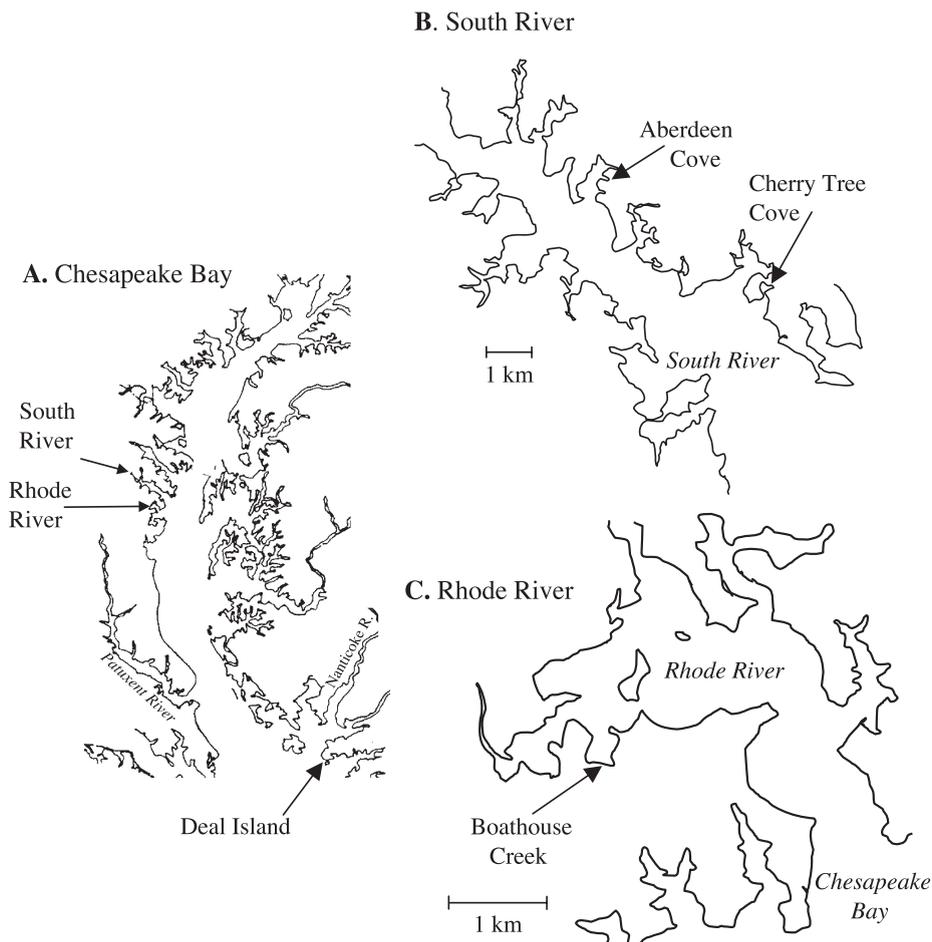


Fig. 1. Study sites in the upper Western Chesapeake Bay. (A) Chesapeake Bay, (B) South River, (C) Rhode River.

comparative mortality rates, provided that the affect of the tether is the same on all treatments (Peterson and Black, 1994).

Tethers were 1 m long, attached on one end to a heavy chain laid at a depth of 30 cm and on the other to crabs with glue. Crabs were tied to the chain at 3-m intervals, with alternating hatchery and wild crabs within each group of 10. Percent survivorship after 3 days was compared between wild and hatchery crabs within each group of 10 crabs using paired *t*-tests. Crabs that had molted, leaving behind intact carapaces and releasing themselves from their tethers, were not included in the analysis. All proportion data were arc-sine square-root transformed prior to analysis.

In addition to the tethering experiment, survivorship of 2,600 hatchery crabs released into the wild was compared to that of 1,300 wild crabs captured, tagged, and re-released into the wild on May 28, 2003. Hatchery crabs were members of Cohorts A and B raised at COMB and ranged in size from 10 to 21 mm CW (mean 16.1 mm CW  $\pm$  1.9 SD). Wild crabs were collected in Laws Thoroughfare, Deal Island, and ranged in size from 7 to 28 mm CW with an average of 16.4 mm CW ( $\pm$  5.3 SD). All crabs were tagged with Northwest Marine Technology (NMT) microwire in the basal muscle of the fifth pleopod (the swimming leg) and with red elastomer tags, also manufactured by NMT, in the first segment of the fifth pleopod (Davis et al., 2004a). To distinguish between wild and hatchery tags, elastomer was placed in the left swimming leg of wild crabs and in the right leg of hatchery crabs.

Crabs were released on May 25, 2003 into Aberdeen Cove, and resampled weekly or once every two weeks until October 2003. Two methods were used for resampling: The first was a 16 m long, 3 m high beach seine with 6 mm mesh, pulled for 25 m parallel to shore in four locations within Aberdeen Cove by two people standing approximately 10 m apart (total sample area 1000 m<sup>2</sup>). The second gear type was a 1 m wide, 50 cm high epibenthic sled with a 2-m long, 6 mm mesh net. The sled was towed by a shallow-water boat for 100 m (total sample area 100 m<sup>2</sup>) at seven sites within Aberdeen Cove. Recaptured tagged crabs were measured and returned to their recapture locations. The number of hatchery and wild crabs recaptured was compared using a chi-squared test.

## 2.2. Color differences

Differences in color between hatchery-raised and wild juveniles of the same size were quantified using analysis of digital photography. Each of 20 pictures featured two hatchery crabs and two wild crabs randomly assembled on a white background. A standardized photographic set-up was established to minimize differences in lighting across photographs; however, the inclusion of both crab treatments (hatchery and wild) within each photograph served to reduce the impact of variation across photographs. Each image file was then analyzed in Adobe Photoshop 6.0 by identifying the hue (shade of color), saturation (amount of hue), and brightness (light vs. dark) of 10 points along a transect from the posterior to the anterior of the carapace of each crab. Values were averaged across these ten points to achieve one value of each per crab.

All crabs were 10–30 mm in carapace width (CW), corresponding to the size at release in the current investigative stock enhancement program. Preliminary analysis indicated that color did not differ between 10–30 mm males and females, so gender was not controlled. Molt stage was controlled, however, with all photographed crabs in the intermolt (C) stage. For hue, which is measured on an arbitrary 360° scale, calculation of circular statistics (mean and confidence intervals) followed Zar (1999). Saturation and brightness were compared between hatchery and wild crabs using *t*-tests, and hue data were compared using the Watson-Williams test for circular data.

To test whether hatchery and wild crabs chose different substrates, potentially based on carapace color differences, we used a laboratory experiment. Crabs were released individually into 1  $\times$  0.5 m tanks covered with 15–20 mm of sand and filled with water to a 10-cm depth. The sand bottom was divided into four quadrants, with two the color of the average wild crab and two the color of the average hatchery crab. The two quadrants of each color were arranged diagonally to each other. Wild-crab-colored quadrants were created with sand collected off the Smithsonian Environmental Research Center (SERC) dock. Hatchery-crab-colored sand was created by mixing five parts of dock sand with one part of white sand (PetSmart™ Vita-sand). Digital photography and

image analysis were used to verify that the color of the sand backgrounds corresponded to the average color of the two crab types.

Individual crabs were added to the center of each tank, where all four sand quadrants met, and allowed 10 min to acclimate. After this period, the position of the crab was noted every 5 min for 60 min. Trials were run only during the day and using only 10–30 mm crabs. A trial duration of 60 min was chosen after a pilot study indicated that substrate color choice did not differ between the first hour and the subsequent 3-h period. Trials longer than this were not considered due to potential for crabs to change color within this time frame.

### 2.3. Color conditioning and effects on survivorship

The ability of hatchery crabs to change color and the time required for such a change was investigated with two trials of a laboratory experiment at SERC. Each of 27 plastic containers (30×45×10 cm) was assigned to one of three bottom coverings: brown sand, brown tank liner, and plain white. Brown sand was collected off the SERC dock, intended to represent wild conditions, and layered 2 cm thick in each container. Brown plastic tank liners were intended to represent a potential hatchery facility conditioning alternative to sand. Plain white bottoms were intended to represent a default hatchery tank (though the tanks at COMB, where these crabs were raised, were light blue). Containers were then assigned to one of three outdoor 0.5 m<sup>3</sup> tanks fed by ambient flowing Rhode River water in a randomized block design.

In the first trial, two crabs, one male and one female, were added to each container, which was then covered with mesh lids to prevent escape. Color (hue, saturation, and brightness) was assessed with image analysis as described above after 2, 5, and 11 days. Each photograph contained the three crabs in a tank row, one from each treatment, such that any effects of photograph lighting or tank lighting would be displayed across treatment type. If any crab of the three that belonged in an image block died, the entire block was discarded. The second experiment was similar to the first, except that only one crab was used per container, and color was assessed after 1, 2, 4, and 7 days. Color data were analyzed as above. Tukey (saturation and brightness) and Dunn's (hue)

multiple comparisons tests were used to distinguish which substrate treatments differed from the others.

To determine whether substrate color affected survivorship when subjected to predators, one field and one laboratory experiment were used. Surviving crabs from the first color conditioning trial were tethered in Boathouse Creek. Three groups of three crabs conditioned with each substrate treatment were used, for a total of 27 crabs. Tethers were placed such that treatments were alternating. Mortality rate was calculated for each group of crabs within a treatment. The laboratory experiment was designed to specifically test effects of substrate conditioning on predation rates by striped bass, an important predator of blue crabs (Orth et al., 1999). Six to seven crabs of each substrate treatment were added to a 2.25 m<sup>3</sup> (1.5 m in diameter by 1.2 m deep) indoor fiberglass cylindrical tank, followed by six striped bass 100–160 mm in total length (TL). Tanks were lined with a thin layer of sand to simulate natural habitat color without allowing crabs to bury. All juvenile crabs were marked with a distinguishing series of external paint marks. Data from both experiments were analyzed using a two-way ANOVA to compare effect of substrate conditioning treatment and effect of experiment type.

### 2.4. Spine length conditioning and effects on survivorship

Differences in lateral spine length between hatchery and wild crabs have been previously demonstrated (Davis et al., 2004b). From May to July 2003, we tested the hypothesis that hatchery-raised crabs could develop longer lateral spines, an anti-predator feature, if they were exposed to predators. We exposed hatchery-raised crabs to one of three predator treatments for 27 days: adult blue crabs, one of the most important predators of juvenile blue crabs (Hines and Ruiz, 1995); striped bass, suggested to be important by Orth et al. (1999); and a control with no predator. Exposure was potentially both visual and chemical, though not tactile, and was created by separating hatchery-raised juvenile crabs from predators with 1 cm mesh barriers.

Ten outdoor 0.5 m<sup>3</sup> tanks were divided in half, with one half of all tanks containing 30 hatchery crabs from 9 to 30 mm CW (mean 19.8 mm CW±0.6 SE). The other half of each tank contained one of the three

treatments: four adult (>120 mm CW) male blue crabs (three tanks), four striped bass 100–160 mm in total length (four tanks), or no predator (three tanks). Juvenile and adult crabs were fed pellet food, and striped bass were fed both pellet food and grass shrimp. We hypothesized that long spines are a defensive mechanism against fish predators, whose gape is a limiting factor, but not blue crabs, which are not limited by the width of the juvenile crab prey but probably by their chelae size. All crabs were measured after 8 days and after 27 days. Both carapace width and spine length were measured to the nearest 0.5 mm. Spines were measured from the tip of the spine to the point at which the spine meets the curve of the carapace (and therefore no longer represents a protrusion). Change in spine length was compared among predator treatments for the two time periods separately by averaging all crabs within a tank, using tank as replicate, with ANOVA and Tukey post-hoc tests.

We exposed survivors of this experiment to predation in the laboratory to determine whether our predator conditioning improved survivorship. Ten crabs of each treatment were individually tagged with paint markers and added to each of three indoor 2.25 m<sup>3</sup> cylindrical tanks. As above, each tank contained six striped bass 100–160 mm TL and a bottom of thin sand. Survivorship rates were compared among treatments with a randomized-block design ANOVA, with tank number as a blocking factor. All percentage data were arc-sine-square-root transformed prior to analysis.

To isolate effects of spine length alone, a similar laboratory experiment was conducted using wild juvenile blue crabs collected from the Rhode and South Rivers. Half of these wild crabs were left intact, but the lateral spines of the other half removed. Two days later, 10 crabs of each treatment were added to each of three 2.25-m<sup>3</sup> indoor cylindrical tanks, six striped bass 100–160 mm TL were added, and mortality rates were compared between shortened and unshortened crabs using a *t*-test.

### 3. Results

#### 3.1. Hatchery versus wild survivorship

Tethered crabs from the hatchery had lower survivorship ( $77.3\% \pm 8.7$  SE) than wild crabs

( $88.5\% \pm 6.5$  SE), though the difference was not significant at the  $\alpha=0.05$  level (paired *t*-test:  $t_7=2.2$ ,  $p=0.061$ ). Recapture rates of the 2600 hatchery and 1300 wild crabs released into the field also suggest a lower survivorship of hatchery crabs. Over the 60-day period following crab release, after which point hatchery and wild crabs were indistinguishable due to elastomer tag loss (Davis et al., 2004a), more total wild crabs were recaptured (35) than hatchery crabs (29), a ratio that was significantly higher than that expected based on 1 wild: 2 hatchery crab release ratio (chi-square test:  $X^2=12.1$ ,  $p=0.002$ , Fig. 2).

#### 3.2. Color differences

Hatchery crabs raised in light blue fiberglass tanks were lighter in color, grayer (lower saturation), and a different hue (more blue/green) than wild crabs (Fig. 3). Hatchery crabs also had a significantly lower variance among brightness measurements within a crab (Fig. 3B), suggesting that they are more uniform and less speckled than wild crabs. This color difference was consistent with results of the substrate color choice experiment. Hatchery crabs spent less time in and on the natural-sand-colored substrate than the lighter-colored substrate, and wild crabs spent more time in and on the natural-colored substrate than the lighter-colored substrate. The amount of total time spent in and on the natural substrate by hatchery crabs ( $47.5\% \pm 4.0$  SE) was significantly lower than that of wild crabs ( $66.9\% \pm 7.4$  SE) (*t*-test  $t_{30}=2.3$ ,  $p=0.029$ ). Hatchery crabs also spent less time buried in the

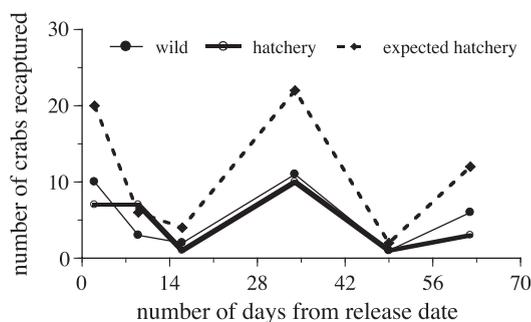


Fig. 2. Number of recaptures of wild and unconditioned hatchery crabs in Aberdeen Cove. The dotted line indicates the expected number of hatchery crab recaptures according to the initial 2:1 release ratio of hatchery crabs: wild crabs. Day 0=May 28, 2003.

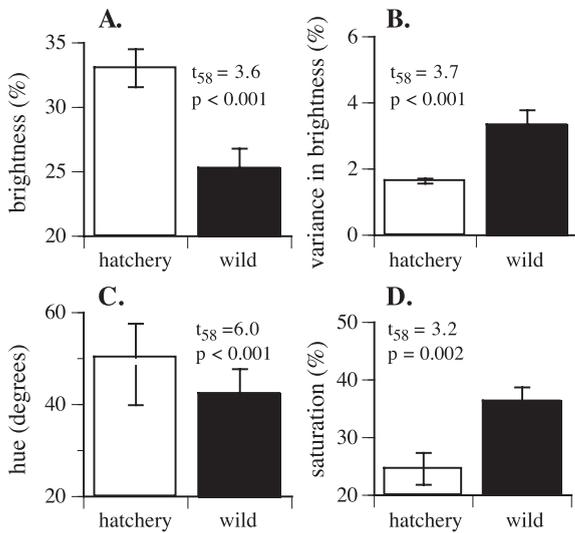


Fig. 3. Differences in (A) brightness, (B) variation in brightness, (C) hue, and (D) saturation between hatchery-raised and wild crabs. SEs are plotted for A, B, and D, and 95% confidence intervals are plotted for hue (C). Hues of 80° and 40° represent green and yellow, respectively. Results of *t*-tests (A, B, and D) and a Watson-Williams test (C) are presented.

sediment (18%±6 SE) than wild crabs (72%±8 SE, *t*-test  $t_{30}=5.3$ ,  $p<0.001$ ).

3.3. Color conditioning and effects on survivorship

Crab carapace color was not fixed and required little time to change. Crabs in the darker sand and tank liner substrates became darker within 1–2 days than those in the white substrate (Fig. 4A and B), patterns consistent with expectations based on differences between hatchery and wild crabs (Fig. 3A). Crabs on the white substrate were expected to differ from crabs on the darker substrates, especially sand, in the same way that hatchery crabs differed from wild crabs (Fig. 3). As expected, crabs in sand became greener in hue whereas “white” and “liner” crabs remained bluer (Fig. 4C and D). However, crabs in sand became less saturated with color than those on the white or brown tank liner substrates (Fig. 4E and F), opposite to expectations based on differences between hatchery and wild crabs (Fig. 3D).

Brown tank liners may not serve the same purpose as sand conditioning. Although the tank liner treatment had a similar impact on crab brightness as sand, the

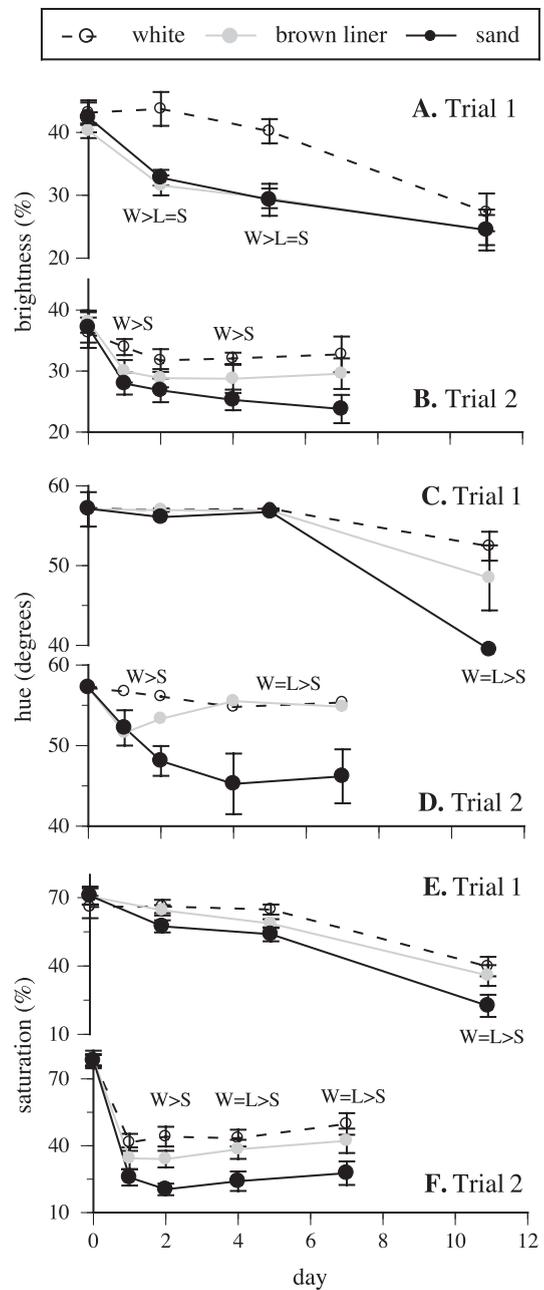


Fig. 4. Effects of substrate type and color on three aspects of crab color: brightness (A and B), hue (C and D), and saturation (E and F), in two trials of a substrate conditioning experiment. Standard errors are presented, and results of Tukey post-hoc tests are listed above each point for which a significant difference among the three treatments existed (ANOVA,  $p<0.05$ ). S=sand, L=brown tank liner, and W=white substrate.

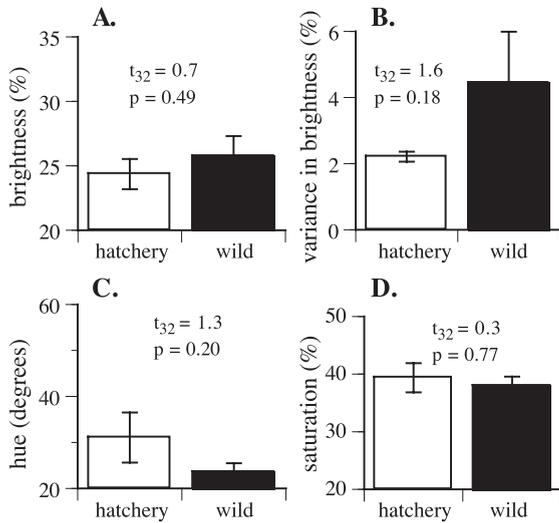


Fig. 5. Lack of differences in (A) brightness, (B) variation in brightness, (C) hue, and (D) saturation between hatchery and wild crabs tethered in the field for 5 days without molting. SEs are plotted for A, B, and D, and 95% confidence intervals are plotted for hue (C). Results of  $t$ -tests (A, B, and D) and a Watson-Williams test (C) are presented.

liner treatment was similar to the white treatment in effects on saturation and hue.

Color change did not appear to require a molt, as only a few crabs molted within the period of 1–2 days. (The average crab in this experiment molted once every 5 days.) Also illustrating this point were 17 wild and 17 hatchery crabs that survived the tethering experiment without molting. Though these wild and hatchery crabs initially differed in color, as they were chosen from the same pool as those in Fig. 3, after 5 days in the field, they no longer differed in any of the four aspects of color (Fig. 5). Hatchery crabs became darker, more saturated with color, more variable in brightness (more speckled), and less blue in hue, and therefore not different from wild crabs.

Despite the hypothesis that the lighter hatchery crabs do not blend in with natural substrate as well as wild crabs and therefore would have higher mortality due to predation, crab color did not significantly affect survivorship. Those conditioned with dark sediment had only slightly higher (and not significantly different) survivorship than those conditioned on a white background (two-way ANOVA, effect of substrate:  $F_{2,13}=1.2$ ,  $p=0.334$ ; effect of

experiment type:  $F_{1,13}=1.9$ ,  $p=0.187$ , Fig. 6). Those conditioned with brown tank liners did not have significantly different survivorship than those of the other two treatments.

### 3.4. Spine length differences

As in 2003, hatchery crabs produced in 2004 also had shorter lateral spines than wild crabs. Of the carapace width of hatchery crabs averaging 16.1 mm  $CW \pm 1.9$  mm SD,  $10.1 \pm 0.4\%$  was made up of spines. This percentage was significantly smaller than that of wild crabs,  $15.5 \pm 0.2\%$ , of the same mean CW ( $16.1$  mm  $CW \pm 5.2$  mm SD) ( $t$ -test:  $t_{151}=8.3$ ,  $p<0.001$ ). We have standardized the mean size of crabs for comparison, as allocation of carapace width to spine slightly changes with crab size. For example, based on measurements of 350, 9–154 mm CW wild crabs from the study site region in 2002 and 2003, 3 mm of the carapace width of a 20-mm crab is spine (17%), while 20 mm of a 120-mm crab is spine (20%).

Like color, spine length was not fixed. Hatchery crabs exposed to striped bass for 27 days (and approximately 4–5 molts) developed longer spines than those exposed to the other two treatments (Fig. 7A). Spines of crabs exposed to striped bass increased by 1.4 mm, significantly longer than spines of crabs that had been exposed to either blue crabs (0.3 mm) or no predators (0.04 mm) (ANOVA:  $F_{2,9}=17.0$ ,  $p=0.002$ , Tukey post-hoc test  $p<0.05$ ). Crabs exposed to striped bass maintained a constant percentage of carapace width composed of spine (about 6.5%), whereas this percentage dropped over time to about

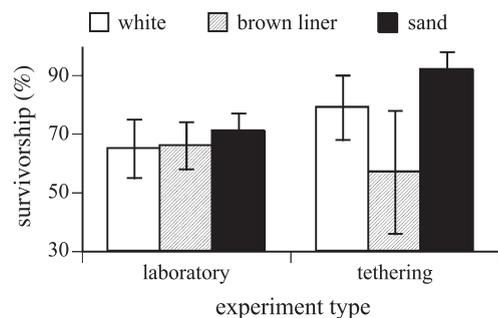


Fig. 6. Survivorship of hatchery crabs in two types of experiments, laboratory and field tethering, depending on substrate conditioning type.

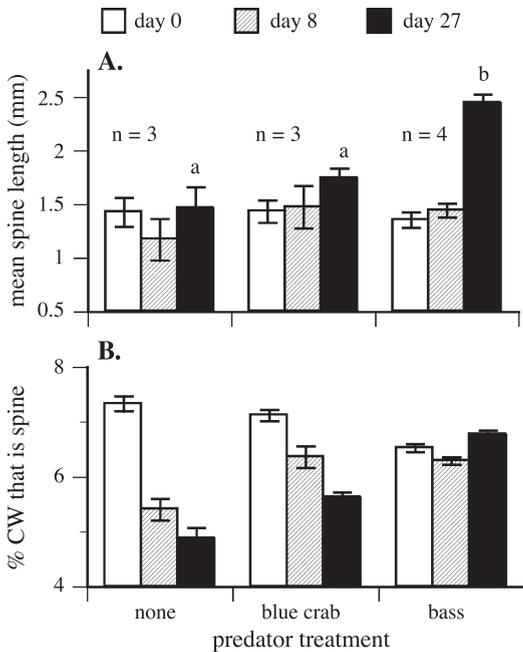


Fig. 7. Effects of predator treatment on (A) spine length and (B) % of carapace width composed of spine after 0, 8, and 27 days of predator exposure. Sample sizes (number of tanks) are listed in (A). All crabs within a tank were averaged to obtain a tank mean. Like letters in (A) indicate treatments that did not differ significantly on day 27 according to Tukey post-hoc tests.

5% for those not exposed to fish predators (Fig. 7B). Differences among treatments were evident after 8 days, but not significant until several weeks after exposure to predators, suggesting that spine length is a less plastic trait than color.

That spine length is related to laboratory conditions is illustrated by differences between wild crabs brought into the laboratory and wild crabs that were never kept in the laboratory. Fifteen wild crabs collected from Cherry Tree Cove on June 2, 2003, and then held in the laboratory until July 10, 2003, had significantly shorter spines at the end of this 38-day period than 15 crabs collected from Cherry Tree Cove on July 10 ( $t$ -test:  $t_{28}=7.1, p<0.001$ ). After the 38-day period, those that were held in the laboratory (mean size  $22.5\pm 1.7$  mm SE) had a mean spine length of  $0.7\pm 0.07$  mm, and spines made up  $6.4\pm 0.5\%$  of the carapace width. On the same day, those captured in the field (mean size  $23.9\pm 1.5$  mm SE) had spines that were more than twice as long

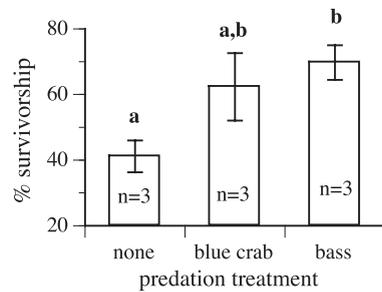


Fig. 8. Effects of predator exposure treatment on rates of survivorship from striped bass predators. Sample sizes (number of tanks) are listed. Like letters indicate treatments that did not differ significantly according to Tukey post-hoc tests.

( $1.9\pm 0.2$  mm), and spines made up  $15.7\pm 1.2\%$  of their carapace width.

### 3.5. Effects on spine length conditioning on mortality

When faced with striped bass predators in the laboratory, hatchery crabs of different conditioning regimes had different survivorship rates (randomized block ANOVA:  $F_{2,4}=6.2, p=0.059$ ). Hatchery crabs previously exposed to striped bass, and therefore attaining longer spines, had lower mortality rates than crabs that had not been previously exposed to predators, but not than crabs that had been exposed to adult blue crabs (Fig. 8).

That this difference in mortality is due to spine length, rather than some other experience factor related to their conditioning, is illustrated with two additional comparisons. First, wild crabs whose spines had been artificially shortened were preyed upon in the laboratory by striped bass at higher rate than those crabs whose spines were left long (paired  $t$ -test:  $t_2=4.4, p=0.048$ ) (Table 1). Second, hatchery crabs that were preyed upon in the tethering experiments of Boathouse and Aberdeen Coves had significantly shorter spines than those that survived ( $t$ -test:

Table 1  
Effects of shortening lateral spines of wild crabs on survivorship when subjected to striped bass predators in a laboratory experiment

Group	CW (mm)	Spine length (mm)	% CW that is spine	% Survivorship
Unshortened	$36.6\pm 1.5$	$2.6\pm 0.2$	$14.1\pm 1.1$	$66.7\pm 17.0$
Shortened	$35.8\pm 1.8$	$0.8\pm 0.05$	$4.7\pm 0.4$	$52.2\pm 20.0$

Mean values $\pm$ SE are presented.

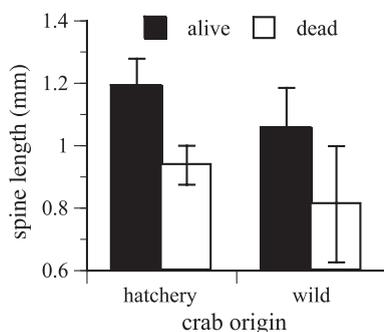


Fig. 9. Differences in spine length between hatchery crabs that survived a field tethering experiment and those that were preyed upon.

$t_{23}=2.4, p=0.027$ ) (Fig. 9). This difference between the two groups (alive and dead) was not related to body size, as measured by carapace width minus spine length. Crabs that remained alive had a mean spine-less carapace width of  $13.6 \pm 0.6$  mm, not significantly different than that for preyed-upon crabs,  $13.1 \pm 0.5$  mm ( $t$ -test,  $t_{23}=0.6, p=0.58$ ). The spine length difference between dead and surviving crabs was not significant for wild crabs ( $t$ -test:  $t_{23}=1.1, p=0.295$ ) (Fig. 9).

#### 4. Discussion

Results of this study indicate that through certain types of conditioning, the morphology and appearance of hatchery-raised organisms can be modified before their release into the field. Such modifications will likely improve hatchery crab survivorship in the field, which at present is lower than that of wild crabs. While the goal is not to create hatchery individuals that can survive better than, and therefore potentially outcompete, wild individuals, conditioning techniques present the opportunity to improve survivorship of hatchery individuals to a level equal to that of their wild counterparts. Unconditioned hatchery crabs can still contribute significantly to local populations in the field (Davis et al., in press); however, the present study shows promise that cost-effective methods exist to improve survivorship of hatchery-raised blue crabs. Should survivorship improve, contributions to local populations and ultimate brood stocks can be even higher.

Hatchery conditions in most types of aquaculture settings by necessity and often by design differ from wild conditions. For example, hatchery organisms are often fed a diet that is enhanced nutritionally but does not permit organisms to learn natural prey handling. Predators are purposely not present, as their presence is generally inconsistent with the goal of maximizing hatchery survivorship. Tank color is often uniform and based on a manufacturer's default. However, results of the present study indicate that simple additions to the methods of hatchery-rearing meant to condition organisms to wild conditions before their release may lead to increased success of stock enhancement programs.

In the present study, exposure to predators led directly to increased spine length, and increased spine length led directly to higher survivorship. Similar spine plasticity has been noted in other invertebrates that rely on external spines for defense against predators. For example, dorsal spines in several *Daphnia* species lengthened after exposure to predators (Grant and Bayly, 1981; Kreuger and Dodson, 1981; Hebert and Grewe, 1985). Spine formation in a subtidal bryozoan increased when nudibranch predators were present (Harvell, 1986). Differences in spine shape and length were linked to habitat, and potentially predators, in queen conch juveniles (Martin-Mora et al., 1995). Other anti-predator devices, such as carapace or shell thickness, have also been related to presence of predators (Lively, 1986; Trussell, 2000). It is possible that other factors than predator exposure can induce spine lengthening, and exact cues spurring these morphological changes are not known. In the present study, crabs did not require an environment in which they were subjected to attacks in order to respond with an increase in spine length. Therefore, conditioning within a hatchery environment on a large scale, or perhaps in an intermediate field pen step, appears possible without an increased threat to juvenile blue crab survivorship. Exposure to predators may also result in behavioral changes that affect, either positively or negatively, survivorship in the field.

In order to improve the efficiency of the conditioning process, future work might focus on the mechanism by which predator-exposure conditioning operates. For example, juvenile crabs were able to both see and smell the striped bass with which they shared the tank, but it is not known which was most important. If only chemical cues are necessary,

perhaps water in which striped bass have been previously held may be enough to induce the spine length change. Future work may also focus on the optimal stage at which to begin such conditioning. Preliminary analysis suggests that hatchery crabs have shorter spines than their wild counterparts as early as the late larval stage (Davis and Natunewicz, unpublished data). Finally, future work may also address whether a molt is required for spine increases to occur.

Crab color was also a plastic trait, able to change within 1 day of a change in background substrate. Although molting allowed a crab to change color more, a molt was not necessary for a color change to occur, suggesting a process dissimilar to that of other crab species that do require a molt (Palma and Steneck, 2001). Aspects of blue crab color have been demonstrated to change to a small degree on an even shorter scale, that of a semidiurnal tidal scale (Fingerman, 1955). Mechanisms of color change in the blue crab are not known. Blue crabs have been shown to recognize color but not detect brightness levels (Burse, 1984). Despite this fact, a change in carapace brightness in response to substrate color was the most dramatic aspect of color change in the present study.

Color was not linked directly to survivorship in the present study, but may be indirectly related to survivorship through behavioral modifications. In the present study, crab color was related to substrate choice, with crabs choosing substrates that most closely matched their carapace color. If habitat choice of newly released hatchery crabs is similarly affected, and different habitats have different suites of predators or different food resources, carapace color may ultimately affect survivorship during the first 24 h before they change color.

The ability to change color may also play a survivorship role in wild populations. As primary nursery habitat types, such as submerged aquatic vegetation, decline in the Chesapeake Bay, juvenile blue crabs may be forced to seek alternative nursery habitats. The ability to change color may aid in quick transitions among habitats.

Methods of conditioning to make certain aspects of morphology more similar to those exhibited by wild individuals will likely improve efficiency of stock enhancement programs. However, morphology may not be the only plastic area in which conditioning can help. Other aspects, such as behavior, may also differ

between hatchery-raised and wild organisms (e.g., Berejikian et al., 1996), including blue crabs (Young-Williams, unpublished data). Such differences potentially call for the incorporation of behavioral conditioning in stock enhancement methodology to complement morphological conditioning. For many recruitment-limited, severely depleted fishery stocks, stock enhancement programs may become the only method by which populations sizes can be returned to sustainable levels. At present, though many programs have successfully enhanced target populations, room exists for increased efficiency in these programs.

### Acknowledgments

We are extremely grateful to Mike Mangold and the U.S. Fish and Wildlife Annapolis office for the frequent use of their microwire taggers, to Freya Hendrickson for countless field hours, and to our human taggers: Rob Aguilar, Mike Goodison, Midge Kramer, Kirah Forman and others. Thanks also to COMB scientists, especially Odi Zmora, Emily Lipman, and John Stubblefield, who worked to raise the crabs. Thanks to Thomas Hallock for providing fish and Cecily Natunewicz for providing wild-caught larvae. This work was supported by the NOAA Coastal Ocean Program, the State of Maryland, Philips Seafood, the Maryland Watermen's Association, and the Smithsonian Fellowships Program.

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