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Linking temperature and salinity tolerance to winter mortality of Chesapeake Bay blue crabs (*Callinectes sapidus*)

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Abstract

Blue crabs (*Callinectes sapidus*) form one of the most important and largest commercial fisheries in Chesapeake Bay. Blue crabs have evolutionary origins in the tropics, although they currently inhabit temperature estuaries that exhibit major fluctuations in diurnal, monthly, and seasonal environmental conditions. Therefore, harsh winter conditions in Chesapeake Bay are a potentially important source of blue crab stock loss. However, this variable has been largely unexamined. To assess the effects of variation in winter environmental conditions on blue crab survival, we measured winter morality of crabs in the field and conducted laboratory experiments to test the interactive effects of low temperature, salinity, and blue crab life stage. Field studies indicated that blue crabs suffered relatively low winter morality rates (≤3%) during five out of eight winters, when bottom water temperature in February was at or above the 8-year average (3.4 °C). However, in years when bottom water temperature fell below the February average, annual mortality rates rose to 6.0–14.5%. Mortality rates were highest in the coldest regions of Chesapeake Bay, and larger crabs and female crabs were most vulnerable to these stressful conditions. Similarly, in the laboratory, mortality was highest in the lowest temperature (1 °C) and salinity (8 ppt) treatments. Mature females were more sensitive to winter conditions than juvenile crabs. Of the juvenile life stages, recruits (<15 mm carapace width) were least tolerant to winter conditions. These results indicate that temperature, salinity, and blue crab life stage are important variables in predicting survivorship over winter months. Because winter mortality may be a significant source of stock loss for blue crabs, especially during severe winters and in low salinity areas of Chesapeake Bay, these predictions can be used to improve management estimates of stock size prior to each summer fishing season.

Keywords: Blue crab; *Callinectes sapidus*; Salinity tolerance; Temperature tolerance; Winter mortality

1. Introduction

Blue crabs (*Callinectes sapidus*) comprise the largest and most important commercial fishery in Chesapeake Bay (Rugolo et al., 1998). Recent studies

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suggest that the Chesapeake Bay blue crab population is at a depressed level after an 85% decline in spawning stock biomass during 1990–2001 (Lipcius and Stockhausen, 2002). To better understand these trends, it is important to identify all sources of natural and anthropogenic mortality.

Past efforts to identify sources of mortality and estimate annual changes in stock size have been elusive. The majority of studies have focused on fishing data, thereby excluding sources of mortality that occurred during the non-fishing season, such as winter-induced mortality (Dudley and Judy, 1973; Cole, 1998). Recent estimates of stock size have been based on several approaches, including fall recruitment estimates (Lipcius and Van Engel, 1990), spatially explicit and multiple life stage models (Miller, 2001, in press), and winter dredge surveys (Schaffner and Diaz, 1988; Volstad et al., 2000). However, these stock predictions have varied in accuracy, in part due to demographic responses to large fluctuations in environmental conditions during the period between when the data are collected and the time when the estimate of stock size is applied. For example, recruitment and stock assessment data collected in the fall and early winter do not include probabilities of winter-induced mortality into stock size estimates for the following spring.

Winter-induced mortality is a potentially important source of mortality for blue crabs due to large fluctuations in winter conditions; however, this variable has been largely unexamined (Van Engel, 1987, 1999). As a species with evolutionary and biogeographic origins in the tropics, and a historical geographic range of Nova Scotia to northern Argentina (Rathbun, 1930), the Chesapeake Bay population is approaching the northern limits of the species' distribution. Crabs in this region may not be well adapted for the most extreme winters, impairing the Chesapeake population. For example, watermen and scientists conducting winter dredge surveys have noted higher rates of mortality during extreme cold winters (Pearson, 1948; Dudley and Judy, 1973; Kennish et al., 1982; Kahn et al., 1998; Davis, 1999).

Within estuarine environments, species distributions are often limited by temperature and salinity tolerance, as estuaries are exposed to large diurnal, seasonal, and yearly fluctuations in salinity, temperature, and dissolved oxygen (Leffler, 1972; Mangum and Towle, 1977). While blue crabs can tolerate a wide range of salinities (Tan and Van Engel, 1966; Ballard and Abbott, 1969; Tagatz, 1971; Guerin and Stickle, 1992), osmoregulation efficiency is lowest and energy demands are highest at low salinities and temperatures, especially for mature females (Tagatz, 1971). In a laboratory study, Tagatz (1969) found that temperature tolerance levels decreased in lower salinity waters. Thus, the physiological stress of low salinities and extreme low temperatures may have a synergistic effect in lowering blue crab tolerance to winter conditions.

To assess the relative importance of severe and moderate winter environmental conditions on blue crab mortality, we examined the combined effects of low temperature and salinity on adult and juvenile survivorship through field sampling of Chesapeake Bay blue crabs and controlled laboratory experiments. The goals of this study were: 1) to assess annual and spatial variation of blue crab winter mortality within the northern, lower-salinity half of the Chesapeake Bay over a period with wide fluctuations in winter severity; 2) to test the interactive effects of temperature, salinity, and crab life stage on blue crab mortality; and 3) to compare levels of stress, as defined by number of autotomized (dropped) limbs, among low salinity–temperature regimes.

2. Methods

2.1. Winter dredge survey

The Maryland Department of Natural Resources (MDNR), Chesapeake Biological Laboratory (CBL), and Virginia Institute of Marine Science (VIMS) have conducted an annual winter dredge survey throughout Chesapeake Bay since 1989 (Volstad et al., 2000; Sharov et al., in press). The survey sampled blue crab populations using a standard 1.83-m-wide Virginia sampling dredge to determine the geographic location, bathymetric distribution, size, and sex of over-wintering blue crabs. The dredge was lined with a 12.7-mm nylon mesh to select for crabs of at least 15 mm carapace width (CW; Sulkin and Miller, 1975). From 1989 to 1995, annual stratified random sampling occurred throughout Chesapeake Bay between mid-December and March, as described in Sharov et al. (in

press). Beginning in 1996, sites in Maryland were sampled once in December or January and those with high crab density were resampled in March. Effects of over-wintering stress are most evident in March, after water temperatures have reached minimum levels and are beginning to rise (Speir et al., 1998). Thus, over-wintering mortality rates—taken as the percent of dead crabs per tow—were calculated only from March 1996 to 2003 surveys. We considered the percent of dead crabs per tow to be the dependent variable.

To estimate annual temperature and salinity conditions within the mainstem of the upper half of Chesapeake Bay, data were gathered from MDNR Water Quality Monitoring Project (B. Romano, personal communication). Twelve stations were monitored from 39.4401°N, 76.0247°W to 37.9118°N, 76.168°W. For each year, we determined the average bottom water temperature from all stations during February, historically the lowest annual monthly temperature. Although duration of exposure is an important variable, as shown in our laboratory experiments (Section 2.2), the field temperature data were only collected a few times each month and, therefore, such values could not be determined. Since the month of lowest salinity varies annually, we determined: 1) the lowest salinity measurement from December–March, and 2) the greatest monthly change in salinity during each winter.

The percent of dead crabs, or over-wintering mortality rate, was calculated from March 1996 to 2003 tow data. Nearby monitoring sites recorded temperature and salinity. The effects of February bottom water temperature, lowest winter salinity, and depth at which crabs were collected were tested using a backward stepwise regression (SAS Institute, 1992). To address the relationship between crab sex and size and the susceptibility to lethal over-wintering conditions, mortality rates between large (60+ mm CW) and small (<60 mm CW) size categories, corresponding to age 0 and age 1+, and male and female blue crabs were examined using the Kolmogorov–Sminov chi-square test (SAS Institute, 1992).

2.2. Laboratory experiments

2.2.1. Experiment I

To examine mortality rates of mature females and juvenile blue crabs within controlled regimes of realistic extreme and moderate winter temperatures and salinities, we conducted a series of full-factorial experiments in the laboratory. During February 1994–2003, average bottom temperature was 3.39±0.46 °C (S.E.) in the upper Chesapeake Bay (Fig. 1). Our experiments were conducted in temperature-controlled walk-in chambers. One chamber was set at 1 °C for 30 days and increased to 3 °C for the following 30 days, representing a cold snap followed by average winter temperature. The second chamber was set to 3 °C for 60 days to represent an average winter. Within each temperature regime, three salinity regimes were tested to represent the different regions of the upper bay: 8 ppt, 12 ppt, and 16 ppt.

To test for differences in responses among life stages, we compared survival rates among three

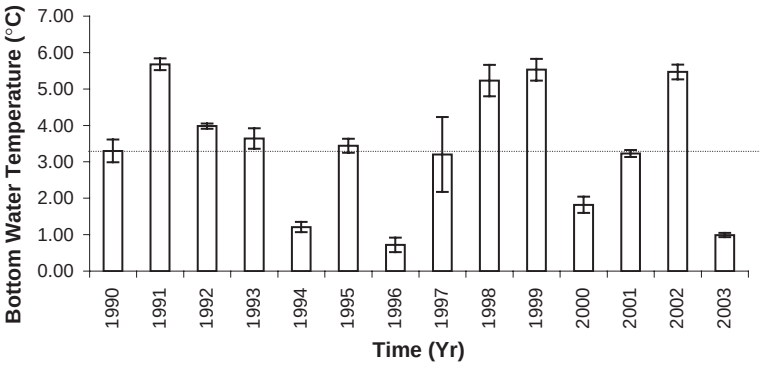

Fig. 1. Mean February bottom water temperature, 1990–2003, collected at 12 stations throughout the upper half of Chesapeake Bay, from 39.4401°N, 76.0247°W to 37.9118°N, 76.168°W. Error bars indicate S.E. Dashed line indicates average February bottom water temperature (3.4 °C).

groups: mature females (>130 mm CW) that form the broodstock the next summer, medium juveniles (80–115 mm CW) that molt to maturity early in the next fishing season and may also contribute to the broodstock, and small juveniles (20–65 mm CW) that represent the young of the year. Crabs were collected in February 2002 so that winter acclimation had already been achieved in the field. MDNR collected experimental juvenile crabs from Tangier Sound, as a part of the winter dredge survey (Section 2.1). Mature females were collected via commercial winter dredges in the same general area. Once collected, crabs were held in temperature-controlled walk-in chambers at approximately 7 °C, a temperature low enough to prevent molting and limit aggressive interactions and yet warm enough to prevent subjecting crabs to extreme thermal stress prior to the experiment. Salinity was lowered by approximately 1–2 ppt/day until it reached test salinities. Temperature was lowered at 1 °C per 2 days until it reached the test temperature. Crabs were held for <2 weeks during salinity and temperature acclimation prior to the experiment.

For each salinity×temperature×life stage treatment, we had three replicates and each replicate consisted of a container with six crabs, for a total of 324 crabs. The containers for mature females were 18 in.×12 in.×3.5 in., for medium juveniles were 18 in.×12 in.×3.5 in., for small juveniles were 14.5 in.×10.5 in.×6 in., and for recruits were 6 in.×5.5 in.×3.25 in. No sediment was added to containers to prevent anoxic conditions and minimize disturbance to crab each time survivorship was assessed. A constant supply of oxygen was maintained within each container. Water was replaced every other day to limit build-up of toxins, and ammonium and nitrate levels were checked regularly. All treatments had a 12 L:12 D light cycle. Survivorship was assessed approximately every 24 h. Crabs were considered dead if appendages were not moving (Tagatz, 1969), or, if after prodding an eyestalk, no subsequent movement was observed. Crabs were not fed during the experiments, as feeding does not occur at these low temperatures.

2.2.2. Experiment II

Experiment II was conducted to examine survivorship rates for juveniles at more mild temperatures than those tested in Experiment I. Methods were the same as in Experiment I, except that walk-in chambers were set to 3 °C, representing average February temperature, and 5 °C, representing a more mild winter. Temperatures were held constant in the two chambers for the full 60-day duration of the experiment. An additional difference was that the mature female life stage was not tested, but a recruit life stage (<15 mm CW) was added. Crabs used in Experiment II were collected from areas near the Potomac River, as a part of MDNR winter dredge survey from late December 2002 to January 2003.

At the onset of the experiment, we had three replicates for each life stage×temperature×salinity treatment, for a total of 324 crabs. We recorded crab size, sex, and limbs missing on day 1 of the experiment. Survivorship was assessed approximately every 24 h. When a crab died, we recorded limbs missing, or rate of autotomy, because no molting, and therefore no limb regeneration, occurs at these low temperatures. Autotomy is a reflex severance of an appendage from a fixed breakage point, which has been primarily attributed as a mechanism to avoid predators and limit wounds (Wood and Wood, 1932; Hopkins, 1993; Juanes and Smith, 1995). However, aggressive interactions and predation would not likely occur within our experimental conditions as locomotor activity ceases below 5.5 °C (Van Heukelem and Sulkin, 1990). Therefore, autotomy rates may be indicative of physiological stress: by releasing limbs, energy can be conserved and used primarily to maintain vital organs.

2.2.3. Data analysis for laboratory experiments

Results of Experiments I and II were analyzed separately by two methods (SAS Institute, 1992). To test for interactive effects of temperature, salinity, and life stage on blue crab survival, we conducted a three-way ANOVA at day 30. We chose to conduct the ANOVA at day 30 since there was approximately one-third mortality in all containers, such that the experiment had proceeded long enough to reveal patterns of survivorship across the experimental factors. The ANOVA model included temperature, salinity, and life stage as main fixed and orthogonal factors. Student–Newman–Keuls (SNK) post-hoc tests were used to compare means of significant factors. When interactions occurred, SNK tests were conducted

within each factor and across the other significant factor, for both interactive factors. Therefore, a Bonferroni correction was applied, and the new α level was set at p=0.025. The second method of analysis, an accelerated failure time model, was utilized to understand how time until death was affected by salinity, temperature, and crab size. Since this model assumes constant conditions, only data from days 1–30 of Experiment I were included, as the temperature was increased after day 30 in the cold snap treatment. Data from all 60 days of the experiment were included for analyses of Experiment II. Alternative error structures were compared using a likelihood ratio test, and hazard functions were estimated for each level of significant treatment factors (Allison, 1995).

Limb autotomy was only recorded in Experiment II. To test whether higher rates of limb loss, or autotomy, occurred with crabs that died compared to those that survived until 60 days, we preformed a one-way ANOVA examining the effect of survival on autotomy rate. Lastly, we conducted a three-way ANOVA examining the interactive effects of temperature, salinity, and life stage on autotomy rate. Student–Newman–Keuls (SNK) post-hoc tests were used to compare means of significant factors. When interactions occurred, SNK tests were conducted across both factors, separately for each level each that factor.

3. Results

3.1. Field studies

From 1990 to 2003, February bottom water temperature averaged 3.4 °C and ranged from 0.7 to 5.7 °C (Fig. 1). In contrast to water temperatures, annual variation in lowest winter bottom water salinity was relatively stable (10.43 ppt–16.65 ppt) and never deviated significantly from a mean value of 13.64 ppt. However, there are relatively large variations in salinity on a monthly timescale (e.g., +8.57 ppt, February–March 1998; −9.03 ppt, January–February 1997).

Mortality rates of over-wintering crabs sampled in the field varied between 0.74% and 14.5% among years from 1996 to 2003 (Fig. 2). Mortality rates were relatively low (<3%) for five out of eight winters of

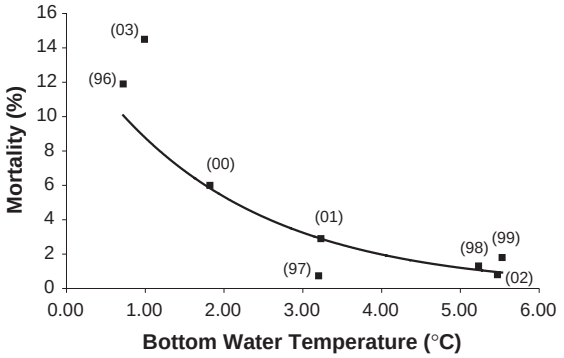

Fig. 2. Annual mortality as a function of February bottom water temperature during MDNR winter dredge surveys, 1996–2003. An exponential curve was fitted to the data yielding the following equation: $y=14.4e^{-0.4963x}$; $R^2=0.7161$. Data for crab size and sex are pooled. The number in parentheses refers to year data were collected.

1996–2003. However, in years when bottom water temperature fell at least 1 °C below the February average (3.4 °C), mortality rates rose to 6.0–14.5%. Mortality tended to be higher in colder, fresher areas of the Chesapeake Bay, such as the northern region of the mainstem and within shallow tributaries (Fig. 3). Similarly, a backwards stepwise regression, examining the multiple effects of temperature, salinity, and depth, indicated that bottom water temperature was a statistically significant factor (Table 1). The analysis produced the following equation:

$$\text{mortality rate} = (0.141 \pm 0.03) - (T^{*}0.011 \pm 0.004) - (S^{*}0.004 \pm 0.002)$$

where T=water temperature and S=salinity.

In addition to temperature, the size of a blue crab significantly impacted mortality rates, as the larger crabs had higher mortality rates (N=5636, χ^2=329.69, p<0.0001). The proportion of age 0 to age 1+ was 1.72:1 for live crabs and 0.18:1 for dead crabs. Sex also significantly impacted mortality rates as females exhibited higher mortality rates than males (N=5636, χ^2=18.88, p<0.0001). This gender effect seemed to be more pronounced at larger sizes.

3.2. Laboratory experiments

In Experiment I, temperature, salinity, and life stage significantly affected survival rates (Table 2,

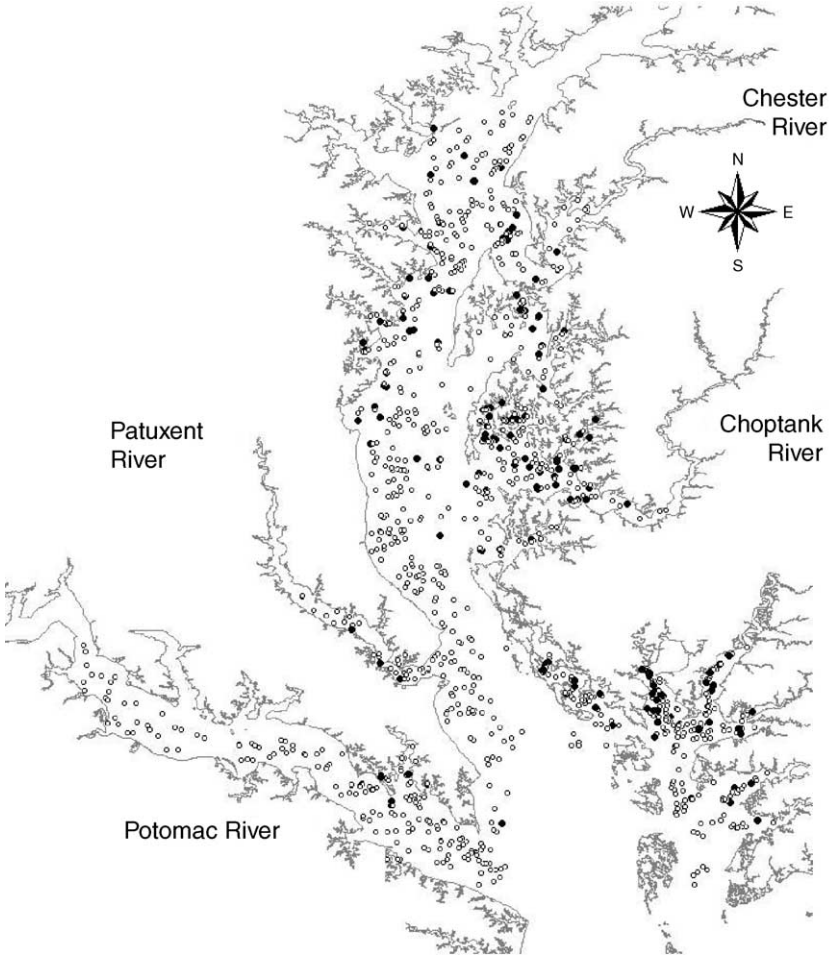

Fig. 3. Distribution of MDNR winter dredge survey sites in March 1996–2003; (●) Dredges with dead crabs present; (○) dredges with all live crabs.

Fig. 4). A significant temperature×life stage interaction indicated that the survivorship was lower in the cold snap treatment as compared to the average (3 °C) temperature treatment for small and medium juveniles, but there was no difference found among mature females. Among the three salinity treatments, the lowest survivorship occurred in the lowest salinity (8 ppt) treatment (Fig. 5a). At both temperature treatments, mature females experience significantly lower survivorship than small and medium juveniles (Table 2). These results indicate that all of the test conditions were highly stressful for mature females, as they suffered invariably high mortality in all treatments (Fig. 5b).

Table 1
Results from a backwards stepwise regression examining the relationship between temperature, salinity, and depth to overwintering morality rates

Factor	*df*	F	p[a]
Temperature	1	8.32	**0.005**
Salinity	1	3.20	0.077
Depth	1	0.01	0.933
Regression	79	6.04	**0.004**

[a] Bold numbers indicate that $p<0.05$.

Maximum likelihood fits of the accelerated failure time model also indicated that time to death was significantly influenced by salinity, temperature, and

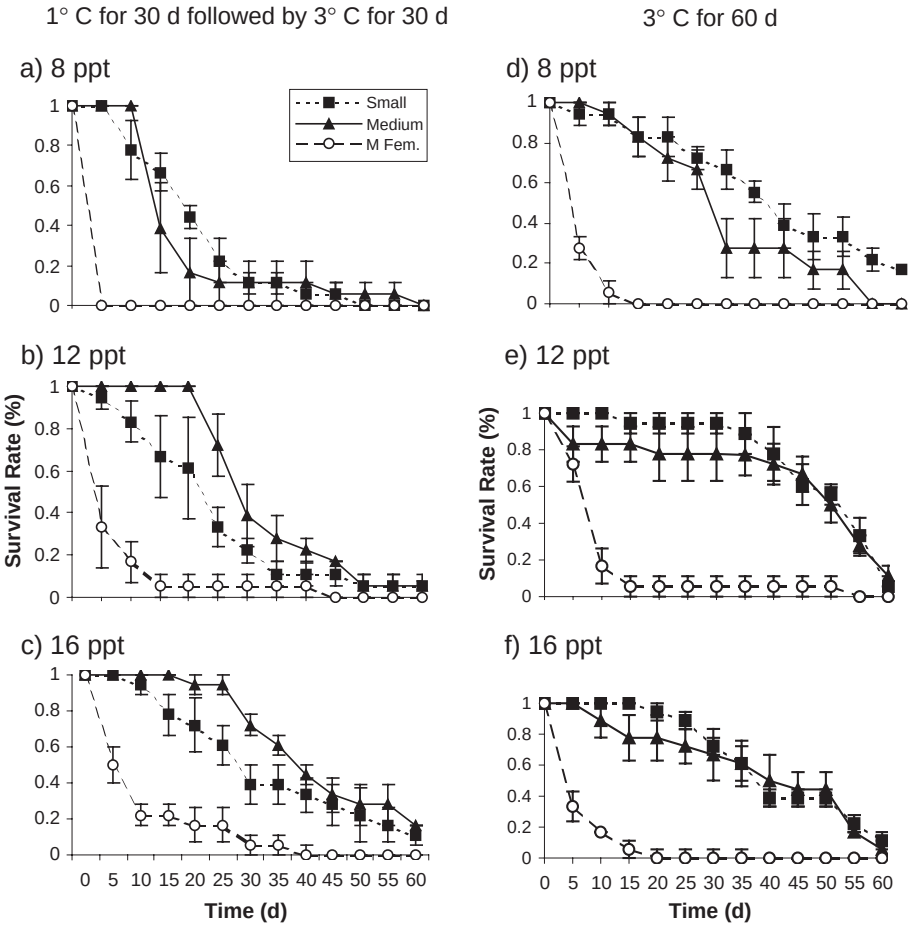

Fig. 4. Time course of blue crab survival for two temperature treatments in combination with three salinity treatments in Experiment I. Cold snap treatment=1 °C for 30 days followed by 3 °C for 30 days; average temperature treatment=3 °C for 60 days; salinities=8 ppt (a and d), 12 ppt (b and e), and 16 ppt (c and f). Figure legend indicates life stage for each panel: small=small juvenile; medium=medium juvenile; m fem.=mature female. Error bars indicate S.E.

blue crab life stage (Table 3). Likelihood ratio tests indicated that a lognormal error structure was the most appropriate. To control for significant effects, hazard functions were run at either the median salinity (12 ppt), 3 °C temperature, or medium juvenile regime. Hazard functions rose steadily during the beginning of the experiment, peaked during the second to third week, and continued to decline at a much lower rate until the end of the experiment (Fig. 6). The highest risk of death varied with treatment: for example, the highest risk of death was 0.0367 on day 26 for small juveniles, 0.0320 on day 30 for medium juveniles, and 0.2020 on day 4 for mature females; 0.0713 on day 8 at 8 ppt, 0.0450 on day 14 at 12 ppt, and 0.0322 on day 7 for 16 ppt; and 0.0571 on day 13 for the cold snap treatment and 0.0470 on day 9 for the average temperature treatment.

In Experiment II, life stage and salinity significantly affected survival rates, but temperature treatment did not, and there were no significant interactions among the three factors (Table 2, Fig. 7). Survival rates were significantly higher at 16 ppt than at 12 ppt or 8 ppt. Recruits were more sensitive to winter conditions than small or medium juveniles. Unlike the results of Experiment I, which used colder temperature regimes, survivorship rates did not differ between the average (3 °C) and mild (5 °C) temperature treatments.

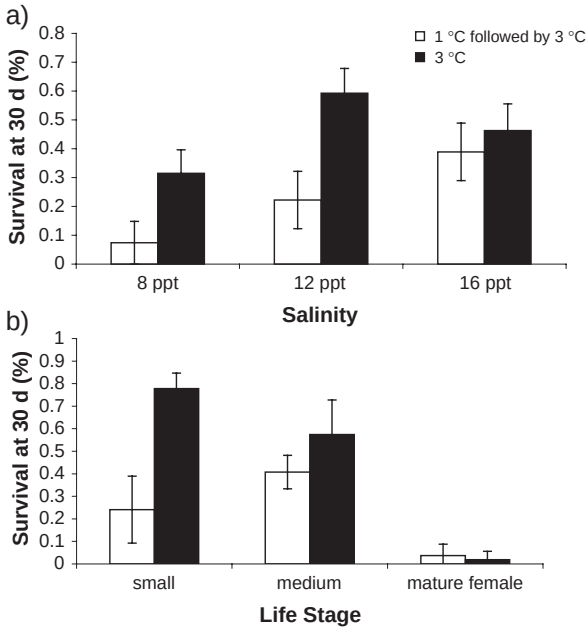

Fig. 5. Survival rates at day 30 of Experiment I. (a) Interaction between temperature and salinity. Histogram indicates means (±S.E.) across all life stages. (b) Interaction between temperature and life stage. Histogram indicates means (±S.E.) across all salinity treatments. Figure legend indicates temperature treatment for each panel: 1 °C followed by 3 °C=cold snap treatment; 3 °C=average temperature treatment.

Maximum likelihood fits of the accelerated failure time model for Experiment II also indicated that time to death was significantly influenced by salinity and life stage, but not by temperature (Table 3). To control for significant effects, hazard functions were run at either the median salinity (12 ppt) or the small juvenile regime. Hazard functions followed similar patterns as in Experiment I and the highest risk of death varied with treatment for significant factors (Fig. 8). For example, the highest risk of death was 0.166 on day 5 for recruits, 0.066 on day 24 for small juveniles, and 0.057 on day 27 for medium juveniles; and was 0.070 on day 7 at 8 ppt, 0.048 on day 5 at 12 ppt, and 0.040 on day 7 at 16 ppt. Likelihood ratio

Table 2
Effect of temperature, salinity, and life stage on blue crab survival during Experiments I and II

Factor	Experiment I				Experiment II		
	df	F	p[a]	Post-hoc[b]	*F*	p[a]	Post-hoc[b]
Temperature	1	13.31	**0.001**	**3 °C>1 °C**[c]	1.315	0.259	
Salinity	2	10.92	**<0.001**	**16.12>8**	7.474	**0.002**	**16>12.8**
Life stage	2	47.94	**<0.001**	**S, M>MF**	6.442	**0.004**	**S, M>R**
Temperature×salinity	2	1.485	0.240		0.478	0.624	
Temperature×life stage	2	5.554	**0.001**		0.561	0.576	
Salinity×life stage	4	1.818	0.147		0.958	0.442	
Temperature×salinity×life stage	4	0.385	0.818		1.524	0.216	

[a] Results from three-way ANOVA at day 30 of experiment; bold numbers indicate that $p<0.05$.

[b] Results from SNK post-hoc test describe conditions where higher survivorship was attained. 1 °C=cold snap (1 °C for 30 days followed by 3 °C for 30 days), 3 °C=average winter temperature (3 °C for 60 days); 16=16 ppt, 12=12 ppt, 8=8 ppt; R=recruit juvenile, S=small juvenile, M=medium juvenile, MF=mature female.

[c] As a result of the life stage×temperature interaction, higher survivorship was only observed in the 3 °C treatment vs. the 1 °C for medium and small juveniles.

Table 3
Accelerated failure time model for Experiments I and II

Parameter	Experiment I			Experiment II		
	Estimate	S.E.	p[a]	Estimate	S.E.	p[a]
Intercept	3.651	0.291	**<0.001**	2.499	0.2531	**<0.001**
Temperature	0.192	0.062	**0.002**	0.0138	0.044	0.7549
Salinity	0.081	0.019	**<0.001**	0.0350	0.0135	**0.0096**
Life stage	−1.030	0.079	**<0.001**	−0.2806	0.1098	**0.0071**
Scale	0.954	0.053		0.7728	0.0323	

[a] Bold numbers indicate that $p<0.05$.

tests indicated that a lognormal error structure was the most appropriate.

In both Experiments I and II, the average temperature treatment (3 °C) was run at all salinities for small and medium juveniles. Survival rates for these two life stages were lower in Experiment II than Experiment I (Fig. 9). Although overall absolute survivorship values differed between the two experiments, the effects of salinity and size class were consistent between experiments.

In Experiment II, crabs that survived the experiment were more often intact (missing no limbs) than those that died before the end of the experiment (day 60; one-way ANOVA: $F_{1,305}=10.96$, $df=1$, $p=0.001$; Fig. 10a). In fact, no crabs that survived until day 60 dropped limbs. Life stage and temperature were significant and interactive factors in describing patterns of autotomy (Fig. 10b, Table 4). At the mild temperature (5 °C) treatment, autotomy rates were significantly higher for small juveniles and recruits than for medium juveniles, while there was no difference by size class at the average temperature (3 °C) treatment (Table 4). For small juveniles and recruits, there were greater rates of autotomy at the mild temperature (5 °C) treatment compared to the average temperature 3 °C treatment (Table 4).

4. Discussion

Winter mortality can be a significant source of stock loss for the Chesapeake Bay blue crab population, especially during extreme winters. Our results suggest that blue crab tolerance to cold temperatures is further reduced at low salinities, as the highest mortality rates were found in the lowest temperature and salinity conditions in both the field and laboratory. To predict winter mortality, temperature, salinity, and blue crab life stage are all important and interactive factors.

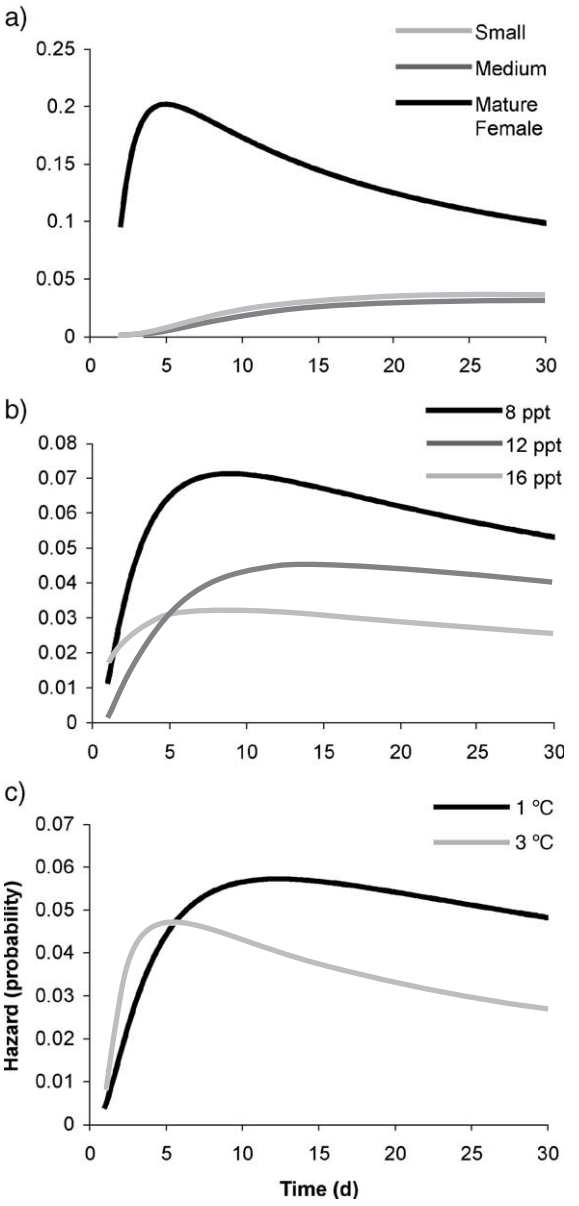

Fig. 6. Hazard function generated from maximum likelihood fits of the accelerated failure time model from days 1 to 30 of Experiment I. (a) Hazard function for small juveniles, medium juvenile, and mature females at 12 ppt and 3 °C. (b) Hazard function for 8 ppt, 12 ppt, and 16 ppt during medium juvenile treatments at 3 °C. (c) Hazard function for the cold snap (1 °C) and average (3 °C) temperature treatments during medium juvenile treatments at 12 ppt.

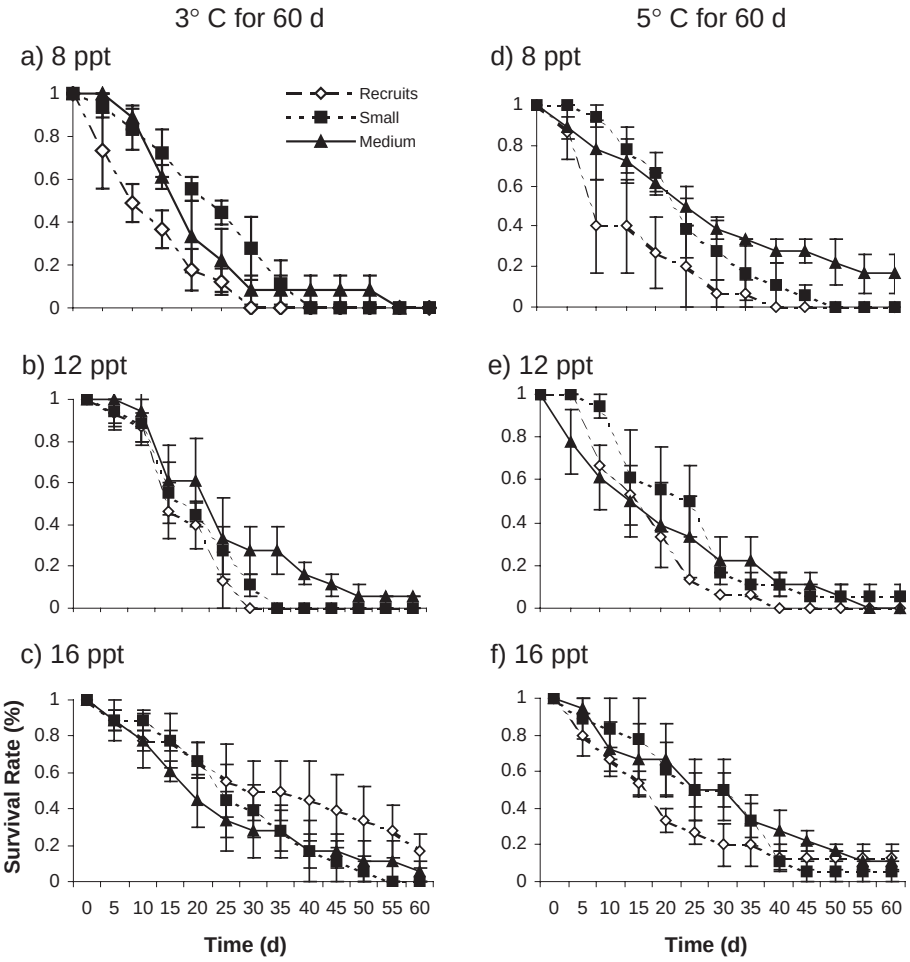

Fig. 7. Time course of blue crab survival for two temperature treatments in combination with three salinity treatments in Experiment II. Average temperature treatment=3 °C for 60 days; mild temperature treatment=5 °C for 60 days; salinities=8 ppt (a and d), 12 ppt (b and e), and 16 ppt (c and f). Figure legend indicates life stage for each panel: recruits=recruits; small=small juvenile; medium=medium juvenile. Error bars indicate S.E.

4.1. Effects of temperature and salinity

Temperature and salinity are often considered two of the most important environmental factors determining species distribution in marine and estuarine environments due to physiological constraints and requirements (Tagatz, 1971; Ballard and Abbott, 1969; Leffler, 1972; Lewis and Roer, 1988). Likewise, in the present study, the lowest temperature and salinity conditions yielded the highest mortality rates. Lower temperature tolerance at lower salinity is most likely the result of higher physiological demands and lower physiological abilities. The stress of maintaining an osmotic gradient in low salinity often adds to the strain on the metabolic activity at lethal or near lethal temperatures (Todd and Dehnel, 1960). Furthermore, Tagatz (1971) found the lowest osmotic concentrations for blue crabs in the lowest salinity and temperature treatments.

We also found significantly lower survivorship rates if water temperatures were below the February average (3.4 °C); however, survivorship was not higher if February temperatures were above average temperatures. For example, in the laboratory, survivorship was significantly lower in the cold snap treatment (1 °C for 30 days followed by 3 °C for 30

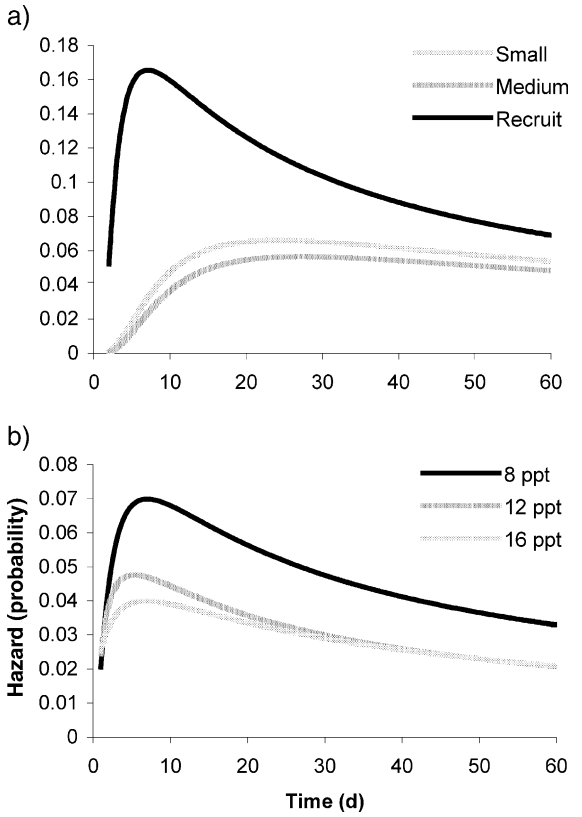

Fig. 8. Hazard function generated from maximum likelihood fits of the accelerated failure time model from Experiment II. (a) Hazard function for recruits, small, and medium juvenile crabs at 12 ppt. (b) Hazard function for 8 ppt, 12 ppt, and 16 ppt during small juvenile treatments.

days) than the average treatment (3 °C for 60 days), but there was no difference between the average (3 °C for 60 days) and mild (5 °C for 60 days) temperature treatments. Similarly, the winter dredge survey showed a relatively constant level of winter mortality (<3%) during most winters, except when temperatures dipped significantly below average. Fishermen and fishery managers have also reported higher levels of mortality during the harshest winters, when February bottom water temperature dropped below 1–2 °C (Pearson, 1948; Dudley and Judy, 1973; Kennish et al., 1982; Davis, 1999). Thus, winter mortality of blue crabs appears to exhibit a marked increase as temperatures drop below a tolerance limit. Hair (1971) found evidence for such a response when examining heat tolerance with a mysid shrimp, *Neomysis awatshcensis*. This species experienced low mortality from 14 °C to 30.5 °C, but higher mortality with each concomitant increase in temperature above 30.5 °C. For blue crab populations, significant mortality may not occur unless water temperatures drop significantly below 3 °C for an extended period of time.

There was a positive relationship between salinity and survivorship during both experiments. At low salinity, osmotic regulation demands are higher as blue crabs exhibit higher energy expenditure (i.e., higher respiration rates), lower energy absorption

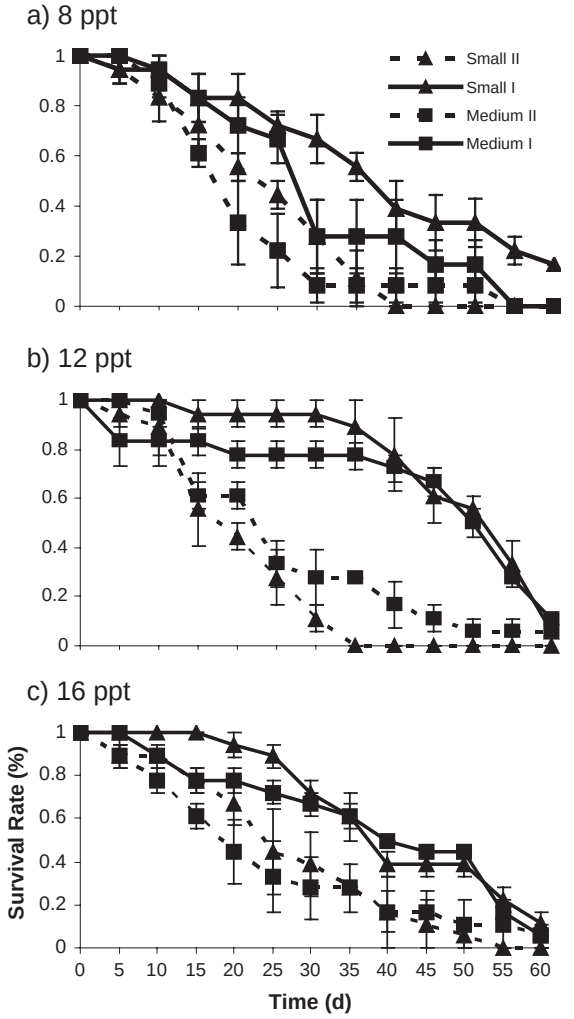

Fig. 9. Comparison of survival rates at the average February temperature treatment (3 °C for 60 days) at (a) 8 ppt, (b) 12 ppt, and (c) 16 ppt for small and medium juveniles during Experiments I and II.

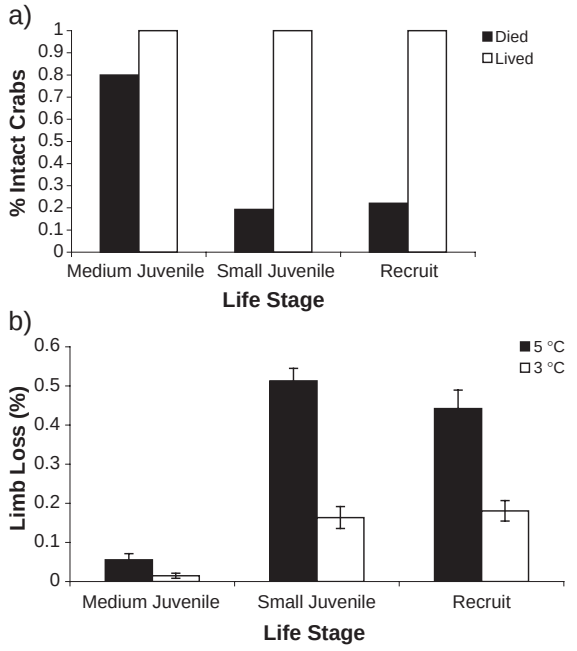

Fig. 10. Frequency of autonomy for experimental crabs. (a) Percent of crabs that remained intact at end of the experiment versus at the time of death during test period. Lived=alive at day 60; Died=died prior to day 60. (b) Percent of limbs that were dropped during experiment for all experimental crabs. Histogram indicates means (±S.E.) across all salinity treatments. 3 °C=average temperature treatment; 3 °C=mild temperature treatment. All data are from Experiment II only.

(i.e., food consumption and absorption efficiency), and lower levels of ATP (Findley et al., 1978; Guerin and Stickle, 1992). The salinity at which these energy demands become lethal may depend in part upon impact from other stresses that also increase physiological demand and lower osmoregulation efficiency. For example, during Experiment I, 12 ppt was not as lethal as 8 ppt, but during Experiment II, 12 ppt was as lethal as 8 ppt (Table 2). The 12 ppt treatment may have been more lethal in Experiment II as these experimental crabs were exposed to more stressful field temperatures prior to the experiment (Fig. 11). Although experimental temperatures were slightly higher during Experiment II, field temperatures were so much lower in 2003 (prior to Experiment II) than in 2002 (prior to Experiment I) that crabs from Experiment II were most likely more stressed during the experiment than crabs from Experiment I. These results suggest that in regions of moderate salinity, the effect of salinity is more lethal during colder winters.

From December–March, crabs and other estuarine crustaceans are often found in lower abundances in lower salinity regions (Broekema, 1941; Miller et al., 1975). Our study suggests that over-wintering mortality is one mechanism that lowers density in these areas, especially during harsh winters. However, it is unclear as to what degree the lower abundance is produced by a direct loss (i.e., over-wintering mortality during that season) or movement out of the area prior to winter. Such a migration pattern is shown by blue crabs (Millikin and Williams, 1984) and could evolve in response to selective pressures of physiological stress.

4.2. Life stage

In addition to temperature and salinity, in our study, life stage was an important predictor of over-wintering mortality. In the field study and Experiment I, mature females had significantly lower survivorship rates than juveniles. In our field studies, larger crabs were less tolerant of winter conditions, as described in other field observations (Pearson, 1948; Van Engel, 1987). Within mid-Atlantic estuaries, males comprise a higher percentage of over-wintering blue crabs in

Table 4
Effect of temperature, salinity, and life stage on autotomy in Experiment II

Factor	*df*	F	p[a]	Post-hoc[d]
Temperature	1	96.719	**<0.001**	**5 °C>3 °C**[b]
Salinity	2	0.101	0.904	
Life stage	1	84.960	**<0.001**	**S, R>M**[c]
Temperature×salinity	2	2.609	0.075	
Temperature×life stage	1	17.979	**<0.001**	
Salinity×life stage	2	1.423	0.226	
Experiment×salinity×life stage	2	1.663	0.133	

[a] Bold numbers indicate that $p<0.05$.

[b] Results from SNK post-hoc test describe condition where greater autotomy was observed. 3 °C=average winter temperature (3 °C for 60 days); R=recruit juvenile, S=small juvenile, M=medium juvenile.

[c] As a result of the temperature×life stage interaction, greater autotomy was observed at 5 °C vs. 3 °C only for the small and recruit size classes.

[d] As a result of the temperature×life stage interaction, greater autotomy was observed for small juveniles and recruits as compared to medium juveniles at 5 °C, but not at 3 °C.

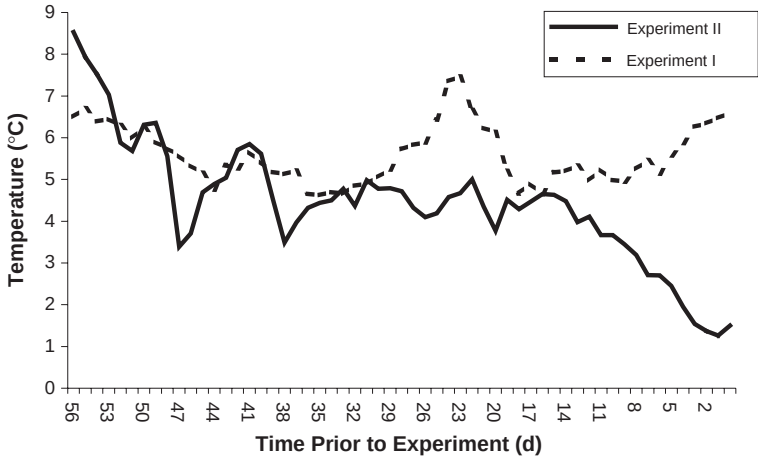

Fig. 11. Exposure to field temperatures for experimental crabs prior to Experiments I and II. Temperatures were collected in the Patuxent River, Baltimore, MD (39°16′N, 76°35′W), a subestuary of Chesapeake Bay and a NOAA long-term data collection site.

fresher waters (Miller et al., 1975; Schaffner and Diaz, 1988). Mature females may avoid fresh waters since they are less efficient at osmoregulation and less tolerant to low salinity and temperature conditions (Tan and Van Engel, 1966; Tagatz, 1971). Therefore, mature females over-wintering in warmer, more saline waters may be subjected to lower osmoregulatory stress and experience lower winter-induced mortality rates than in colder, fresher waters.

The extremely low survival rates experienced by mature females in Experiment I may in part be explained by this fall migration. In other words, few mature females over-winter in such low temperatures (1 °C and 3 °C) and low salinities (8 ppt and 12 ppt) as exposed to in Experiment I. Similarly, juveniles may more regularly over-winter in these areas and therefore be better suited for surviving such harsh winter conditions.

After females spawn during the summer–fall in the lower portion of Chesapeake Bay, recruits migrate from that area to the upper, more northern regions of the bay, often within subestuaries. Therefore, recruits settle along a gradient of salinity levels as they travel north, and move varying distances into tributaries. Lower salinity areas, especially within shallow tributaries, can offer important resources and optimal physical conditions for juvenile crabs, such as areas with predation refuges (Ruiz et al., 1993; Hines and Ruiz, 1995), higher food concentrations (Mangum and Towle, 1977), and conditions for faster growth (Van Engel, 1958; Tagatz, 1969; Leffler, 1972; Perkins-Visser et al., 1996). While movement to these areas may be optimum for summer or fall conditions, this may result in a higher risk of mortality during the winter. Our research showed a significant temperature×salinity interaction, by which mortality was highest within the shallow tributaries. Laboratory studies also showed that survival was significantly lower if crabs were less than 15 mm CW. Likewise, Kennish et al. (1982) attributed higher winter-induced mortality rates among the smallest crabs. However, if recruits can grow greater than 20 mm CW before the onset of winter, over-wintering location may be less important in terms of winter-induced mortality.

4.3. Limb loss

Autotomy has been primarily attributed as a mechanism to avoid predators and limit wounds, although these hypotheses have rarely been tested experimentally in the field (Juanes and Smith, 1995). Our data suggest that physiological stress may also impact autotomy rates, as extremely high rates of limb autotomy were observed in crabs that died and because experimental temperatures were low enough to prevent fighting and cannibalism (Van Heukelem and Sulkin, 1990). By dropping limbs, energy can be conserved and used primarily to maintain vital organs. Thus, autotomy may be a “last resort” response to physiological stress, which may be correlated with longer

survival. Temperature conditions fluctuate in the field, and additional time and conserved energy from dropping limbs may be sufficient to survive through a temporary drop in water temperature. In the laboratory, we may not have observed crabs that autotomized limbs and survived the experiment since temperature and salinity conditions were held constant.

While the low experimental temperatures prevented fighting and cannibalism (Van Heukelem and Sulkin, 1990; M. Rome, personal observation), we occasionally observed crabs latched onto one another, especially in the mild temperature treatment. This increase in activity may have influenced autotomy in conjunction with physiological stress, as autotomy rates were significantly higher in the mild temperature treatment (5 °C) than the average temperature treatment (3 °C). At over-wintering sites with extremely high crab density, latching behavior may cause an increase in autotomy due to the interactive effects of physical contact and physiological stress. These results also suggest sublethal differences between the two temperature treatments: during milder winters, crabs are more active and more aggressive interactions occur.

Small juveniles and recruits had significantly higher rates of limb autotomy than medium juveniles. Arnold (1988) suggested that autotomy occurs when benefits outweigh costs. Recruits experienced higher levels of stress, as measured by a lower survival rate, such that the benefit of autotomy (conservation of energy for life-sustaining organs) may be greater for recruits than for medium juveniles. However, small and medium juveniles experienced similar mortality rates, suggesting that the benefit of autotomy was also similar for both life stages. Multiple lines of evidence suggest that losing a limb is less costly for small juveniles and recruits than for medium juveniles. For example, regeneration potential is inversely related to size, indicating that smaller crabs can regenerate limbs faster than medium juveniles (Cheung, 1973; Miller and Watson, 1976). In addition, mortality rates of tethered, small crabs (<60 mm) did not vary with prior limb loss, although medium crabs (61–110 mm) showed a positive correlation between limb loss and mortality rates (Smith, 1990a, 1995). Thus, vulnerability to predation prior to regeneration may be another cost that is greater for larger juveniles.

Future studies should determine the likelihood, frequency, and impact of physiological stress on autotomy rates. The majority of studies examining autotomy in the field rely upon fishing gear that require organisms to be active and not over-wintering (Krouse, 1976; Shirley and Shirley, 1988; Smith and Hines, 1991a). Thus, autotomy as a result of severe thermal and physiological stress may be overlooked. Autotomy can have several potential costs at the individual and population levels: limb loss can reduce growth and regeneration rates (Smith, 1990b), feeding (Smith and Hines, 1991b), and competitive ability (Sekkelsten, 1988; Smith, 1992), especially when more than one limb is lost and if it is a claw or swimmeret that is dropped. Our study is one of the first to suggest that physiological stress may be a previously overlooked factor that can influence autotomy rates.

4.4. Conclusions and implications

Experimental treatments represented realistic winter conditions and had lethal effects upon experimental blue crabs. According to projected hazard functions, risk of death increased steadily, generally peaked during the second or third week of the experiment, and continued throughout the experiment at a lower rate (Figs. 6 and 8). These results suggest that in the field, after the onset of a stressful event, there is a high rate of mortality for the least tolerant crabs during the first few weeks. After that point, there will be a slower rate of mortality events among the more tolerant crabs. Therefore, these results imply that a few extreme cold snaps of 2–3 weeks may be more lethal than continuous cold temperatures. Furthermore, both the magnitude (e.g., lowest temperature) and duration of stressful events are important variables in predicting the total impact of winter conditions on the blue crab population.

Risk of mortality was higher in the laboratory experiments than field surveys. Laboratory mortality rates might be inflated for several reasons. For example, initial capture of crabs from over-wintering sites might cause higher levels of stress than that of undisturbed crabs (e.g., those from winter dredge survey). To limit disturbance to crabs in the laboratory (e.g., digging through sediment to find crabs and assess survival), we did not include sediment in containers. However, inclusion of sediment might

have provided more field-like conditions and prevented inflation of mortality rates. In addition, we found high levels of autotomy, especially in the mild temperature treatment. Limb loss may lead to a higher probability of death during stressful conditions, as was suggested by Willis et al. (1982); however, blue crab studies have been unable to assess the importance of prior limb loss on survival rates when larger predators are not a factor (Juanes and Smith, 1995). Thus, the relative influence of autotomy on winter-induced mortality is unclear. The presence of disease should also be tested for in the future.

Prior field exposure may also influence survival rates found in the laboratory, as Experiment II yielded significantly higher mortality rates than Experiment I (Fig. 10). Crabs collected from the field immediately prior to Experiment I were exposed to much milder conditions than those collected immediately prior to Experiment II. For example, in the Patuxent River, a subestuary of Chesapeake Bay, crabs were exposed to temperatures at or below experimental temperatures (5 °C) for nearly 2 months before Experiment II, but for only a few weeks before Experiment I (Fig. 11). Thus, crabs in Experiment II were exposed to more thermal stress prior to the experiment, which may in part explain the higher mortality rates. Nonetheless, relative measures of survival from laboratory studies in combination with field surveys predict that the highest rates of mortality occur during the most extreme winters, and in low salinity, colder waters, especially for mature females.

Our study clearly demonstrates that the duration of stressful temperature and salinity events, as well as blue crab life stage, is an important variable in predicting winter mortality. Including probability of death as a function of crab life stage and environmental conditions in a spatially explicit stage-based model offers the potential of prorating stock estimates developed from the winter dredge survey to improve predictions of subsequent fishery yield. For example, Miller (2001) has developed a stage-based modeling approach for the Chesapeake Bay blue crab that allows for stock predictions based upon crab life stage, and seasonal and spatial variability. Other factors that may effect mortality rates and improve prediction accuracy include: 1) other physical conditions (e.g., dissolved oxygen, nutrients, and pH; Van Engel, 1987); 2) pollutants, biocides, and chemicals (e.g., Van Engel, 1987); 3) nutritional history of crabs (Winget et al., 1976; Kennish et al., 1982); and 4) over-wintering habitat (e.g., Schaffner and Diaz, 1988).

The Chesapeake Bay blue crab is a heavily exploited fishery (Rugolo, 1998) and currently assumed to be at a depressed level (Lipcius and Stockhausen, 2002). Our study indicates that that winter mortality can be an important source of stock loss that is based upon the severity of stressful weather conditions, spatial location within the Chesapeake Bay, as well as the size and sex of the crab. Management success for a sustainable fishery may depend upon including estimates of winter mortality when predicting stock size prior to the summer fishing season.

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