ABSTRACT

Title of Document: WINTER MORTALITY OF THE BLUE CRAB (*CALLINECTES SAPIDUS*) IN THE CHESAPEAKE BAY

Laurie J. Bauer, Master of Science, 2006

Directed By: Dr. Thomas J. Miller
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Over its range, the blue crab (*Callinectes sapidus*) is exposed to a wide range of environmental conditions. At mid-latitudes, within its range, overwintering mortality may play an important role in regulating local blue crab populations. A 121-day 2x2x2 factorial experiment was used to test for the effects of temperature (3°C, 5°C), salinity (10, 25) and sediment (sediment, no sediment) on survival. An accelerated failure time model was fit to the survival data. Time to death significantly increased with increasing temperature, salinity, and crab size. I applied a temperature and salinity-dependent survival model to empirical temperature and salinity data to explore spatial and interannual patterns in overwintering mortality in the Chesapeake Bay. Predicted survival was highest in the warmer, saline waters of the lower Bay and decreased with increasing latitude. There was also significant interannual variation in that predicted survival was lowest after the severe winters of 1996 and 2003.

WINTER MORTALITY OF THE BLUE CRAB (*CALLINECTES SAPIDUS*) IN CHESAPEAKE BAY

By

Laurie J. Bauer

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Advisory Committee:
Professor Dr. Thomas J. Miller, Chair
Dr. Victor S. Kennedy
Dr. David H. Secor

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Chapter 1: Introduction

The blue crab, *Callinectes sapidus*, is distributed broadly along the eastern coast of the American continent, typically ranging from Massachusetts to Uruguay, although in warm years they can be found as far north as Nova Scotia (Williams 1984). Throughout this range, the blue crab experiences dramatically different climatic conditions (tropical – boreal) that have important consequences on life history patterns. Additionally, throughout much of its range, the blue crab is commercially exploited. Approaches to assessing the sustainability of these fisheries require the development of population dynamic models of individual blue crab stocks. Current models and stock assessments of the blue crab assume constant winter mortality, but inter-annual fluctuation in winter severity may lead to varying degrees in mortality. The goal of this thesis is to quantify and describe patterns in the overwintering mortality of blue crabs in Chesapeake Bay. These estimates can ultimately be used by fishery biologists and managers to more accurately model blue crab natural mortality, thereby improving stock assessments.

The blue crab supports valuable commercial and recreational fisheries. From 1950 to 2003, the annual U.S. commercial catch has averaged 75,811 metric tons (MT).[1] During this same time period, the blue crab's economic value has risen substantially, although it has dropped off slightly in the last seven years (Figure 1.1). The largest fishery for the blue crab, both historically and today, is on the Chesapeake Bay in Maryland and Virginia (Figure 1.2). Prior to 1950, the Chesapeake Bay accounted for over 75% of the total U.S. blue crab harvest (Stagg and Whilden 1997). Since 1950, the annual combined Chesapeake Bay harvest has averaged 32,735 MT.

[1] Data from NOAA's Fishery Statistics and Economics Division, available online at http://www.st.nmfs.gov/st1/

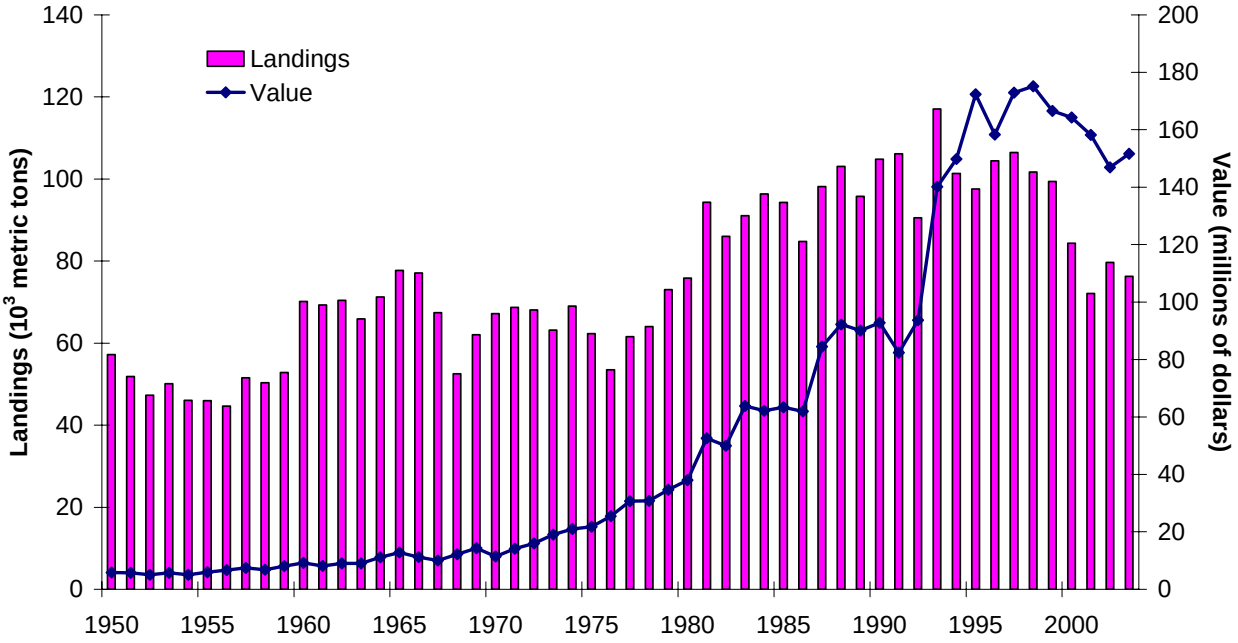


Figure 1.1. Total U.S. blue crab landings and economic value, 1950-2003. Data from NMFS website (http://www.st.nmfs.gov/st1/commercial/index.html).

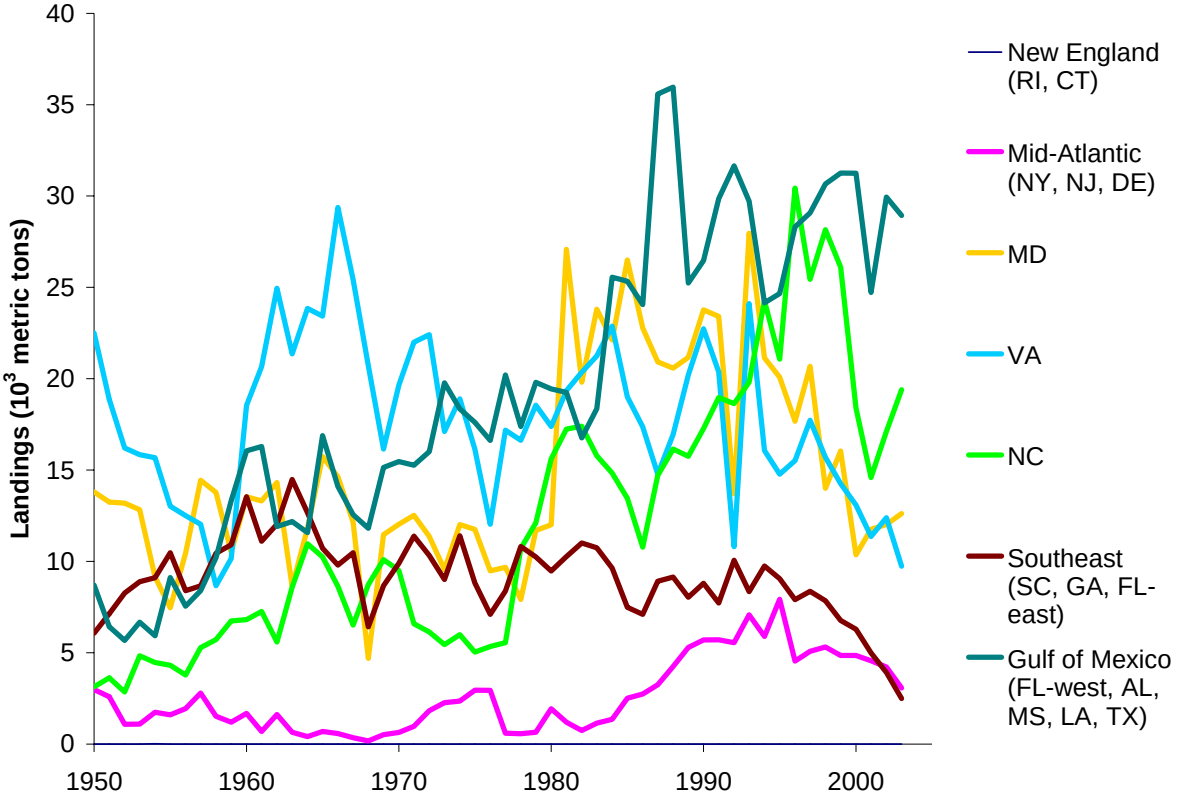


Figure 1.2. Blue crab landings by region, 1950-2003. Data from NMFS website (http://www.st.nmfs.gov/st1/commercial/index.html).

However, as landings in Maryland and Virginia have declined in recent years and the fishery has expanded in other regions such as Louisiana and North Carolina (Figure 1.2), the share of the Chesapeake Bay region to the total U.S. harvest has decreased to around 30% of the total landings in the past several years (Figure 1.3). In addition to the drop in landings in the Chesapeake Bay, since the early 1990s there has been a decline in total abundance (Miller et al. 2005), spawning stock biomass (Lipcius and Stockhausen 2002), recruitment (Lipcius and Stockhausen 2002) and size of adult blue crabs (Abbe 2002, Lipcius and Stockhausen 2002) in the Chesapeake Bay. Despite the decrease in abundance, exploitation rates have remained high, raising concern over the sustainability of the stock (Miller et al. 2005).

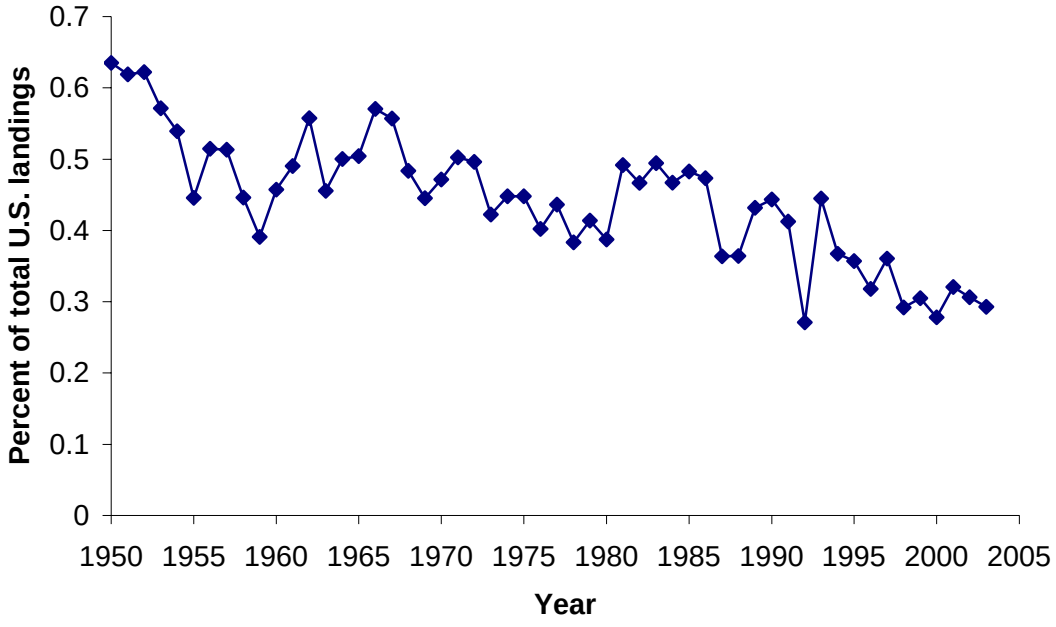


Figure 1.3. Proportion of Chesapeake Bay harvest (Maryland and Virginia combined) to total U.S. harvest, 1950-2003. Data from NMFS website (http://www.st.nmfs.gov/st1/commercial/index.html).

The blue crab has an estuarine-dependent life cycle. In Chesapeake Bay, mating occurs after the female crab's terminal molt (Van Engel 1958). Subsequently, females migrate to high salinity waters in the lower bay, while males and immature crabs typically overwinter in the upper bay and tributaries (Van Engel 1958). The timing of migration depends on the location of mating. Females from the upper Bay likely do not spawn until the season after mating, while females from the lower Bay may produce a brood in the same season that they mate (Turner et al. 2003). Spawning occurs from late spring through the summer months (Jones et al. 1990). Females extrude and fertilize a brood of eggs (a sponge) with stored sperm and then move to the mouth of the bay to release larvae (Tankersley et al. 1998). Females may produce multiple sponges from a single mating (Hines et al. 2003). The released larvae, termed zoea, are advected offshore to the continental shelf where they go through seven to eight zoeal stages and one megalopal stage (Costlow and Bookhout 1959). Upon the final molt, megalopae return to the Bay and are retained in the estuary by tidally rhythmic, vertical migration (see review by Epifanio 1995). The megalopae settle in seagrass habitats or other structurally-complex, shallow nursery areas to undergo further development (Lipcius et al. 1990, Olmi 1995).

Once juveniles reach the fifth instar, they begin to disperse throughout the estuary to forage and grow (Pile et al. 1996). Juvenile crabs may experience high mortality from cannibalism by adult blue crabs (Dittel et al. 1995, Hines and Ruiz 1995) and predation by fish, including striped bass, *Morone saxatilis* and Atlantic croaker, *Micropogonias undulatus* (von Montfrans, Virginia Institute of Marine Science, pers. comm.). Small crabs spend most of their time in shallow waters to avoid predation and gradually move

into deeper water as they grow bigger (Dittel et al. 1995, Hines and Ruiz 1995, Hines et al. 1995). In addition to consuming smaller conspecifics, blue crabs also eat bivalves (particularly the Baltic clam, *Macoma balthica*), fish, and other crustaceans (Hines et al. 1990, Mansour and Lipcius 1991). Thus, in addition to supporting the most lucrative remaining fishery in the Chesapeake Bay, the blue crab serves a critical ecological role in the Bay ecosystem (Baird and Ulanowicz 1989).

Blue crabs are efficient osmoregulators and are found in a wide range of salinities. Occasional populations have been noted in freshwater lakes (Cameron 1978) and hypersaline lagoons (Guerin and Stickle 1992). As previously noted, mature females in estuaries undergo seasonal migrations, thus crabs are often spatially-segregated by life stage and sex. In Chesapeake Bay, *C. sapidus* maintains its hemolymph (blood) hyperosmotic to the external medium at salinities below 25 and isosmotic to the medium at salinities above 25 (Lynch et al. 1973). The blue crab has been referred to as a "strong regulator" for its ability to control its hemolymph composition regardless of the salinity of its environment (Pequeux 1995). There are, however, apparent metabolic costs of osmoregulation. Laboratory studies have demonstrated that blue crab respiration increases with decreasing salinity (Engel and Eggert 1974, Guerin and Stickle 1992). There is also evidence that female blue crabs are less efficient osmoregulators at low salinities (Tan and Van Engel 1966).

Over its range, the blue crab is exposed to a wide range of environmental conditions that could potentially cause latitudinal variations in their life history. The growth and metabolic rates of blue crabs are positively related to water temperature (Leffler 1972). Molting, and thus growth, ceases when the temperature falls below a

minimum temperature threshold (T_{min}) of ~ 9-11°C (Smith 1997, Brylawski and Miller in press). Above this threshold, the intermolt period is a function of temperature (Brylawski and Miller in press). Additionally, in regions where winter temperatures decline predictably below this threshold, such as in the mid-Atlantic region, blue crab assumes a quiescent state and buries in the sediment, likely in an effort to reduce metabolic costs. As a result of these two temperature-induced patterns, the time, and therefore age, to maturity also varies with latitude. Crabs in the southern latitudes can grow year round and have been observed to mature in less than a year in the St. John's River, FL (Tagatz 1968) and the Gulf of Mexico (Perry 1975). In contrast, due to both overwintering and the shorter growing season, blue crabs in Chesapeake Bay may take up to 18 months to mature (Van Engel 1958, Ju et al. 2003).

Overwinter-induced stress and mortality have been documented in a wide range of taxa. During wintertime, food is scarce and aquatic organisms must cope with this energy deficiency by migrating to warmer habitats, or reducing physiological activity, or both (Conover 1992). The ability of organisms to survive this period can influence recruitment dynamics and ultimately limit the northern range and distribution of a species (Johnson and Evans 1990). Overwintering mortality has been documented in a variety of taxa, including turtles (Costanzo et al. 2004), insects (Lombardero et al. 2000), mollusks (Strasser et al. 2001, Thieltges et al. 2004), and many freshwater, estuarine, and marine fish species (Post and Evans 1989, Hurst and Conover 1998, Hales and Able 2001, Lankford and Targett 2001, McCollum et al. 2003, Finstad et al. 2004).

The bioenergetic costs of overwintering are thought to be one of the main causes of winter mortality, particularly during the first year of life (Johnson and Evans 1990).

Smaller individuals tend to have less fat stored, yet metabolize resources faster than larger individuals, putting them at greater risk for energy depletion (Shuter and Post 1990). This size-dependent winter mortality has been observed in numerous fishes, including white perch, *Morone americana* (Johnson and Evans 1990), yellow perch, *Perca flavescens* (Post and Evans 1989), and striped bass, *Morone saxatilis* (Hurst and Conover 1998). Consequently, juveniles born earlier in the growing season that can reach a larger size by the onset of cold weather have a better chance of surviving the winter (Conover 1992)

However, the size-dependent mortality pattern is not universal. Often there is considerable variation in size selectivity on an interannual basis (Hurst and Conover 1998), and in some cases a reverse size-selective pattern has been observed where mortality was greatest for larger fish (Lankford and Targett 2001). Even where smaller fish have been observed to experience greater mortality than their larger conspecifics, the cause cannot always be attributed to starvation (Hurst et al. 2000). Further, if deaths were caused solely by energy depletion, one would expect reduced mortality at lower temperatures due to slower metabolic rates (Johnson and Evans 1996). In fact, mortality is often temperature-dependent, with more individuals dying at low temperatures or during prolonged, severe winters, suggesting that other factors such as thermal and osmotic stress are also important regulators of winter mortality (Lankford and Targett 2001, Hurst and Conover 2002). For several fish species, mortality becomes significant when temperature falls below 3-4° Celsius for a prolonged period of time, suggesting that this temperature may be a critical threshold for survival (Johnson and Evans 1996, Lankford and Targett 2001, McCollum et al. 2003).

As an organism approaches its lower lethal temperature limit, the costs of maintaining an osmotic gradient increases, which may be particularly important for estuarine species that inhabit a wide range of salinities. Striped bass exhibit higher overwinter survival at intermediate salinities compared to full freshwater and seawater (Hurst and Conover 2002). Tolerance to low temperatures decreases at low salinity among Atlantic croaker (Lankford and Targett 2001). One possible reason for this enhanced stress is the apparent breakdown of osmoregulatory capabilities at extremely cold temperatures that disrupts normal ion balance (Hochachka 1988). Osmoregulatory failure has also been suggested to be the cause of mortality at low temperature for white perch (Johnson and Evans 1996), and white crappie, *Pomoxis annularis* (McCollum et al. 2003). While polar and boreal ectotherms have evolved mechanisms for maintaining ion fluxes at near lethal temperatures (Hochachka 1988), species living in temperate or tropical latitudes may not possess the same adaptations. However, northern populations are often more tolerant of cold temperatures than southern populations of the same species, as has been observed for Atlantic silverside, *Menidia menidia* (Schultz et al. 1998), and summer flounder, *Paralichthys dentatus* (Malloy and Targett 1994).

The potential importance of winter mortality in blue crab has been noted by numerous watermen and scientists (Pulmier 1901, Pearson 1948, Dudley and Judy 1973, Kahn and Helser 2005) and recently has been highlighted by the results of a fishery-independent survey for blue crab in the Chesapeake Bay. The Winter Dredge Survey (WDS) has been conducted annually since 1990 to estimate baywide blue crab abundance, describe the size and sex composition of the population, and estimate exploitation and fishing mortality rates (Sharov et al. 2003). In 1996, an unusually large

number of dead crabs were found during the survey, coincident with a cold winter, which raised concern about the potential effects of severe winters on the crab stock. Since then, high crab-density sites have been resampled by Maryland Department of Natural Resources to estimate over-wintering mortality (Sharov et al. 2003). High levels of winter mortality were observed in the survey in 2002/2003, again after an unusually harsh winter (Rome et al. 2005). It seems likely that inter-annual variability in winter temperature induces inter-annual variability in overwintering mortality. Explicitly, accounting for this source of natural mortality would improve abundance estimates obtained from the WDS, which are used by resource managers to set targets for commercial and recreational crabbing. This estimate will also improve calculations of the exploitation rate, or the number of crabs harvested relative to the number of crabs available.

Modeling the population dynamics of blue crabs is challenging due to the discontinuous nature of crab growth, in addition to both the spatial and temporal variation in blue crab life history, ecology, and distribution (Miller and Smith 2003). Furthermore, the exploitation patterns similarly vary spatially and temporally. Miller (2001) developed a stage-based matrix model of blue crab life history that accounts for the discontinuous nature of crab growth. Recently, Miller (2003) expanded the stage-based modeling approach to include spatial and temporal variability. Survival probabilities varied to reflect patterns in fishery exploitation, but did not vary to reflect important environmental gradients present in the Chesapeake Bay. Inclusion of temperature- and salinity-dependent overwinter survival rates would improve the reliability of the model results.

Despite the occasional evidence of temperature-dependent winter mortality in the field, experiments to test the effects of low temperature on blue crabs and other crustaceans are sparse. Tagatz (1969) examined the acute (48h) thermal tolerance of juvenile and adult blue crabs and found that crabs were less tolerant of extreme temperatures at low salinity. Although the tolerance limits for adults and juveniles were similar, juveniles were slightly less tolerant to cold than adults (Tagatz 1969). Recent work by Rome et al. (2005) has also indicated that temperature and salinity are important regulators of winter mortality, and that mature females and small recruits <15 mm may be particularly sensitive to low temperature-salinity combinations. Although their experiments were carried out over an extended duration (60 days), winter conditions in the Chesapeake Bay and other mid-latitude regions may persist for a much longer duration, and there is a need to more thoroughly investigate blue crab survival over longer exposure times.

Objectives

My thesis has two primary objectives:

Objective 1: Quantify the probability of blue crab mortality as a function of its exposure history to temperature and salinity

This objective was addressed in laboratory experiments where juvenile crabs were exposed to temperature and salinity combinations reflective of wintertime conditions in the Chesapeake Bay. The laboratory results were then used to generate a function describing temperature- and salinity-dependent winter survival. The effects of additional factors, including size, presence of sediment, sex, light, and crab origin, on survival were also investigated. The results from this work will be presented in Chapter 2. The chapter

is written as a draft manuscript for intended submission to the Journal of Experimental Marine Biology and Ecology.

Objective 2: Quantify spatial and interannual trends in crab overwintering mortality in Chesapeake Bay

In Chapter 3, I demonstrate the consequences of temperature- and salinity-dependent mortality on blue crab abundance and fishery landings in the Chesapeake Bay. Environmental time series were modeled to provide an estimate of the temperature and salinity exposures at individual locations throughout the bay. These data were combined with output from the accelerated failure time analysis (Objective 1) to predict spatial and interannual patterns in survival probability for years 1990-2004. These estimates were then compared to those observed in the field by Maryland DNR during the Winter Dredge Survey. Finally, I investigated how the baywide expected mortalities would alter observed abundance and the exploitation fraction. This chapter is written as a draft manuscript intended for submission to Estuaries.

Chapter 2: Temperature, salinity, and size-dependent winter mortality of juvenile blue crabs (*Callinectes sapidus*)

Abstract

At mid-latitudes within its range, overwintering mortality may play an important role in regulating local blue crab (*Callinectes sapidus*) populations. While previous laboratory work demonstrated the significance of low temperature and salinity on crab survival, experimental mortality rates were much higher than levels observed in the Chesapeake Bay under similar conditions. I conducted a 121-day experiment to improve estimates of winter mortality by incorporating more realistic temperature acclimation periods and light-levels than in previous studies. The 2x2x2 factorial experiment tested the effects of temperature (3°C, 5°C), salinity (10, 25) and sediment (sediment, no sediment) on the survival of juvenile crabs. The crabs, ranging from 14-68 mm carapace width (CW), were obtained from field surveys in the Patuxent River and from a hatchery at the Center for Marine Biotechnology, Baltimore, MD. The presence of sediment did not significantly improve crab survival. Hatchery raised crabs experienced significantly lower survivorship than wild caught crabs, which suggests that crabs of hatchery origin may be less winter-hardy than wild crabs. Observed survival of crabs of both origins was 71% at high salinity (25) under both temperature regimes, but only 40% at 3°C and 10 salinity. I fit an accelerated failure time model to the survival data, and found that time to death significantly increased with increasing temperature, salinity, and crab size. These results suggest that winter survival varies with winter severity, is spatially dynamic, and that small juveniles are more at risk of dying over the winter than their larger cohorts.

Introduction

Aquatic organisms are exposed to multiple temperature-dependent stressors during winter. For example, extreme low temperatures itself can cause mortality (Shuter et al. 1980, Storey and Storey 1996). Further, low temperatures combined with a shorter photoperiod leads to decreased production in temperate systems, so that food resources also may be limited (Shuter and Post 1990, Pangle et al. 2004). In combination, these stressors may act to limit the range and distribution of a given population (Johnson and Evans 1990, Shuter and Post 1990, Thieltges et al. 2004). Within population ranges, variability in the degree of winter severity can lead to variability in the level of winter mortality (Post and Evans 1989, Fullerton et al. 2000, Thieltges et al. 2004).

During winter, feeding, growth, and metabolism of ectotherms are generally low, so that many organisms store lipids during the growing season in preparation for the winter (Hagen et al. 1996, Schultz and Conover 1997). Exhaustion of energy reserves is thought to be one of the main causes of winter mortality, particularly during the first year of life (Post and Evans 1989, Johnson and Evans 1990, Fullerton et al. 2000). Survival of juveniles during this critical overwintering period can ultimately influence recruitment dynamics (Santucci and Wahl 2003). Variation in winter severity has been linked to variable recruitment and year class strength in a number of invertebrates (Strasser and Pieloth 2001, Thieltges et al. 2004) and fishes (Post and Evans 1989, Lankford and Targett 2001). Long winters may also delay the production or settlement time of the next generation (Beukema 1992).

During the growing season, juveniles must balance the trade-off between allocating energy to growth and storing reserves for times of food scarcity (Post and

Parkinson 2001). Timing and duration of periods of low winter temperature greatly influence starvation-induced mortality. An early onset of cold weather would mean less time for individuals to grow and store reserves, and a longer winter requires organisms to use more lipid resources. Smaller individuals tend to have less fat stored, yet metabolize resources faster than larger individuals, putting them at greater risk for energy depletion (Shuter and Post 1990). In most cases, juveniles born earlier in the growing season may reach a larger size by the onset of cold weather and have a better chance of surviving the winter (Conover 1992). Thus, size-dependent winter mortality is often thought to be starvation-induced (Oliver et al. 1979, Post and Evans 1989, Johnson and Evans 1990).

If deaths were caused solely by energy depletion, however, one would expect higher survival at lower temperatures due to slower metabolic rates that reduce energy use (Johnson and Evans 1996). In fact, mortality is often temperature-dependent, with more individuals dying at low temperatures or during prolonged, severe winters (Lankford and Targett 2001, Hurst and Conover 2002). Further, size-dependent survival patterns are not always straightforward. Often there is considerable variation in size selectivity across a latitudinal gradient (Garvey et al. 1998) or on an interannual basis (Hurst and Conover 1998). In some cases, a reverse size-selective pattern has been observed where mortality was greatest for larger individuals (Lankford and Targett 2001, Sharov et al. 2003). Even where smaller individuals have been observed to experience greater mortality than their larger conspecifics, the cause cannot always be attributed to starvation alone (Hurst et al. 2000). Smaller individuals may be more at risk for predation or cannibalism if the winter is mild and predators are active (Garvey et al.

1998). In addition, size-dependent migration success may play an important role in contributing to winter mortality (Munch et al. 2003).

As the lower lethal temperature range of an organism is approached, starvation may become less important than mechanisms that operate under cold stress. One possible reason for enhanced stress is the apparent breakdown of osmoregulatory capabilities at extremely cold temperatures that disrupts normal ion balance (Hochachka 1988). Johnson and Evans (1996) noted that while age-0 white perch (*Morone americana*) were more susceptible to starvation at 4ºC, the cause of death at lower temperatures was more likely osmotic failure. Impaired osmoregulatory capabilities may lead to high overwinter mortality for coastal and estuarine inhabitants that may experience a wide range of salinities such as striped bass, *Morone saxatilis* (Hurst and Conover 2002) and Atlantic croaker, *Mircopogonias undulates* (Lankford and Targett 2001). In addition, temperature affects oxygen delivery, the carbon dioxide system, and pH in aquatic poikilotherms (see review by Cameron and Mangum 1983).

Although there are numerous reports of "winterkills" or decreased abundance of aquatic invertebrates after cold winters (Beukema 1991, 1992, Thieltges et al. 2004, Smith et al. 2005), there have been considerably fewer laboratory studies to quantify the effects of cold temperature on invertebrates in comparison to fish. One candidate species in which winter mortality may play an important ecological role is the blue crab, *Callinectes sapidus*. The blue crab is distributed broadly along the eastern coast of the American continent, typically ranging from Massachusetts to Uruguay (Williams 1984). Throughout its range, the blue crab experiences different climatic conditions (tropical – boreal) that have important consequences on life history patterns. For example, molting,

and thus growth, ceases when the temperature falls below a minimum temperature threshold (T_{min}) of ~ 9-11°C (Smith 1997, Brylawski and Miller in press). At southern latitudes where the temperature never falls below the T_{min}, crabs can grow year round and have been observed to mature in less than a year in the St. John's River, FL (Tagatz 1968) and the Gulf of Mexico (Perry 1975). In contrast, in temperate regions where winter temperatures regularly fall below this threshold, crabs overwinter in the sediment, during which time no growth occurs (Mauro and Mangum 1982). This cessation in growth extends times to maturity such that blue crabs in the Chesapeake Bay may take up to 18 months to mature (Van Engel 1958, Ju et al. 2003).

Norse (1977) suggested that the distribution of most *Callinectes* species (Family Portunidae) in the Atlantic is limited by the summer temperatures required for egg hatching and zoeal survival. However, the blue crab is the only species of *Callinectes* that ranges far into seasonably cool temperate regions, and at mid-latitudes within its range, overwintering mortality may play an important role in regulating local populations and the range of this species. In support of this, larger-than-normal numbers of dead crabs have been observed in the Maryland portion of the Bay during particularly cold years (Sharov et al. 2003, Rome et al. 2005). Similar observations have been made in other Mid-Atlantic estuaries (Kahn 2003). Thus, spatial and interannual variation in winter severity may lead to varying degrees of winter mortality of the blue crab in Chesapeake Bay.

The incipient lethal temperature technique is commonly used to quantify acute temperature tolerance (Fry 1971, Beitinger et al. 2000). In this approach, groups of organisms are moved from a variety of acclimation temperatures into a series of constant

test temperatures. Similar to the median lethal dose methodology (LD_{50}), mortality levels at each temperature are estimated to determine the lower and upper temperatures lethal to 50% of the fish at selected exposure time intervals. The endpoints can then be used to generate upper and lower tolerance limits and temperature tolerance polygons over a range of acclimation and test temperatures. Employing this approach, Tagatz (1969) determined the 48-hour upper and lower tolerance limits of blue crabs at both low (6.8) and high (34) salinity. Upper and lower tolerance limits increased as the acclimation temperature increased and crabs showed a wide range of thermal tolerance (Tagatz 1969). In addition, crabs were less tolerant of temperature extremes at low salinity, and juveniles were slightly less tolerant to cold than adults. The findings indicated that blue crabs could tolerate acute periods of freezing temperatures (0 ºC was the lowest temperature tested).

However, acute thermal tolerance tests are limited in their application. The sudden transfer from the acclimation to experimental temperature in incipient lethal temperature studies results in an abrupt change that is seldom experienced in nature (Beitinger et al. 2000). In addition, the short duration of the exposure in Tagatz (1969) makes it difficult to predict crab tolerance during chronic periods of low temperature. An alternative to dose-response methods is survival analysis, which make more effective use of mortality data by using both the number and timing of deaths to describe the distribution of mortalities (Newman and Dixon 1996). In addition, survival analysis accounts for censored observations (i.e., observations for which the time to death is unknown). Individuals that are still alive at the end of exposure are “right-censored.” For cases where only a window of time rather than an exact time of death is known, the

observations are "interval-censored." Survival analysis has been employed often in the industrial and medical field and is increasingly being used in ecological studies (Muenchow 1986, Chambers and Leggett 1989, Barbeau et al. 1994, Newman and McCloskey 1996, Borsuk et al. 2002). The approach is more useful for modeling the effects of a stressor than are dose-response methods.

A preliminary experiment conducted in 2004 (Appendix A) and recent work by Rome et al. (2005) both indicated that crabs are sensitive to low temperature and salinity. However, the duration of these experiments was shorter than the typical length of winter and mortality rates were higher than those observed in the field. Winter severity is likely a function of both the minimum temperature and its duration. Therefore, in laboratory experiments testing cold tolerance, it is crucial to simulate typical winter length. Thus, the purpose of this research was both to quantify the temperature, salinity, and size-dependent mortality of crabs over a longer exposure time using survival analysis and to investigate the possibility that other factors, such as sediment and light, help to regulate blue crab winter mortality.

Methods

Experimental design

Juvenile crabs ranging from 14 to 68 mm carapace width (CW) in size were obtained from the Center of Marine Biotechnology (COMB), Baltimore, MD and from field sampling in the Patuxent River, MD in October 2004. Initially, crabs were kept in flow-through tanks with ambient filtered seawater from the Patuxent River from October to January to ensure that crabs experienced a seasonal temperature decline at the rate they would be exposed to in the field (Figure 2.1). Crabs from COMB and the Patuxent River

were kept in separate tanks during the acclimation period and were tracked separately throughout the experiment. During the acclimation period, crabs were fed a mixture of Ziegler® shrimp pellets and fresh squid *ad libitum*. Observations indicated that feeding decreased as the temperature dropped below 10°C, and ceased shortly thereafter. Accordingly, crabs were not fed during the experiment.

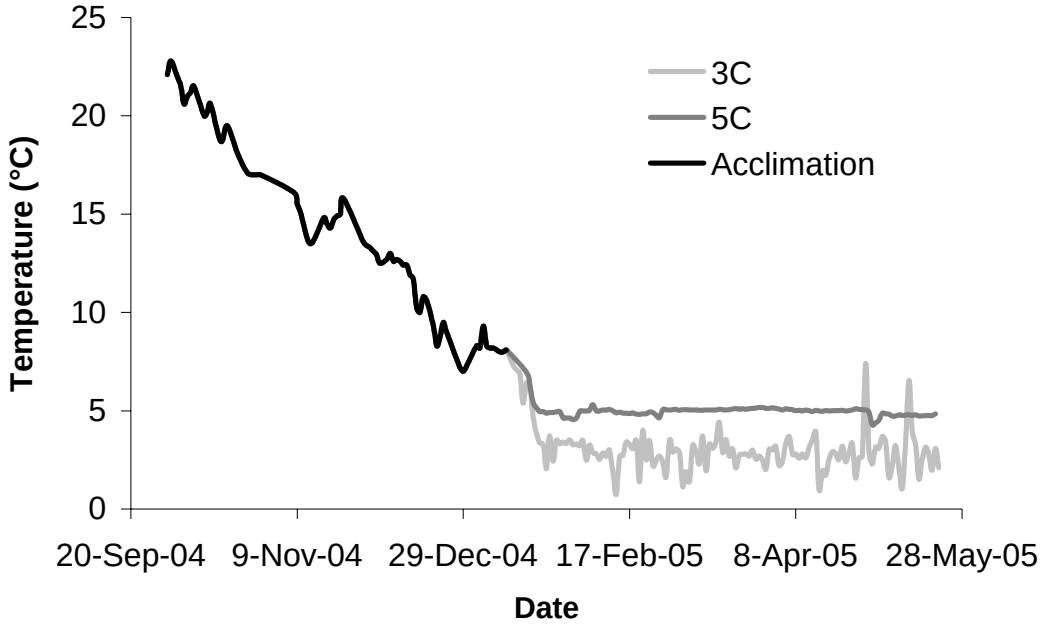


Figure 2.1. Observed temperature record during the acclimation period and simulated winter temperatures for the two experimental temperature treatments.

The experiment was designed as a 2x2x2 factorial to test for the effects of temperature (3°C, 5°C), salinity (10, 25) and sediment (sediment, no sediment) on survival. Clean sand, sieved through 1-mm mesh to remove large particles, was used for several reasons. First, concerns over the biological oxygen demand over the four-month test period prevented the use of field substrates. Furthermore, inspection of maps of crab wintertime distribution (Jensen and Miller 2005) and sediment data from the Chesapeake

Bay Program database[2] indicated that crabs overwinter in a diversity of sediment types ranging from clay to sand (personal observation). Finally, crabs that were placed in containers with sand during the acclimation period readily buried into the substrate.

Two hundred and twenty crabs were used in the experiment; 63 wild and 157 hatchery-raised (Figure 2.2). Crabs from both sources were included because hatchery crabs in a previous experiment (Appendix A) exhibited similar survival patterns to wild crabs in Rome et al. (2005). In addition, including crabs from both origins allowed us to compare survival across a wider range of sizes (Figure 2.2). Within each temperature-salinity combination, 15 crabs were assigned to the no-sediment treatment and 40 crabs were assigned to the sediment treatment. Crabs were randomly assigned to each treatment combination. In January 2005, when the ambient water temperature reached approximately 8°C, crabs were moved into constant temperature rooms to complete the acclimation. Temperatures in the constant temperature chambers were lowered approximately 0.5°C per day until the test temperature was met and salinity was increased at 2 units per day for the high salinity treatment. The low salinity treatment was similar to ambient salinity and thus only minor adjustment was necessary. During the acclimation period, the light regime was gradually shifted to a longer dark period until crabs were maintained in constant darkness once the experiment began.

During the experiment, crabs were held in individual 3-L Prolon circular containers, and five containers each were placed in one larger 33-L (62.2cm x 45.1cm x 18.1cm) water bath of the appropriate temperature and salinity treatment combination. An aerator was placed in each bath and two 1-cm and four 0.5-cm diameter holes were

[2] Available online at http://www.chesapeakebay.net

drilled in each crab container to improve water circulation. The 33-L baths were randomly assigned to shelves within the temperature control rooms.

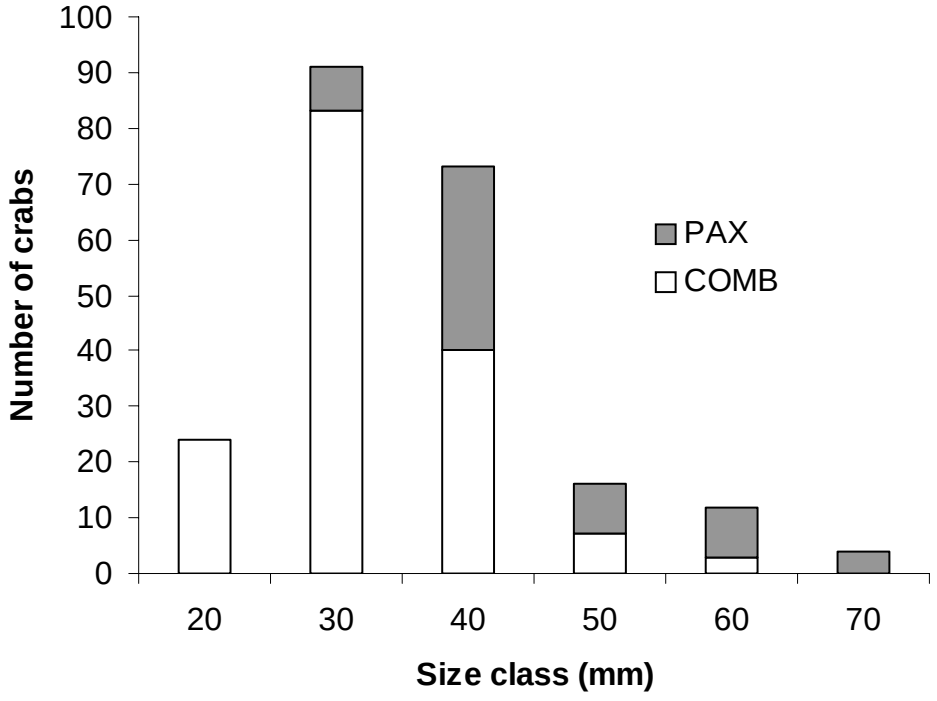


Figure 2.2. Size distribution, grouped by 10 mm intervals, for crabs used in the experiment from the Patuxent River (PAX) and the Center for Marine Biotechnology (COMB).

The experiment was conducted for 121 days, equivalent of the typical length of winter in much of Chesapeake Bay. The water temperature, salinity, and dissolved oxygen were recorded in each bin every day, and crabs were checked for mortality. Fifty percent of the water was changed every 2 d, and ammonia levels were monitored throughout the experiment to ensure that adequate water quality was maintained. All sampling was conducted by aid of a headlamp with a red filter, as blue crabs are insensitive to red light (Cronin and Forward 1988). Crabs that were suspected of being dead were gently probed with a glass rod, and if they did not respond to stimulation they

were removed and placed in a petri dish for further observation. In a few instances, crabs were in a cold-induced torpor and began to move when they were moved to ambient room temperatures; these crabs were returned to the water. Crabs that did not move after being held at ambient temperature for several minutes were considered dead.

The burial status of each crab was monitored throughout the experiment. To avoid disturbing crabs that were buried or partially buried, four crabs from each temperature-salinity combination were randomly selected to be sampled every 11 days. To minimize disturbing fully buried crabs during this sampling procedure, we lined each container with plastic mesh and this was gently pulled up to "sieve" the crab out of the sediment. Thus, each buried crab was sampled once during the duration of the experiment, and again when the experiment ended.

Data analysis

All data analyses were conducted in SAS version 8.2 (SAS Institute, Cary, NC). To determine whether survival to the end of the experiment differed among crabs of different sex, origin, and sediment treatment, log-rank tests were used to test for homogeneity among groups (Proc Lifetest, Allison 1995). Specifically, the null hypotheses were tested that juvenile blue crab survival is independent of sex [H_o: $S(t)_{Male} = S(t)_{Female}$], crab origin [$H_o$: $S(t)_{COMB} = S(t)_{PAX}$], and presence of sediment [H_o: $S(t)_{No_Sediment} = S(t)_{Sediment}$].

Survival analysis was used to quantify the pattern of mortality during the experiment. This approach relies on estimates of the time to death of individual crabs that died in the experiment. Central to survival analysis is the mortality probability density function, f(t), which provides an estimate of the probability that an individual will

die in a small time interval (t, t+dt). The cumulative distribution function of mortality, F(t), represents the proportion of individuals that are dead at time t,

(1) $$F(t) = \frac{\text{Number dead at time t}}{\text{Total number exposed}}.$$

The survival function, S(t), is the probability that an individual survives past time t, or the proportion of the population still alive at time t,

(2) $$S(t) = 1 - F(t).$$

Survival begins at one and decreases over time to zero. The hazard function, h(t), or the instantaneous mortality rate, is the probability that an individual will die in the interval (t, t+dt) conditional on an individual reaching time t:

(3) $$h(t) = \frac{f(t)}{S(t)}.$$

The survival function can also be expressed as the exponentiated function of the integral of the hazard function:

(4) $$S(t) = \exp\left[- \int_{t}^{0} h(t)\, dt \right].$$

To model the effects of temperature, salinity, and size on the time to death, an accelerated failure model was fit to the data. The variables were treated as continuous covariates. All main effects and two-way interactions were included and the model was reduced using backward elimination. The accelerated failure model is a class of parametric survival models that are fit with maximum likelihood methods using the function of the covariates and candidate distributions for error. The general form of the accelerated failure model is:

(5) $$\ln t_i = f(x_{ik}) + \varepsilon_i,$$

where t_i is a random variable denoting the event time for the *i*th individual in the sample, x_{ik} are the values of k covariates, and ε_i is the random error term. Covariates have a multiplicative effect over time so that they serve to "accelerate" death (Hosmer and Lemeshow 1999).

The underlying shape of baseline mortality is determined by the specified error distribution. The model was fit with all supported distributions (exponential, Weibull, lognormal, gamma, log-logistic). The appropriate model was selected using the Akaike's information criterion (AIC) statistic, log-likelihood ratio tests, and graphical diagnostics. Log-likelihood statistics cannot be compared directly because the models differ in the number of estimated parameters (Newman and Dixon 1996). The AIC statistic adjusts the log-likelihood statistic for the number of parameters in the candidate model and the model with the lowest AIC is deemed to have the best fit. Log-likelihood ratio tests compare nested models (Allison 1995). For example, the exponential and Weibull models are both special cases of the generalized gamma model, and the null hypothesis of the likelihood ratio test is that the particular restriction of the nested model is true (Allison 1995). Linearization transformations for the candidate distributions were performed on the data (Newman and Dixon 1996) and examined visually. The transformation that yielded the straightest line was selected.

Results indicated that the Weibull function was the most appropriate baseline mortality function. Although the generalized gamma had a slightly lower AIC statistic, the likelihood ratio test between the Weibull and the generalized gamma indicated that the Weibull model could not be rejected ($0.10<p<0.05$). Additionally, the Weibull model

is computationally simpler to use and examination of the graphical diagnostics indicated that the Weibull model best fit the data.

The Weibull distribution is a power function, indicating that the risk of death increases over time:

(7) $$h(t,x,\beta,\lambda) = \lambda t^{\lambda-1}\exp[-\lambda(\beta_0 + \beta_1 x_1 + ... + \beta_i x_i)],$$

where λ is the Weibull shape parameter, β_0 is the intercept parameter, and β_1 to β_i are the covariate parameter estimates (Hosmer and Lemeshow 1999). The corresponding survivorship function for the accelerated failure form of the Weibull model is

(8) $$S(t,x,\beta,\lambda) = \exp\left\{-t^{\lambda}\exp\left[-\lambda(\beta_0 + \beta_1 x_1 + ... + \beta_i x_i)\right]\right\}.$$

Results

During the experimental period, the temperature averaged 2.9 ± 0.9 (mean ± SD)°C and 5.0 ± 0.2 °C in the low and high temperature treatments, respectively. In the 3°C treatment, mechanical difficulties in the constant temperature room caused the temperature to fluctuate more widely than in the 5°C room. In particular, there were two instances late in the experiment where the room temperature spiked and the average water temperature jumped to 7°C (Figure 2.1). The temperature was immediately lowered again to the treatment temperature.

Initially, overall survival was high with over 90% of the crabs remaining alive after two months (Figure 2.3). However, by the end of the four-month trial, overall survival fell to 62%. Crabs in the treatment with no sediment available had a slightly lower survival than those for which sediment was available, although this difference was not significant (χ^2 =1.93, df =1, Pr = 0.16, Figure 2.4). The burial behavior of the crabs

was tracked throughout the experiment. At the onset of the experiment, about 80% of the crabs buried in the sediment; however the proportion of crabs that were buried decreased to less than 30% by the end of the four month trial (Figure 2.5). A higher percentage of crabs was buried at higher temperature. Most crabs were partially visible rather than completely buried. No buried crabs that were uncovered for intermittent sampling were dead upon recovery, therefore no individuals were interval censored.

Survival of male and female crabs were not statistically different ($\chi^2 = 0.16$, df = 1, Pr = 0.68). However, while the overall survival rate for male and females crabs was similar (Figure 2.6a), the sexes did not respond the same way to the temperature and salinity levels ($\chi^2 = 22.8$, df = 7, Pr = 0.002; Figures 2.6b,c). At the end of the experiment, the proportions of male and female crab survivors were under 50% in the low temperature, low salinity treatment. However, the survivorship of females at high temperature under both salinity regimes was high, while males had higher survivorship than females at the low temperature, high salinity treatment.

Hatchery crabs had a significantly lower survival rate than wild-caught crabs ($\chi^2 = 14.21$, df =1, Pr = 0.0002, Figure 2.7). At the termination of the experiment, only 54% of the COMB crabs remained alive, compared to 82% of the Patuxent crabs. However, the average size of the hatchery crabs was smaller than the average size of the wild-caught crabs, and the observed survival of hatchery crabs at 121 d increased with increasing size class (Figure 2.8).

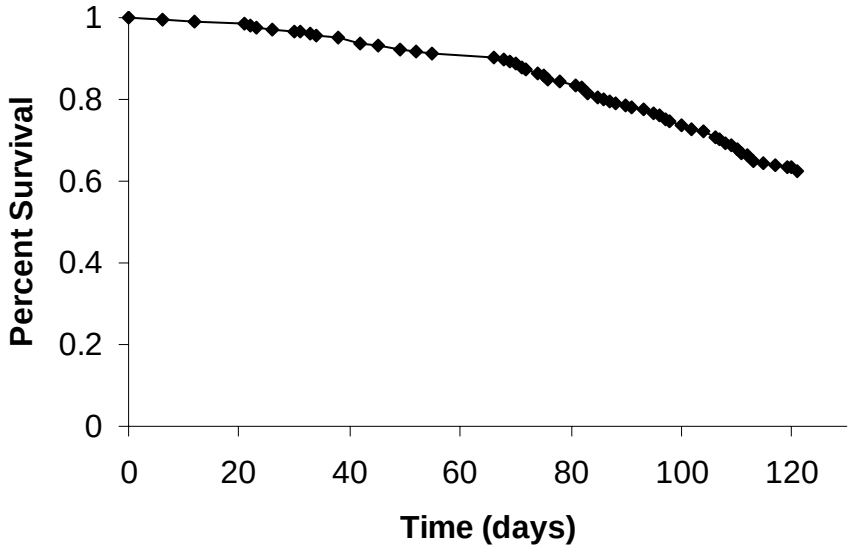


Figure 2.3. Observed proportion survival over the 121-day experiment. Survival times were estimated using the Kaplan-Meier product limit estimator (Proc Lifetest).

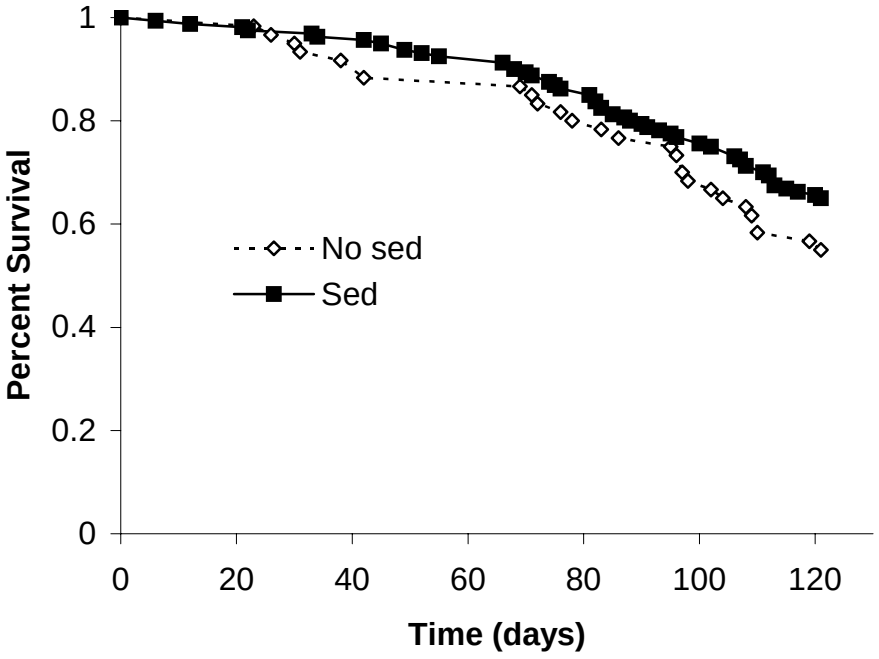


Figure 2.4. Observed proportion survival of juvenile blue crabs grouped by sediment treatment (sediment or no sediment). Survival times were estimated using the Kaplan-Meier product limit estimator (Proc Lifetest).

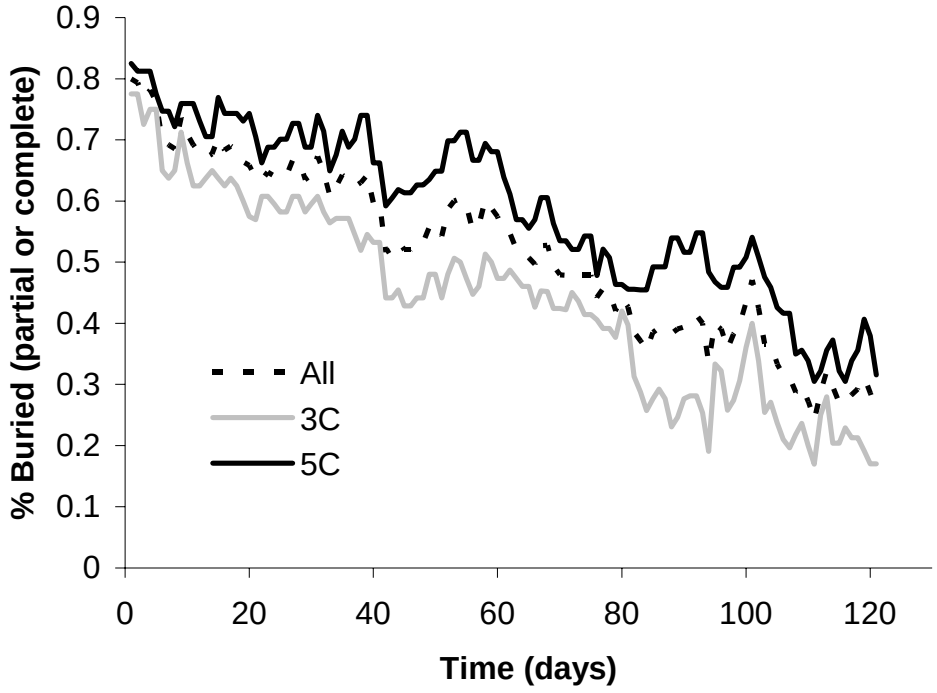
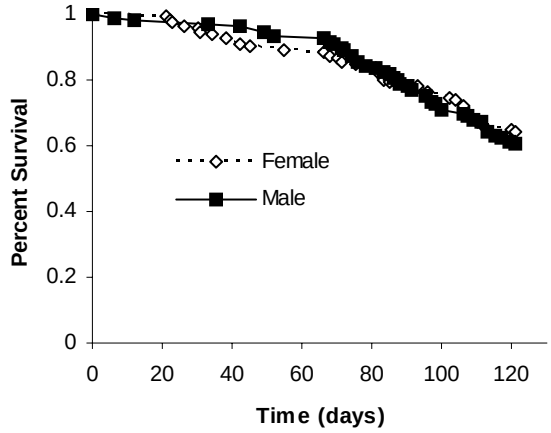


Figure 2.5. Percent of blue crabs that were either partially or completely buried over time.

a)



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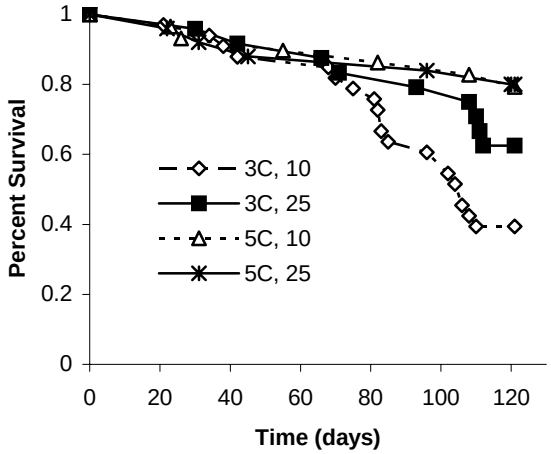


c)

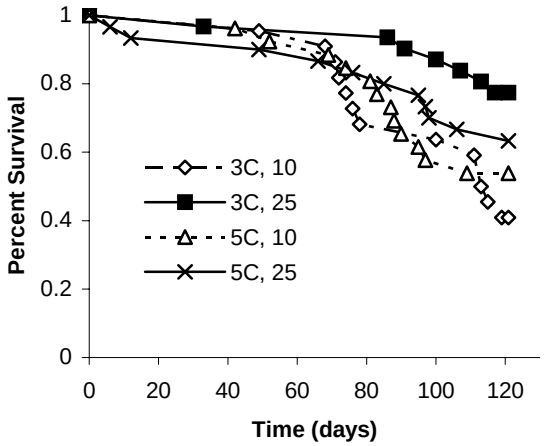


Figure 2.6. a) Observed proportion survival of juvenile blue crabs grouped by sex and observed proportion survival of female (b) and male (c) crabs by treatment. Survival times were estimated using the Kaplan-Meier product limit estimator (Proc Lifetest).

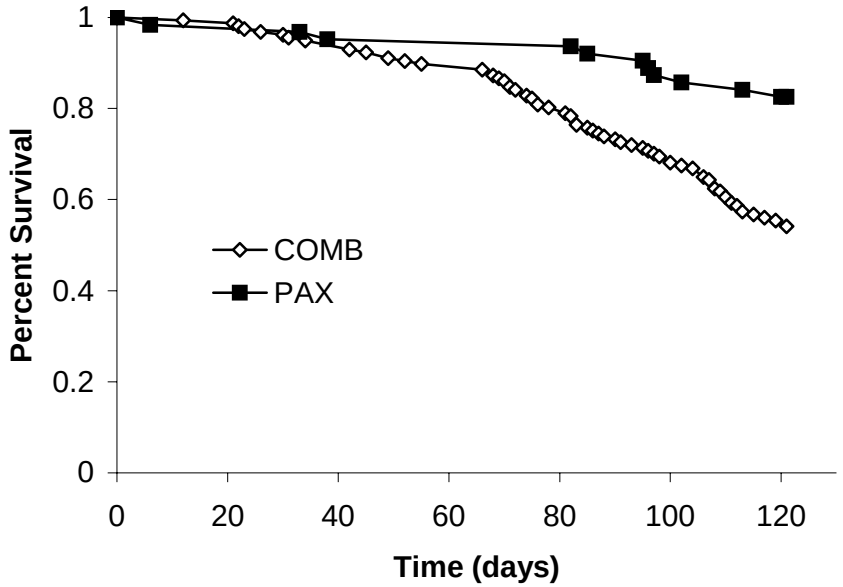


Figure 2.7. Observed proportion survival of juvenile blue crabs by origin (PAX = Patuxent River, COMB = Center for Marine Biotechnology). Survival times were estimated using the Kaplan-Meier product limit estimator (Proc Lifetest).

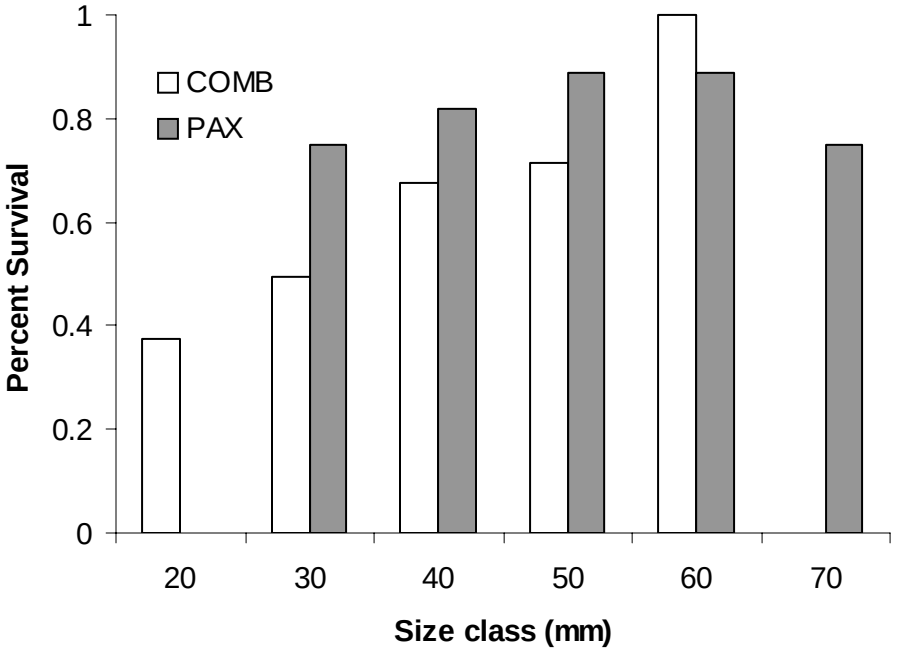


Figure 2.8. Observed 121-day survival of juvenile blue crabs, grouped into 10 mm size classes, from the Patuxent River (PAX) and the Center for Marine Biotechnology (COMB).

The accelerated failure model indicated that temperature, salinity, and crab size all significantly affected blue crab time to death (Table 2.1). No two-way interactions were significant at the 0.05 level and were removed from the model. To compare observed and predicted survival, hazard and survival functions were run for each temperature and salinity treatment at mean crab size. The instantaneous mortality rate increased over time and was significantly higher at low temperature, low salinity combinations (Figure 2.9a). The parameter estimates for temperature and salinity were both positive, indicating that as temperature and salinity increase, the time to death increases. Accordingly, predicted survival was highest in the 5°C, 25 salinity combination and lowest at the 3°C, 10 salinity combination (Figure 2.9b).

Table 2.1. Temperature, salinity, and size-dependent survival of blue crabs exposed to two winter temperatures and two salinity regimes (Proc Lifereg). Parameter estimates are for a fitted Weibull distribution All interactions were insignificant at the 0.05 level and were removed from the model.

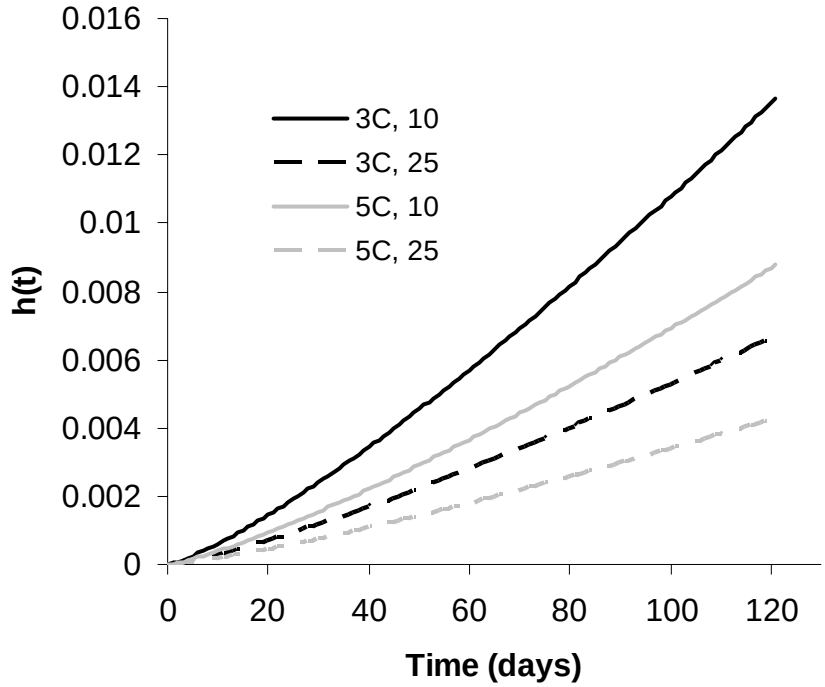
Parameter	df	Estimate	SE	Lower 95% CL	Upper 95% CL	Wald Chi-Square	Pr>ChiSq
Intercept	1	3.59	0.31	2.97	4.20	131.18	<.0001
Temperature	1	0.10	0.05	-0.0001	0.20	3.83	0.05
Salinity	1	0.02	0.007	0.007	0.03	9.17	0.003
Size	1	0.03	0.007	0.01	0.04	15.07	0.0001
Scale	1	0.45	0.05	0.36	0.54		

The fit of the survival model to the observed data varied among treatments. In all treatments, periods of higher mortality rate alternated with periods where few deaths were observed (Figure 2.9b). This was particularly evident at the low temperature, low salinity treatment, where the mortality rate was highest during two periods (t = 68-85 and t = 96-119 days) separated by a week with no mortality (Figure 2.9b). In addition, predicted survival in this treatment was slightly higher than observed survival at Day 121.

Observed survival at Day 121 was 70% at both temperatures in the high salinity treatment; however the model overpredicted the survival rate in the high temperature, high salinity treatment.

Overwintering survival was size-dependent, as survivorship significantly increased with increasing crab size (Table 2.1). In both the mild (5°C) and severe (3°C) winter scenarios, smaller crabs have a higher risk of death than larger individuals (Figures 2.10, 2.11). The effect of size-dependent mortality is predicted to be greatest at low temperature and salinity, which agrees with observed survival patterns. Crabs ≤ 30mm (n=115) had a 121-day survival of 49%, compared with 77% survival of crabs greater that 30 mm (n=105). The average size of surviving crabs increased from 31.4 mm at the beginning of the experiment to 33.6 mm at the end (Figure 2.12). No molting occurred during the experiment. The only two crabs greater than 45 mm died at day 6 and 33, after which no large crabs died (Figure 2.13).

a)



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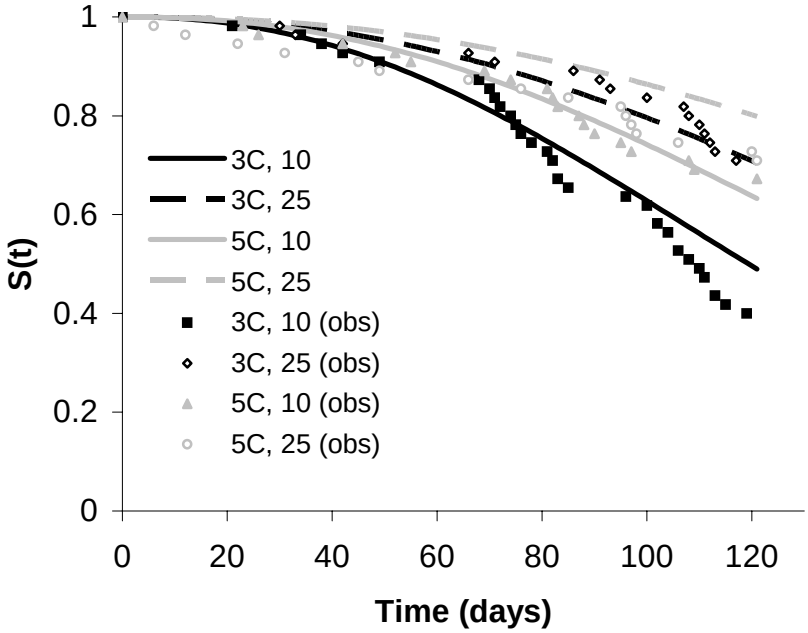
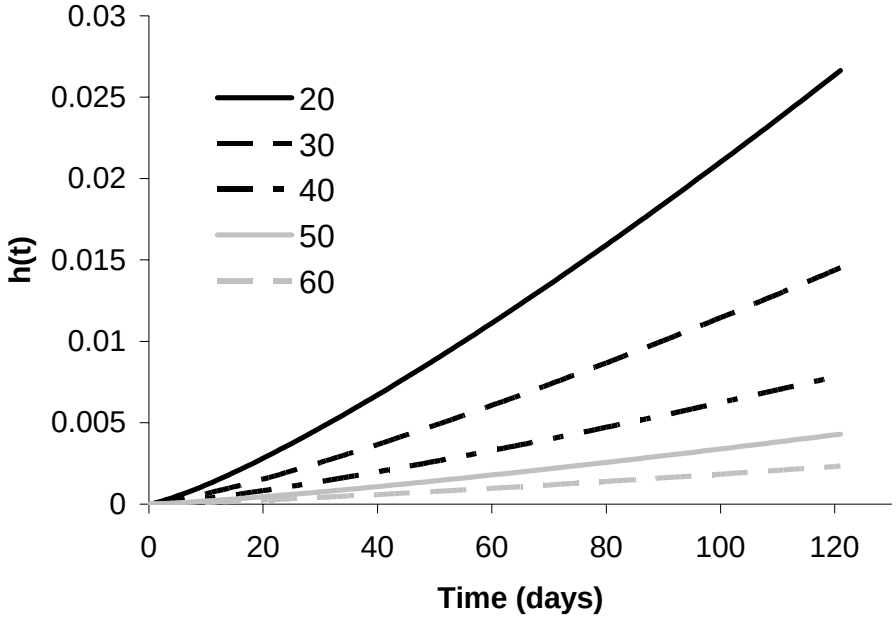


Figure 2.9. a) Hazard function (h(t), instantaneous mortality rate) of averaged-size blue crabs at different experimental temperature and salinity combinations. Parameter estimates for the fitted Weibull distribution are in Table 1. b) Observed proportion survival (reduced from event times using the KM product limit estimator) and estimated survival function (S(t)) for the Weibull hazard functions in a).

a)



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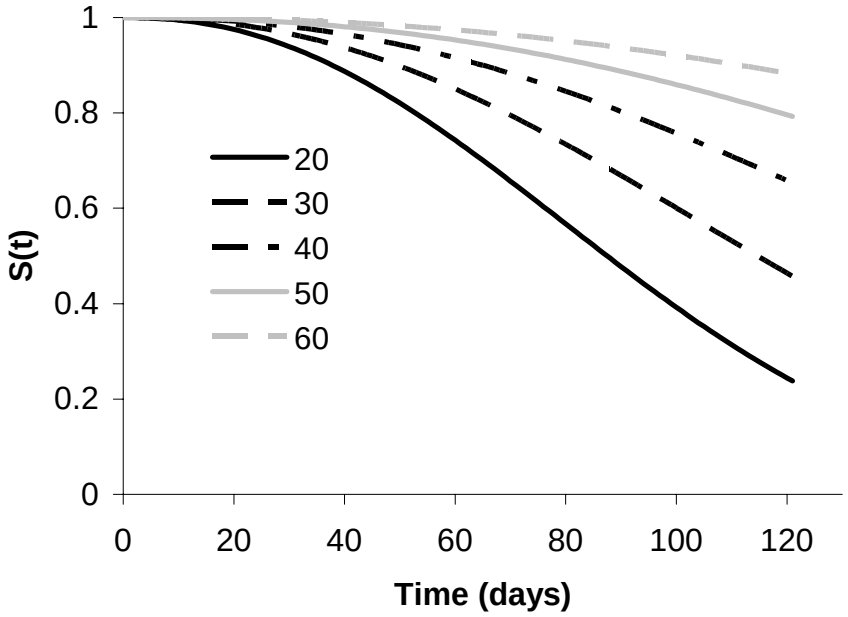
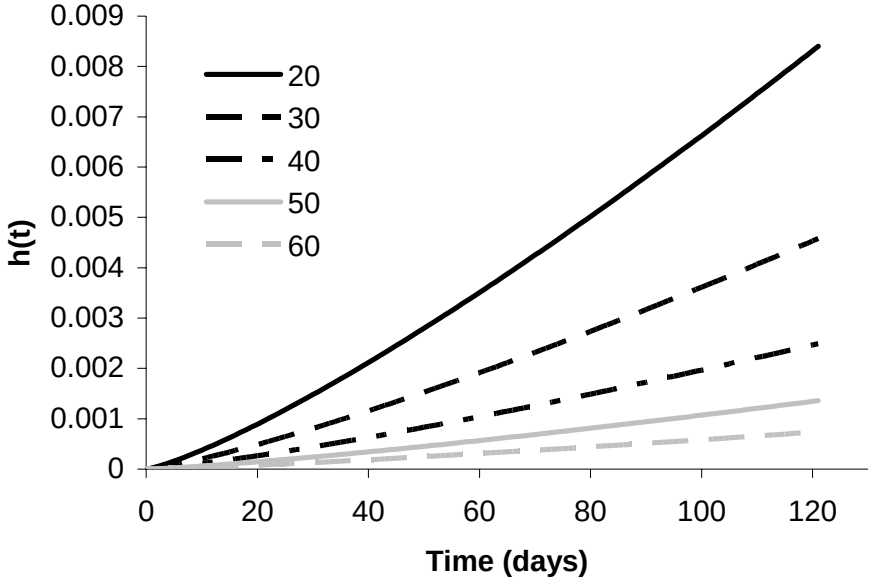


Figure 2.10. Size specific hazard (a, h(t)) and survival (b, S(t)) functions at low temperature (3°C) and low salinity (10) for five size classes.

a)



b)

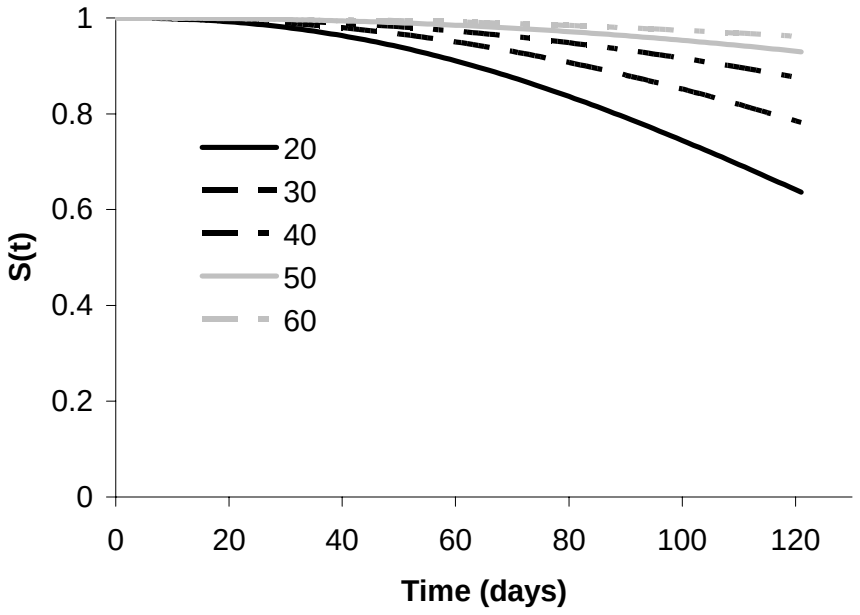


Figure 2.11. Size specific hazard (a, h(t)) and survival (b, S(t)) functions at high temperature (5°C) and high salinity (25) for five size classes.

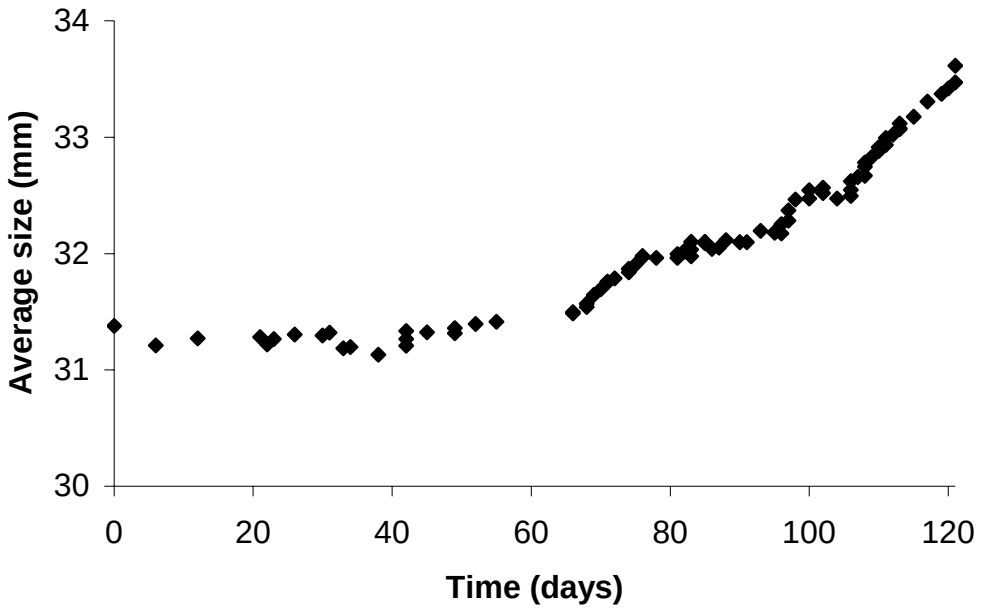


Figure 2.12. Change in the average size (carapace width in mm) of survivors over the duration of the experiment.

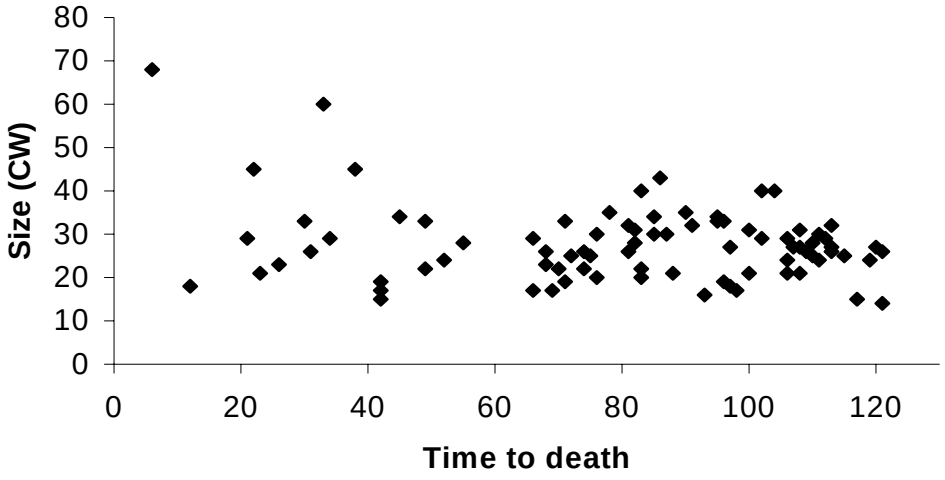


Figure 2.13. Size of blue crabs (carapace width [CW] in mm) by their time to death.

Discussion

Temperature was a significant determinant of overwinter mortality in blue crab in the experiment. The selected temperatures had direct relevance to the ecology of blue crab in Chesapeake Bay. Five degrees is reflective of the average temperature in much of the Chesapeake Bay during a normal winter, but during cold years an average temperature of 3°C is not unusual, especially in northern portions of the Bay (Rome et al. 2005, Chapter 3). Mortality at 3°C was higher than at 5°C, however this effect was most pronounced at low salinity. At high salinity, the survival of crabs in the low temperature exposure was similar to that in the 5°C treatment. In addition, salinity did not appear to have an effect on crab survival in the mild temperature treatment. This suggests that crabs overwintering in tributaries and the mainstem of the upper bay, where temperature and salinity would be lowest, have the greatest risk of death.

Previous studies have also indicated the importance of temperature and salinity on the time to death of blue crabs. Rome et al. (2005) observed higher 30-d mortality among crabs exposed to 1°C than 3°C and increased risk of death at low salinity. However, in a second experiment, the authors found no difference in crab mortality between 3°C and 5°C, which contrasts with my results. The difference may be partially explained by differences in acclimation procedures in the two studies. In Rome et al.'s (2005) experiment, crabs were collected later in the winter and had already experienced harsher conditions prior to the laboratory study. It is likely that they would have already depleted an appreciable amount of their energy stores by the time the experiment began. In contrast, in our study, crabs were brought into the lab earlier to allow time to acclimate to laboratory conditions and minimize disturbance. The experiment began as soon as

ambient water temperature declined to an appropriate level. In addition, crabs were provided ample food until the start of the experiment, although feeding and molting generally ceased below 10°C.

Hazard functions generated by Rome et al. (2005) indicated a high risk of mortality early in their experiment, suggesting that short, extreme cold snaps may be more lethal than continuous low temperatures. In contrast, we observed a hazard rate that increased over time, with most mortality occurring in the second half of the experiment. This would imply that overwintering mortality risk is greater over longer winters. The differences in the shapes of the hazard functions in the two studies may again reflect differences in acclimation periods in the two studies.

Increased lethality at low temperatures may be attributed to a number of causes, including the switch from aerobic to anaerobic metabolism. At low temperature, heart and ventilation rates decrease, the hemocyanin-oxygen affinity becomes so high that delivery to the tissues is impaired, and O_2 is transferred entirely in free form (Mauro and Mangum 1982). Therefore, total oxidative metabolism is reduced which may necessitate hibernation (Mauro and Mangum 1982). Similarly, in another warm-water decapod, the spider crab (*Maja squinado*), the aerobic scope for activity decreases when temperature falls below the lower bound of the optimal temperature range (Frederich and Portner 2000). Frederich and Portner (2000) suggest that oxygen-limited thermal tolerance may in part limit the geographic distribution of marine invertebrates. In addition, the inability of brachyuran crabs to regulate magnesium at low temperatures has been hypothesized as a reason for their exclusion from polar areas (Frederich et al. 2000).

The highest mortality we observed was in the low temperature, low salinity combination. Compared to other portunid crab species, the blue crab is an efficient osmoregulator and has been referred to as a "strong regulator" for its ability to control hemolymph composition regardless of the salinity of the environment (Dorgelo 1981, Pequeux 1995). In Chesapeake Bay, *C. sapidus* maintains its hemolymph hyperosmotic to the external medium at salinities below 25 and isosmotic above 25 (Lynch et al. 1973). Osmoregulation is metabolically expensive due to increased activity of the ATPase transporter (Towle et al. 2001). Towle et al. (1976) demonstrated that the activity of the $Na+/K^{+}$ ATPase in gill microsomes of crabs acclimated to 5 salinity was nearly twice that of crabs acclimated to 34 salinity. Accordingly, blue crab respiration and oxygen consumption increases at low salinity (Engel and Eggert 1974, Laird and Haefner 1976, Guerin and Stickle 1992). Thus, in my experiment, crabs in the high salinity trial would accrue virtually no osmoregulatory costs, while crabs held at low salinity were required to hyperosmoregulate to maintain a proper ionic gradient, and likely would accrue higher metabolic costs. Further, while effects of temperature on osmoregulation are not fully understood (Pequeux 1995), there is evidence that osmoregulation breaks down at low temperatures due to a disruption of the balance between passive and active transport processes (Hochachka 1988). Specifically, the function of ion pumps decline at low temperatures due to temperature sensitivity, which can lead to a decoupling with passive ion channels (Hochachka 1988). The relatively low mortality rate of crabs held at 3°C, 25 salinity compared to 3°C, 10 salinity, would indicate that this breakdown begins to occur in this temperature range.

The sensitivity of both sexes to low temperature and salinity indicates that they both face an increased risk of death in low salinity areas of the bay when the temperature drops to or below 3°C. However, the differential response of males and female blue crabs to the other temperature and salinity combinations cannot be fully explained. There is evidence that mature female crabs are less efficient osmoregulators (Tan and Van Engel 1966); however in my study, juvenile females had an 80% survival rate at 5°C regardless of salinity. However, the extreme temperature fluctuations in the experimental chamber during the last month of the low temperature trial may have also caused the increase in mortality.

In the preliminary experiment (Appendix A), crabs experienced a rapid drop in temperature (1-2°C per day from 20°C) during acclimation that would not be observed in natural conditions. I hypothesized that the subsequent high mortality rate in this preliminary experiment may have been partially induced by an unrealistic temperature decline during acclimation. The importance of acclimation has been noted in other studies. Malloy and Targett (1994) found that at faster rates of temperature decline, summer flounder *Paralichthys dentatus* experienced an increased mortality rate. This indicates that a sudden cold snap that leads to a faster drop in temperature could be detrimental to some organisms. The rate of decline at of the onset of low temperatures can cause death if organisms are unable to accommodate to the lower level (Brett 1956).

The rate of temperature decrease may be important for several reasons. At low temperature, more mitochondria are needed to support the maintenance of sufficient aerobic activity, and sufficient time is needed for mitochondria proliferation (Frederich and Portner 2000). Cooling of water temperature may serve as a cue to switch energy

allocation from growth to storage (Dratnal et al. 1993) or to begin necessary migrations (Conover and Murawski 1982). Mature female blue crabs migrate to the lower bay, and although juveniles do not undergo such a long-range migration, they do move from shallow to deeper areas where the temperature is more stable (Hines et al. 1995). A rapid temperature decline could incapacitate organisms before they could complete migration (Beitinger et al. 2000).

In general, the activity level of crabs during the experiment was low, although individuals at the warmer temperature trial tended to be more active and alert than those at the cooler temperature (personal observation). This apparent state of cold shock was dubbed "chill-coma" by Tagatz (1969). Antennae movement was usually visible in live crabs even when their other appendages were immobile. Often, crabs flipped upside down and became rigid prior to death. Unusual pre-death behaviors have been exhibited by fish under simulated winter conditions, including lethargy, loss of equilibrium, uncoordinated swimming, and protruding eyeballs (Schultz et al. 1998, Hales and Able 2001, Lankford and Targett 2001).

Mortality was size-dependent, with larger juveniles surviving significantly longer than smaller individuals. The accelerated failure model indicates that large juveniles have a high survival rate regardless of winter severity. Survival probability decreases as size decreases. This pattern could result from the lower lipid stores and higher metabolic rate of smaller individuals (Shuter and Post 1990). The relatively high mortality rate in the second half of the experiment in comparison to the first half suggests that starvation may become important as winter progresses. However, no attempt was made to quantify energy use during the experiment. Only two crabs > 45 mm carapace width died and

these deaths occurred early in the experiment and likely were not attributed to starvation. Rome et al. (2005) also observed lower survival of recruits < 15mm than for larger juveniles. Our results indicate that it may be important for crabs to reach a minimum size in order to survive the winter. Thus, crabs spawned later in the fall may not grow to such a minimum size and may be at a disadvantage. Individuals that are spawned and settle early in the summer experience warmer water temperatures, faster growth, and reach a larger size than those settling later in the season (Metcalf et al. 1995). However, the exodus of predators later in the season, coupled with density dependent effects, could favor late settlers (Metcalf et al. 1995). Thus, despite the increased mortality risk, a substantial portion of new recruits likely overwinter at a small size. Early onset of winter would allow crabs less time to grow, thus putting them at greater risk.

In an annual winter dredge survey conducted in Chesapeake Bay, observed mortality is generally higher among larger crabs (Sharov et al. 2003, Rome et al. 2005). Two caveats regarding the relationship between this observation and my experimental results should be noted. The winter dredge mortality estimate is based on dead specimens, and it is unknown whether live and dead crabs are equally vulnerable to the dredge survey. In addition, the crab dredge used in the survey is designed to catch crabs > 15 mm (Sulkin and Miller 1975). Thus field estimates of winter mortality of small crabs are potentially underestimated. However, we do not have parallel experimental data on survival of large, adult crabs under winter conditions with which to compare to the winter dredge data. Thus, a full understanding of the size-dependence of mortality across the entire size range of this species is still lacking.

Contrary to my expectations, the presence of sediment did not significantly improve crab survival, although there was slightly reduced mortality in containers with substrate. We used fewer replications for the non-sediment treatment because we anticipated that most crabs would bury, which would reduce our ability to tell whether they were alive. However, although most crabs buried initially, the percentage of buried crabs decreased over the duration of the experiment, and crabs that did stay buried were usually only partially covered. Clean sand was chosen to minimize chances of the sediment becoming anoxic, but there is evidence that crabs prefer a muddy-sand habitat (Schaffner and Diaz 1988). In addition, Barshaw and Able (1990) observed that blue crabs in the lab buried more readily in mud than in sand. Therefore, in future experiments it would be beneficial to compare sediment types and grain sizes. Although ideally it would be better to use field sediment, the substrate would have to be clean and sterilized to minimize organic content that would increase biological oxygen demand.

Dissolved oxygen levels in the water remained high throughout the experiment, but we were unable to measure oxygen levels in the sediment. Although the water level above the sand was only ~15 cm high and each container had several holes to allow for water circulation, there was no current flowing over the surface that would have increased the replenishment of oxygen to the sediment. Although there were no indications that the sediment became anoxic (e.g., the sand never discolored), it is possible that oxygen levels in the sediment became too low over time. However, in contrast to burrow-dwelling taxa, hypoxia generally does not pose a problem to burying crabs since in most cases they have access to oxygenated water above the sediment

(Bellwood 2002) The buildup of other compounds in the sediment that might discourage burying cannot be ruled out.

It is possible that the burial behavior observed in this experiment can be attributed to conditions in the laboratory that would render burial unnecessary, such as the lack of a water current and predators. Burying behavior is seen mainly as a means of predator avoidance in aquatic crustaceans and blue crabs generally bury shallowly in a horizontal position close to the surface (Barshaw and Able 1990). Whether burying offers protection in high energy or unstable environments is not well known (Bellwood 2002). In warmer months, many portunid crab species remain buried during the day and emerge at night to feed (Bellwood 2002). If light plays any role in cuing burial, then the constant darkness during the experiment could have discouraged typical burial behavior.

In the winter, light is reduced in both intensity and duration. Photoperiod has been demonstrated to influence or control the timing of processes such as reproduction, growth, molting, metabolism, and migration (Demarch 1977, McFarland 1986, Waddy and Aiken 1999, Hamasaki et al. 2004). In the lab, mature female blue crabs have been induced to ovulate and release larvae out of season by manipulating the temperature photoperiod (Zmora et al. 2005). In our 2004 preliminary experiment (Appendix A), blue crabs exhibited poor survival and we speculated that if light did indeed serve as a cue for crabs to reduce their metabolism or store energy for winter, simulating a light regime more similar to their natural environment would improve survivorship. In addition to reduced daylight in the winter, crabs usually reside in deeper channels of the bay where conditions would be dark. Although the effects of a slower temperature decline and light cannot be separated or quantified, the improved survival suggests that at least one of the

factors played a role in improving winter survival under laboratory conditions. Future experiments could be designed to test the effects of varying degrees of light while controlling for the other factors.

While wild-caught crabs exhibited high survival regardless of treatment level, hatchery raised crabs were particularly sensitive to low temperature and salinity. The difference in survival could partially be confounded with the size range of crabs from each source. Many of the crabs from COMB were small compared to those from the Patuxent River (Figure 2.2). However, in the 40 and 50 mm size classes, which were almost equally represented by crabs from each source, a higher percentage of wild-caught than hatchery crabs survived until the end of the experiment (Figure 2.8). This implies that hatchery raised crabs may not be as hardy as field crabs. The results should be interpreted with caution, however, due to the lower genetic diversity of the hatchery raised crabs compared to the wild population. It is possible that the pattern we observed could be result of the condition of one or two broods of crabs rather than hatchery raised crabs in general.

Although our estimates of blue crab winter mortality are a significant improvement on previous efforts (Appendix A), limitations to field application exist. The survival model treats temperature and salinity as continuous variables; however we only tested for two levels of each factor. The assumption may not be valid if temperature and/or salinity exhibit a threshold response on survival. The number of temperatures tested was limited due to laboratory space; future experiments should expose crabs to a wider range of temperature and salinity combinations, and expand the size range to include adult crabs. In addition, our model assumes that winter survival is most

dependent on average temperature. However, temperature variability may influence temperature-dependent survival. In the field, both abiotic and biotic interactions are important, particularly during mild winters. Under warmer conditions, higher metabolic costs often demand active foraging and predation is common (Garvey et al. 2004). Further, disease may contribute to episodes of winter mortality (Messick et al. 1999), as infected crabs may be weakened by parasites and more likely to perish under stressful conditions such as low temperature (Shields 2003).

The mechanisms behind winter mortality in blue crabs and other crustaceans, such as osmoregulatory failure and starvation, warrant further investigation. For example, it is unknown whether the reduced survival at low salinity is a product of osmoregulatory failure or if the higher metabolic costs associated with osmoregulation resulted in faster starvation in those individuals. Further, the mortality rate increased after the first two months and the delayed mortality suggests that depletion of energy reserves may have been more important than a minimum temperature threshold. Foraging patterns in the winter vary among taxa. Consumption is often suppressed with increased winter severity (Fullerton et al. 2000) but many species will feed, albeit to a lesser extent, if resources are available (Johnson and Evans 1991, Pangle et al. 2004). Juvenile Atlantic salmon, *Salmo salar*, suppress their appetite as winter approaches and spend the winter under stones of a stream bed, but as they deplete their reserves will begin to feed again to replenish their stores (Metcalfe and Thorpe 1992). We observed that blue crabs ceased to feed as the water temperature declined. Moreover, the densities of many prey species, such as the clam *Macoma balthica*, may be depleted during the fall and winter (Blundon and Kennedy 1982). However, it cannot be ruled out that they occasionally forage over the

winter. Future experiments should track energy loss and include a treatment where crabs are fed to investigate to what degree mortality is starvation-induced. It is likely that a combination of mechanisms, both biotic and abiotic, regulates blue crab winter survival in the field.

In summary, my study suggests that the winter survival of juvenile blue crabs is dependent upon the combined effects of average water temperature, winter duration, salinity, and size. Additionally, I found a significant hatchery/brood effect. Interannual variability in winter severity may influence blue crab recruitment success and year class strength. All overwintering crabs are assumed to enter the exploitable stock at some point during the following summer or fall (Miller et al. 2005). Thus an occasional severe winter may reduce abundance and catch in the subsequent harvest season. Further, the lower portion of Chesapeake Bay typically experiences a shorter, less severe winter and higher salinity than the upper Bay and tributaries (Chapter 3). Thus winter mortality may vary over space and disproportionately affect individuals in less favorable habitats.

Chapter 3: Spatial and interannual variability in winter mortality of the blue crab (*Callinectes sapidus*) in the Chesapeake Bay

Abstract

The blue crab, *Callinectes sapidus*, is an ecologically- and economically-valuable species in Chesapeake Bay. Field surveys and laboratory experiments indicate that blue crab mortality is significant during severe winters. We applied a temperature- and salinity-dependent survival model to empirical temperature and salinity data to explore spatial and interannual patterns in overwintering mortality. Harmonic regression analysis and geostatistical techniques were used to create spatially-explicit maps of estimated winter duration, average temperature, average salinity, and resulting crab survival probability for the winters of 1990-2004. Predicted survival was highest in the warmer, saline waters of the lower Bay and decreased with increasing latitude up Bay. There was also significant interannual variation in that predicted survival was lowest after the severe winters of 1996 and 2003. Similar patterns of survival were observed in a Bay-wide fisheries independent survey; however our experimentally-derived survival estimates are consistently lower than survival observed in the field. We combine the survival probability maps with maps of blue crab abundance to show how winter mortality may reduce abundance in different parts of the bay and affect estimates of exploitation in the commercial fishery.

Introduction

Factors influencing a species' biogeography are complex and include a suite of environmental variables and dispersal processes that affect individual- and population-level processes (Brown et al. 1996, Zacherl et al. 2003). At temperate latitudes, one such factor is overwintering mortality (Shuter and Post 1990). The importance of winter severity may influence a species' abundance and distribution in several ways (Chapter 2). The ability to survive the winter may limit the range or restrict northern expansion of many aquatic species (Johnson and Evans 1990, Thieltges et al. 2004). As an organism's lower thermal tolerance level is approached, normal functions such as osmoregulation (Hochachka 1988) and oxidative metabolism (Mauro and Mangum 1982) are impaired.

In addition, severe winters have been shown to regulate the interannual abundance of aquatic species within their range (Beukema 1991, Strasser et al. 2001, Thieltges et al. 2004). Smaller rather than larger conspecifics of some fish species may be particularly vulnerable to starvation-induced mortality (Shuter and Post 1990) and this may apply to other organisms. Recruitment rates are often negatively correlated with the winter severity that the young-of-the-year stages of fish experience (Hurst and Conover 1998), and severe winters have been linked to reduced larval production of some invertebrates in successive seasons until the population recovers to its original levels (Strasser and Pieloth 2001, Thieltges et al. 2004). Conversely, in some bivalve species, enhanced recruitment has been observed after severe winters due to reduced epibenthic predation (Beukema et al. 2001, Strasser and Gunther 2001). Effects of winter mortality tend to be most pronounced as the species approaches the extent of its range (Shuter et al. 1980, Thieltges et al. 2004).

Here, we examine the role of winter mortality on the population dynamics of the blue crab, *Callinectes sapidus*, in Chesapeake Bay, a mid-Atlantic estuary. The blue crab is an important benthic predator and scavanger in estuarine ecosystems (Hines et al. 1990, Eggleston et al. 1992) and serves as a link between the benthic and pelagic communities (Baird and Ulanowicz 1989). The species is also economically valuable and supports important commercial and recreational fisheries throughout its range. Historically, Chesapeake Bay has produced the largest share of the total U.S commercial landings. Martell and Miller (in prep.) have assessed abundance trends in the Chesapeake Bay's population of blue crab. These authors indicate that the abundances of pre-recruit and fully recruited crabs varied by three-orders of magnitude and by a factor of four respectively between 1965-2005. Understanding the sources of the observed variability in abundance over time is an important challenge to ensuring the long term sustainability of this species and the regional fisheries it supports. However, modeling the population dynamics of the blue crab in Chesapeake Bay is challenging due to the spatial and temporal variation in blue crab life history, ecology, and distribution (Miller and Smith 2003).

The blue crab exhibits a complex life history (sensu Wilbur 1980). Females spawn in high salinity waters near the mouth of the Bay from June to September (Jones et al. 1990, Tankersley et al. 1998). The zoea are advected offshore into shelf waters, where they molt through seven to eight zoeal stages and one megalopal stage (Costlow and Bookhout 1959). Following the penultimate larval molt, megalopae reinvade the estuary and settle in structurally-complex, shallow nursery areas, including sea grass (Lipcius et al. 1990, Olmi 1995) where they molt into the first juvenile crab stage. As juvenile crabs

grow larger they disperse throughout the estuary to forage and increasingly utilize deeper habitats (Pile et al. 1996). In Chesapeake Bay and other mid-latitude regions, growth and activity ceases over winter as temperature falls below the minimum threshold for growth of about 10-11ºC (Smith 1997, Brylawski and Miller in press). Juvenile crabs often move to deeper channels to overwinter (Hines et al. 1995), although small juveniles (<25mm carapace width (CW)) may continue to utilize vegetated shoal habitats throughout the winter (Orth and van Montfrans 1987). Adult male crabs tend to remain in the upper Bay and tributaries, whereas after mating, mature females migrate to the lower Bay to overwinter. Growth and activity resume in the spring.

Numerous abiotic and biotic factors influence the abundance of crabs at each stage of their life cycle. Initial transport of megalopae into estuaries is believed to be dependent upon southward wind events and tidal currents (Epifanio 1995, Epifanio and Garvine 2001) and may be influenced by the passage of tropical cyclones (Etherington and Eggleston 2000). The survival of new settlers is affected by their size and habitat type (Pile et al. 1996), as juvenile crabs are susceptible to predation by larger conspecifics (Dittel et al. 1995, Hines and Ruiz 1995) and predatory fish including sciaenid and moronid species (J. von Montfrans, Virginia Institute of Marine Science, pers. comm.). Density-dependent mortality of recruits can have a substantial impact on adult stock size the following year (Pile et al. 1996)

Juvenile survival during their first winter may further influence the subsequent entry of blue crabs into the fishable stock. Blue crab winter mortality can be expected to vary due to interannual variation in temperature and secondarily salinity. The results of a fishery-independent survey for blue crab in Chesapeake Bay have highlighted the

potential importance of winter mortality in blue crab. A fisheries-independent winter dredge survey has been conducted annually since 1990 to estimate Bay-wide blue crab abundance, describe the size and sex composition of the population, and estimate exploitation and fishing mortality rates (Sharov et al. 2003). In most years, observed mortality is low, but it has been found to be significant during occasional severe winters (Sharov et al. 2003, Rome et al. 2005). For example, Sharov et al. (2003) estimated that the overall winter mortality rate was 11.9% during the winter of 1996, but was as high as 56.5% of crabs > 120 mm CW. High mortality was again observed in 2003 following another unusually harsh winter (Rome et al. 2005). Similar patterns of reduced blue crab survival after cold winters have been observed in other mid-Atlantic estuaries. The Delaware Bay experiences reductions in blue crab abundance and depressed landings after severe winters (Kahn and Helser 2005). Dredge samples taken from the Delaware Bay during the late winter of 1996 found 47% mortality, with mature females experiencing differentially high rates of mortality (Kahn and Helser 2005). Heavy overwintering mortality in 2003 reduced biomass to the extent that, when combined with commercial harvest, the calculated spawning stock biomass index was zero for the fifth time since 1978 (Kahn 2003, Kahn and Helser 2005).

In addition to interannual variation, winter mortality may exhibit significant spatial variation within a single year. This variation can range from large scale differences among populations across a latitudinal gradient (Fullerton et al. 2000), to small-scale patterns, such as differential mortality of bivalves based on their position in an intertidal zone (Strasser et al. 2001). Within estuaries, both temperature and salinity characteristics may influence the preferred habitat for overwintering and mortality

patterns. For example, Hurst and Conover (2002) demonstrated how the extent and location of optimal winter habitat for striped bass *Morone saxatilis* in the Hudson River estuary varied from year to year dependent upon the salinity structure of the estuary.

Previously (Chapter 2), I demonstrated that survival of juvenile blue crabs is dependent upon temperature, salinity, and size. Crabs were least tolerant of low temperature at low salinity, and survival significantly increased with increasing temperature, salinity, and crab size. Here, I combine the survival model generated in Chapter 2 with empirical data on bottom winter temperatures and salinities measured in Chesapeake Bay to generate spatially-explicit estimates of winter survival in the blue crab. These spatially-explicit estimates are then combined with maps of blue crab abundance and distribution (Jensen and Miller 2005) to estimate how overwintering mortality varies spatially and interannually. To assess the precision and accuracy of model predictions, I compare estimates of predicted survival probability with observed survival estimates in the field.

Methods

Temperature and salinity modeling

To model the duration and magnitude of winter severity for the Chesapeake Bay, I generated maps of average winter bottom temperature and salinity and duration for the years 1990-2004. Temperature and salinity data were obtained from the Chesapeake Bay Program Water Quality database.[3] The Chesapeake Bay Program, established after the 1983 Chesapeake Bay Agreement, is a cooperative effort between federal, state, and

[3] Available online at http://www.chesapeakebay.net/wquality.htm

local agencies to direct restoration efforts in the Chesapeake Bay (CBP 1993). Its Water Quality Monitoring Program has been conducted since June 1984 and monitors 19 water quality variables at stations located throughout the bay. Bottom water temperature is measured with an in-situ thermister and bottom salinity is either measured directly using a Hydrolab Surveyor II, or computed later from conductivity and water temperature when using a CTD (CBP 1993). I used data from all stations in the mainstem of the Bay and adjacent tributaries. The number of stations varied slightly from year to year but ranged from 119 to 150. The distribution of the stations was generally even across the bay (see Figure 3.1 for example). Data were available for all months for most of the stations, although a few stations were only sampled once or twice over the winter months.

For each year, I generated a harmonic regression model of average Bay temperature from November 1 – April 30, which included all recorded temperatures from all stations (see Figure 3.2a,b for example). Harmonic or sinusoidal models have been used previously to describe periodic and seasonal patterns in environmental parameters (Miller et al. 1995, Bloomfield 2000). I used least-squares techniques (REG Procedure, SAS v8.2 SAS Institute, Inc., Cary, North Carolina) to provide estimates of the equation:

(1) $\text{Temperature} = \beta_1 \sin(\omega) + \beta_2 \cos(\omega) + \beta_3(X) + \beta_4(Y)$,

where ω is $(2\pi t_i/365)$, t_i is the day of winter from Nov. 1 (i=1-181 or 182 during a leap year), and X and Y are the coordinates of each station (Universal Transverse Mercator (UTM) zone 18). Variables that were not significantly different from zero at the α=0.05 level were removed from the model. The assumption of homogeneity of variances among treatments was verified by visual inspection of residual plots. The assumption of

normally distributed error was checked with a Shapiro-Wilk's test and visual inspection of the normal probability plot (Proc Univariate, SAS).

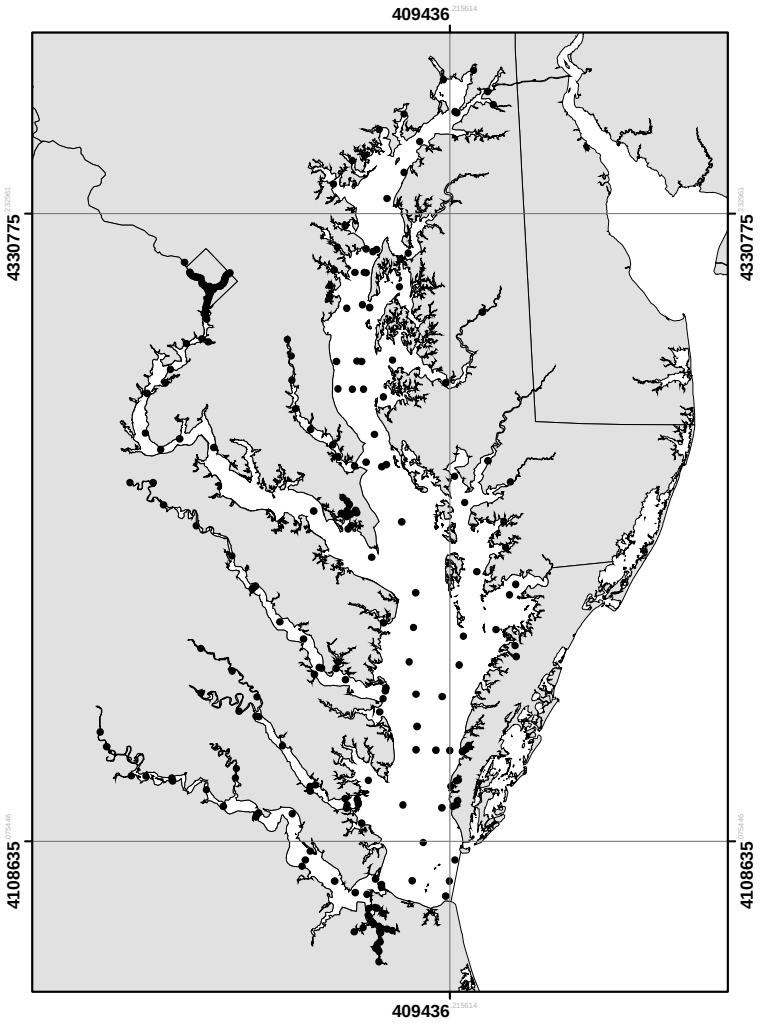


Figure 3.1. Stations sampled by the Chesapeake Bay Program's Water Quality Monitoring Program from November 2002 to April 2003. The map is projected in Universal Transverse Mercator (UTM) coordinates.

Based on the overall regression, I calculated the daily temperature history for each station. First, I generated a daily temperature curve for individual stations by substituting station specific X and Y coordinates into Equation 1. For each station, I then calculated the average difference between the observed and modeled temperatures over the six-month time period,

$$\text{(2)} \qquad \text{Average residual} = \frac{\sum_{n} T_{\text{observed}} - T_{\text{predicted}}}{n},$$

where n is the number of observed temperatures recorded at that station. To account for this deviation, I adjusted each curve by adding the average residual to the predicted daily temperature (Figure 3.2c). I defined the duration of winter as the number of days where the modeled temperature was below 10ºC, which is comparable to the minimum temperature required for blue crab growth (Figure 3.2d; T_{min} – Smith 1997, Brylawski and Miller in press). Average winter temperature was then defined as the average modeled temperature during the period below the T_{min}.

Unlike temperature, there was no clear trend in winter salinity; therefore, salinity was not modeled using harmonic regression techniques. Average salinity for each station was calculated from the bottom salinity values recorded during the defined winter period. Thus, for each CBP station, I developed an estimate of winter duration in days, average winter temperature, and average winter salinity.

Next I used a raster based map of Chesapeake Bay, with a 1 km^2 resolution, to develop predictions of the winter temperature cycle for any location within the Bay. I used kriging, a geostatistical procedure, to predict duration, average temperature, and average salinity for all possible locations within the Chesapeake Bay for each winter.

In this approach, the spatial covariation among all possible sample points (variogram) is used to develop an estimate of the temperature and salinity at an unsampled location based on appropriate weightings of observed values at neighboring sites. I checked for trends due to spatial position using a regression with backwards elimination (α=0.05) to fit a first order trend with interactions (Proc Reg, SAS v8.2). If the trend was significant, variogram modeling and kriging were performed on the residuals.

Robust empirical variograms (Cressie 1993) were calculated in SAS (Proc Variogram, SAS v8.2). Gaussian, spherical, and exponential variogram models were fit to the empirical variograms using non-linear least squares (Proc Nlin, SAS v8.2) and the best fitting model (lowest mean squared error) was used for kriging, except in cases where the model failed to converge or resulted in unrealistic parameters. Subsequently, ordinary kriging was conducted (Proc Krige2d, SAS v8.2) to make predictions over a 1 km grid scale, and the trend was added back to the kriged predications. Interpolated winter duration in days, average bottom temperature, and average bottom salinity were mapped in ArcView v8.1 (ESRI Corp., Redlands, California).

Survival prediction

To calculate survival probability, the mapped winter environmental parameters were combined with a survival model generated from a laboratory experiment (Chapter 2). Briefly, juvenile blue crabs, ranging from 14 to 68 mm in size, were exposed to a combination of one of two temperature (3ºC, 5ºC) and salinity

(10, 25) treatments for four months (121 days), typical of average wintertime conditions and length of winter in Chesapeake Bay. The survival data were modeled using an accelerated failure model with a Weibull distribution (Proc Lifereg, SAS v8.2) to estimate hazard and survival functions. The survival probability at time t is a function of temperature, salinity and size, given by

$$(3)\ S(t) = \exp\left\{-t^{\lambda}\exp\left[-\lambda(3.59 + 0.10(\text{Temperature}) + 0.02(\text{Salinity}) + 0.03(\text{Size})\right]\right\},$$

where t is the winter duration in days and λ is a rate parameter. For each winter from 1990-2004, the survival probability of crabs in each 1 km x 1 km cell in Chesapeake Bay was calculated by inputting the equation and estimates for temperature, salinity, and winter duration into the raster calculator in ArcView v8.1. Predictions were made for average crab size used in the experiment (31mm).

The geostatistical model described above predicts the survival probability of crabs in any location within the Chesapeake Bay. These predictions were compared with field estimates of mortality from the Winter Dredge Survey (WDS). The survey uses a stratified random design (Sharov et al. 2003). At each station a 1-min tow of a 1.83m wide Virginia crab dredge is taken at a speed of 5.4 $km.h^{-1}$ (Sharov et al. 2003). Since 1996, high density sites are resampled in the Maryland portion of the Bay by Maryland Department of Natural Resources to estimate over-wintering mortality. Mortality is estimated as the percent of dead crabs from the resampled stations. For 1996 to 2004 I estimated the correlation (Proc Corr) between predicted average survival from my approach and field survival estimates (Source: G. Davis, Maryland Department of Natural Resources).

I quantified the impact of the predicted mortality on population abundance by multiplying the location-specific estimates of survival with parallel location specific estimates of abundance developed by Jensen and Miller (2005) for years 1990-2002. Map cell densities were transformed to cell-specific abundance by multiplying the density by the cell area (1 km^2). The local abundance estimates were summed across all mapped cells to estimate adjusted Baywide abundance. Although no formal methods are yet available to calculate uncertainty for this technique (M. Christman, University of Florida, personal communication), I calculated 95% confidence intervals for the adjusted abundance estimates for each year using a bootstrapped standard error (Haddon 2001). In the bootstrap, one hundred estimates of the total annual abundance for the year were calculated as the sum over all stations of the station abundance multiplied by a probability of survival was drawn randomly from the distribution of survival probabilities for that year.

The new abundance estimates (N) were combined with catch (C) data (Miller et al. 2005) to calculate new exploitation fraction (U) estimates:

(4) $U = C/N$.

Because original exploitation fraction estimates reported in Miller et al. (2005) are based on abundance from traditional design-based approaches rather than the geostatistical approach, I also calculated exploitation fraction based on original geostatistical abundance for comparison.

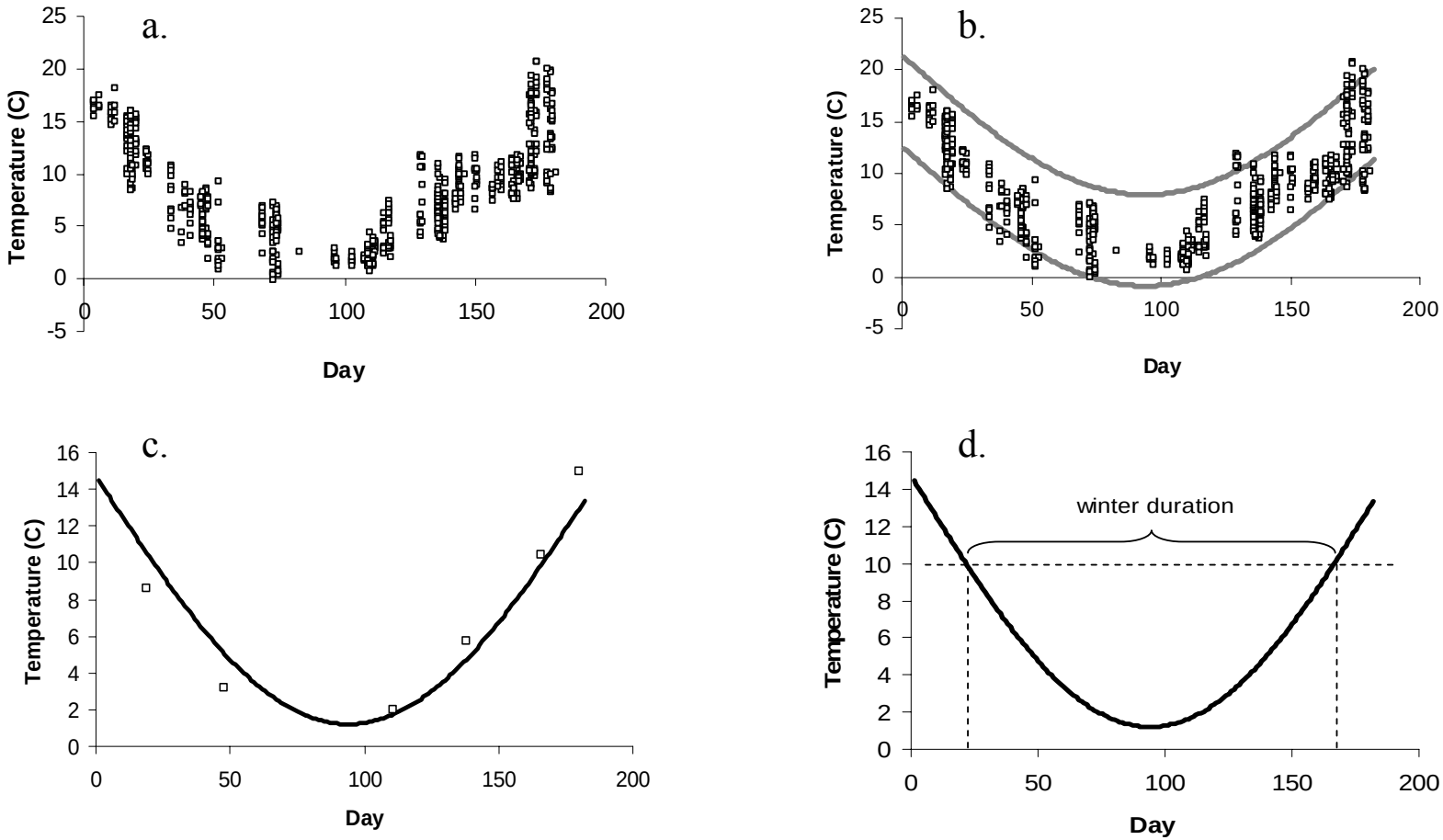


Figure 3.2. Example of how harmonic regression technique was used to estimate winter duration and temperature at each station from the Chesapeake Bay Program data set. Observed temperatures from all stations from Nov. 1, 2003 to April 30, 2004 (a) were included in the harmonic regression model in Equation 1 to generate a range of daily temperature profiles (b; upper and lower limits only) dependent on the X and Y coordinates of each station. The average deviation of each station from the model (Equation 2) was added to the predicted daily temperature to create each station specific profile (c; □ observed, – predicted). The winter duration for each station was defined as the number of days that the modeled temperature was below 10°C (d).

Results

Harmonic regression was able to accurately model patterns in winter temperature (Table 3.1). The sine parameter of the harmonic regression was significant every year, while the cosine parameter was significant in all but three years. When both parameters were significant, there was always a stronger sine than cosine trend, as indicated by the coefficient estimates. In addition, there was a significant north-south (UTM Y) trend in all years, and a significant east-west (UTM X) trend in 12 out of 15 years. The adjusted R^2, a measure of model fit to the observed data, was high (>0.7) in all years.

Table 3.1. Summary statistics and parameter estimates of the seasonal pattern of average Chesapeake Bay temperature from November 1- April 30 for years 1990-2004. Parameters were estimated from Equation 1 in SAS v8.2. NS indicates a non-significant variable at the alpha= 0.05 level; all other variables are significant. Parameter estimates for UTM X and UTM Y are E-06. df= degrees of freedom; prob= probability, Adj= adjusted.

Year	df	F value	prob>F	Intercept	Sin(w)	Cos(w)	UTM X	UTM Y	Adj. R^2
1990	879	541.9	<0.0001	65.0	-11.2	-0.75	-18.1	-10.3	0.71
1991	848	605.7	<0.0001	63.7	-9.1	-0.32	-8.24	-10.9	0.74
1992	850	567.4	<0.0001	62.5	-8.9	0.27	-12.3	-10.4	0.73
1993	827	555.5	<0.0001	53.4	-9.0	0.66	-8.94	-8.78	0.73
1994	770	10001.4	<0.0001	49.9	-15.8	0.76	-8.72	-6.88	0.84
1995	776	881.2	<0.0001	56.0	-13.0	1.03	-9.54	-8.45	0.82
1996	697	909.7	<0.0001	58.4	-11.8	NS	-10.1	-9.78	0.80
1997	736	545.8	<0.0001	54.1	-9.7	-1.02	-6.22	-8.96	0.75
1998	750	440.3	<0.0001	53.9	-9.0	-1.12	-5.72	-8.94	0.70
1999	731	472.8	<0.0001	61.7	-10.3	0.87	-9.39	-10.2	0.72
2000	823	1093.3	<0.0001	55.0	-11.5	NS	-11.0	-8.33	0.80
2001	812	878.3	<0.0001	60.5	-12.8	-0.32	-13.8	-9.46	0.81
2002	796	627.1	<0.0001	77.4	-11.5	-0.34	-9.48	-13.4	0.76
2003	526	581.8	<0.0001	60.6	-12.4	NS	-6.42	-10.4	0.77
2004	751	673.6	<0.0001	62.5	-12.9	0.60	-10.5	-10.2	0.78

First order spatial trends in winter duration, average temperature, and average salinity were significant ($p<0.05$) in all years. Variogram models for winter duration, average temperature, and average salinity are summarized in Tables 3.2-3.4. No single variogram model was appropriate for all years. Spherical models were chosen in most years for winter duration, while for average temperature and salinity, Gaussian variogram models provided a better fit most years.

Table 3.2. Parameters for variogram models of winter duration, as estimated in SAS v8.2. N= number of stations. The range is the maximum distance (meters) at which correlation is observed. The sill, which is the sum of the partial sill and the nugget, represents large-scale variability in the observations, while the nugget represents variability below the sampling resolution or measurement error.

Year	N	Model	Nugget	Partial sill	Range (km)
1990	124	Spherical	5.78	75.98	50,464
1991	124	Spherical	10.71	84.23	39,560
1992	124	Spherical	17.18	120.98	71,999
1993	122	Exponential	12.93	107.23	24,529
1994	122	Spherical	15.07	54.32	48,666
1995	120	Spherical	12.79	97.34	73,352
1996	125	Gaussian	15.59	62.53	32,177
1997	125	Spherical	20.84	121.04	95,879
1998	126	Gaussian	9.95	87.38	13,886
1999	126	Spherical	23.75	77.23	24,468
2000	134	Gaussian	36.24	68.14	21,428
2001	133	Exponential	10.79	43.49	14,030
2002	135	Spherical	17.90	72.97	34,841
2003	125	Gaussian	20.98	35.48	34,695
2004	126	Exponential	2.57	37.76	11,810

Table 3.3. Parameters for variogram models of average winter temperature as estimated in SAS v8.2. N= number of stations. The range is the maximum distance (meters) at which correlation is observed. The sill, which is the sum of the partial sill and the nugget, represents large-scale variability in the observations, while the nugget represents variability below the sampling resolution or measurement error.

Year	N	Model	Nugget	Partial sill	Range (km)
1990	124	Gaussian	0.048	0.23	24,352
1991	124	Gaussian	0.019	0.15	14,346
1992	124	Gaussian	0.061	0.22	34,638
1993	122	Exponential	0.051	0.23	24,456
1994	122	Gaussian	0.048	0.30	16,086
1995	120	Gaussian	0.099	0.34	35,695
1996	125	Gaussian	0.060	0.22	32,679
1997	125	Gaussian	0.055	0.26	34,390
1998	126	Exponential	0.018	0.18	13,339
1999	126	Gaussian	0.029	0.19	11,295
2000	134	Exponential	0.056	0.20	13,107
2001	133	Exponential	0.056	0.17	12,828
2002	135	Gaussian	0.037	0.17	11,986
2003	125	Exponential	0.041	0.14	18,669
2004	126	Gaussian	0.039	0.14	15,335

Table 3.4. Parameters for variogram models of average winter salinity as estimated in SAS v8.2. N= number of stations. The range is the maximum distance (meters) at which correlation is observed. The sill, which is the sum of the partial sill and the nugget, represents large-scale variability in the observations, while the nugget represents variability below the sampling resolution or measurement error.

Year	N	Model	Nugget	Partial sill	Range (km)
1990	128	Gaussian	2.69	23.3	29,362
1991	128	Spherical	0.48	19.9	50,057
1992	128	Gaussian	1.35	29.4	35,293
1993	128	Gaussian	1.98	22.0	31,199
1994	132	Gaussian	1.82	22.2	31,143
1995	129	Gaussian	2.20	27.4	32,968
1996	126	Gaussian	2.80	23.1	28,371
1997	126	Spherical	0.79	20.6	53,123
1998	131	Gaussian	7.71	20.8	30,739
1999	134	Gaussian	1.43	24.6	31,941
2000	146	Gaussian	2.10	28.0	32,651
2001	147	Gaussian	0.77	26.0	33,473
2002	144	Gaussian	0.91	25.9	36,707
2003	142	Spherical	0.19	22.9	61,602
2004	138	Gaussian	2.22	23.2	21,795

Winter duration varied spatially and among years. Winter duration was several weeks shorter in the lower tributaries and mainstem near the Bay mouth than in the upper Bay (Figure 3.3). Similarly, average temperature increased with latitude, and in most years the difference between the minimum and maximum average temperature ranged between 3 to 4°C (Figure 3.4). Winter severity and duration varied between years (Figures 3.3 & 3.4; Table 3.5). Average Bay-wide winter duration varied from 109 (1996) to 150 (2002) days and average Bay-wide winter temperatures varied from 4.4 (1996) to 7.0 (1991, 2002) °C over the time series. The shortest, warmest winter in the time series occurred in 2002, while 1996 was characterized by the longest winter duration and coldest average temperature.

Generally, cold winters also tended to last longer; however, duration was not always predictive of average winter temperature. For example, 1993 was characterized by the second longest winter duration in the time series, but was mild in terms of average temperature. Unusually long, cold winters appear to occur every 3 to 4 years. Additionally, winter temperature decreased and duration increased with latitude.

Salinity varied spatially and values predictably decreased with distance from the Bay mouth. Average salinity ranged from ~30 at the mouth of the Bay to zero at the head of the Bay and upper tributaries (Figure 3.5). Small interannual variation in salinity was also present, although to a lesser extent than the variability in temperature (Figure 3.5, Table 3.5). The lowest and highest average winter salinity occurred in 1997 and 2002, respectively. In most years, average Baywide salinity was about 15, but was higher in years of dry years and lower in wet years. Winters with above average salinity tended to be characterized by lower streamflow from the Susquehanna River, whereas winters with lower salinity were characterized by higher streamflow (streamflow data from USGS, www.usgs.gov). In some years, such as 2002, mild temperatures coincided with high salinities, creating favorable winter conditions for blue crabs.

Predicted survival reflected spatial and interannual variations in winter severity. Survival probability was highest in the lower Bay, and decreased with increasing latitude up Bay (Figure 3.6). Predicted survival was highest in 2002 and lowest in 1996, with several years of intermediate survival rates (Figure 3.6, Table 3.5). There was a significant positive correlation between laboratory-derived survival probabilities and field estimates (Figure 3.7, $\rho = 0.7$, $p = 0.04$). The two years of

lowest predicted survival, 1996 and 2003, were also winters when poor survival was observed in the field. However, in all years the laboratory-derived estimates of survival were exceeded by the field estimates (Figure 3.7).

Table 3.5. Chesapeake Bay-wide means and ranges of winter duration, average wintertime temperature and salinity, and survival probability for 1990-2004. Mean values for each winter were calculated by averaging all of the cells in the Bay-wide map grids in Figures 3.3-3.6.

Year	Winter duration	Average temperature	Average salinity	Survival probability
1990	133 (86 – 150)	5.7 (4.7 – 8.1)	15.4 (-0.8 – 31.2)	0.52 (0.13 – 0.80)
1991	123 (87 – 158)	6.9 (5.2 – 8.4)	15.4 (-0.6 – 32.0)	0.64 (0.11 – 0.88)
1992	132 (74 – 165)	6.6 (5.2 – 8.8)	19.0 (-0.3 – 33.2)	0.63 (0.18 – 0.84)
1993	145 (113 – 172)	6.0 (4.5 – 7.4)	15.6 (-0.6 – 31.0)	0.48 (0.06 – 0.75)
1994	126 (105 – 144)	4.5 (3.0 – 6.1)	15.1 (-0.4 – 31.4)	0.46 (0.08 – 0.73)
1995	120 (71 – 139)	5.8 (4.6 – 8.5)	17.3 (-0.6 – 33.3)	0.62 (0.22 – 0.85)
1996	150 (121 – 170)	4.4 (3.3 – 6.2)	15.3 (-0.5 – 32.0)	0.33 (0.02 – 0.72)
1997	135 (98 – 160)	6.1 (4.9 – 7.9)	12.4 (-0.5 – 29.9)	0.48 (0.09 – 0.82)
1998	132 (103 – 166)	6.5 (4.8 – 7.8)	15.2 (-0.7 – 30.4)	0.57 (0.19 – 0.84)
1999	122 (88 – 148)	6.6 (5.0 – 8.1)	17.7 (-0.2 – 30.6)	0.66 (0.23 – 0.87)
2000	124 (96 – 147)	6.1 (4.6 – 7.6)	17.8 (-0.3 – 32.4)	0.62 (0.26 – 0.83)
2001	134 (90 – 157)	5.0 (3.6 – 7.6)	18.2 (-0.1 – 30.6)	0.50 (0.12 – 0.75)
2002	109 (57 – 137)	6.9 (5.3 – 9.1)	19.8 (-0.3 – 33.5)	0.75 (0.27 – 0.95)
2003	141 (124 – 159)	4.7 (3.6 – 5.8)	16.7 (-0.2 – 30.8)	0.43 (0.04 – 0.75)
2004	132 (110 – 160)	5.1 (3.7 – 6.5)	14.3 (-1.1 – 31.5)	0.46 (0.07 – 0.78)

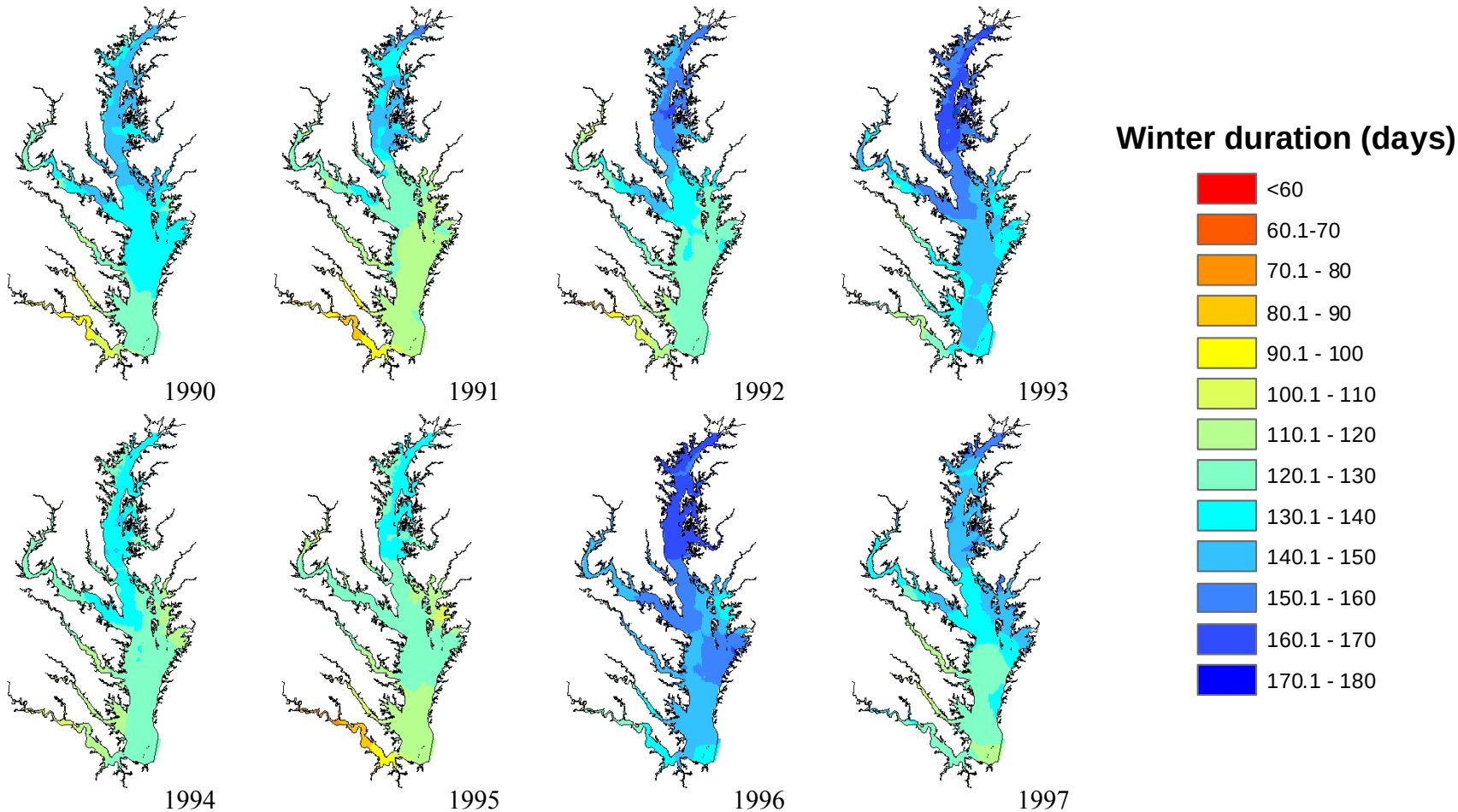


Figure 3.3a. Maps of length of winter duration (days) in Chesapeake Bay for years 1990 to 1997 based on interpolated values from sinusoidal regression of Chesapeake Bay Program water quality data. The year corresponds to the year in which the winter ended.

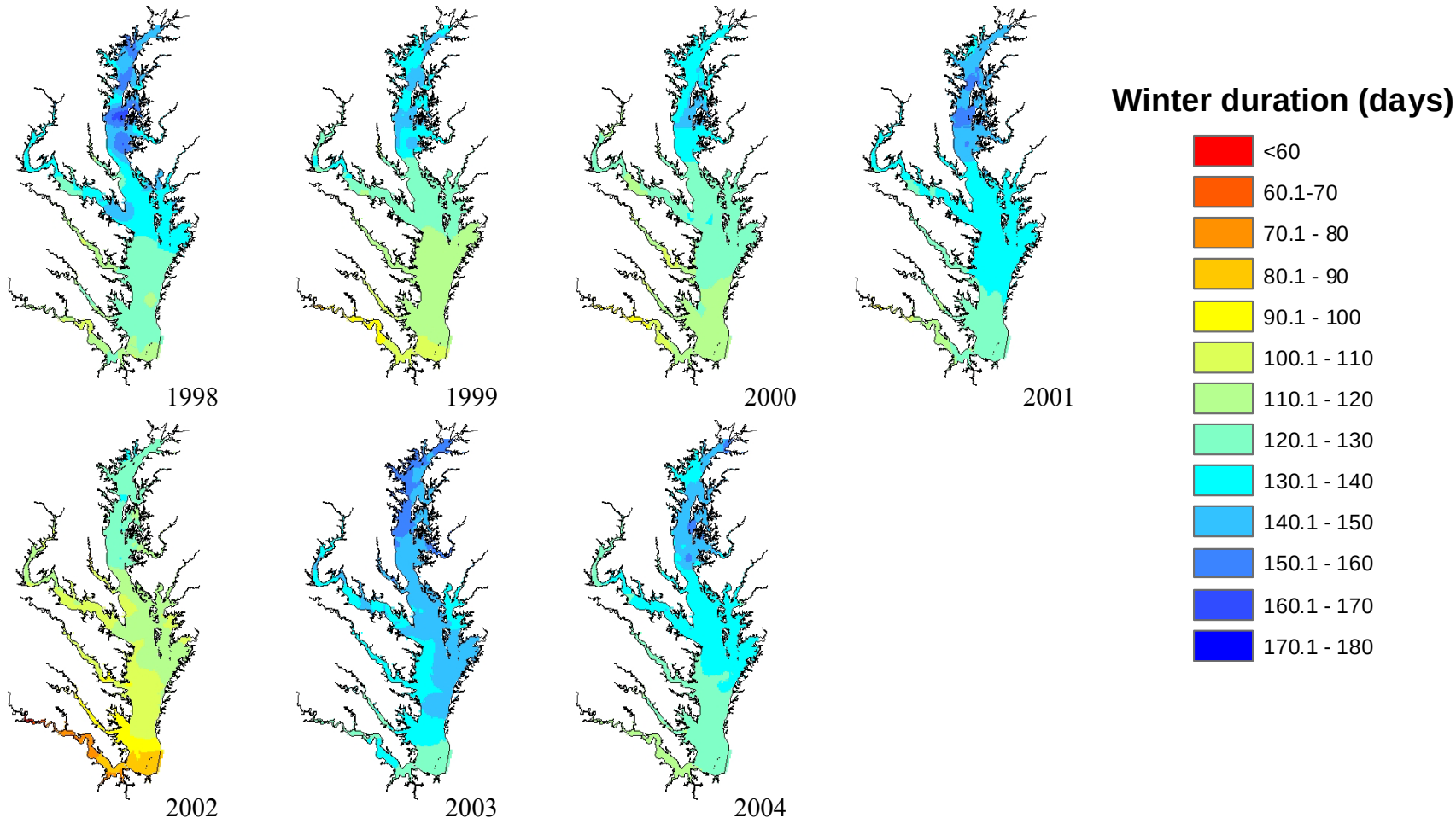


Figure 3.3b. Maps of length of winter duration (days) in Chesapeake Bay for years 1998 to 2004 based on interpolated values from sinusoidal regression of Chesapeake Bay Program water quality data. The year corresponds to the year in which the winter ended.

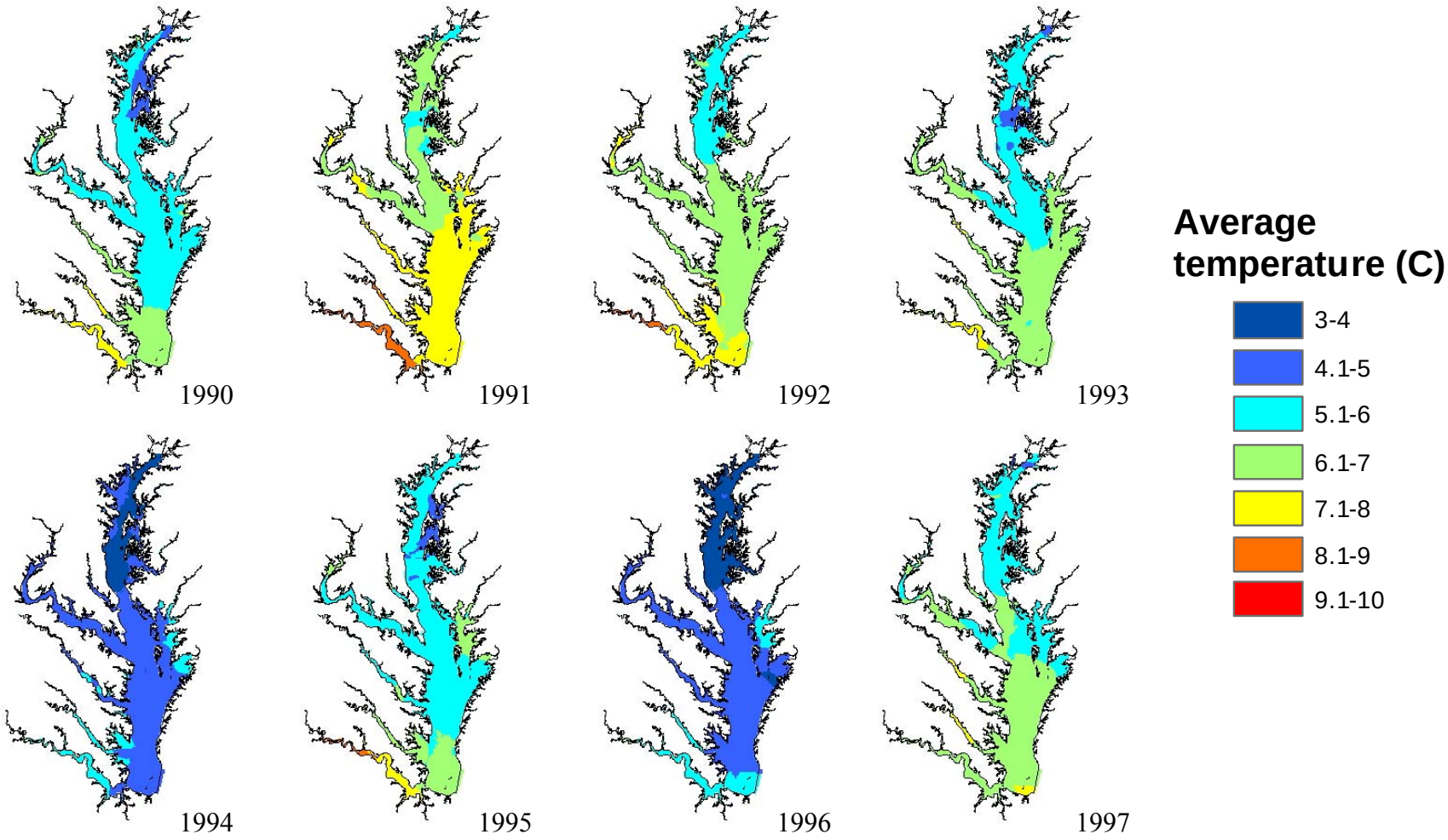


Figure 3.4a. Maps of average wintertime bottom water temperature (°C) in the Chesapeake Bay for years 1990 to 1997 based on interpolated values from sinusoidal regression of Chesapeake Bay Program water quality data.

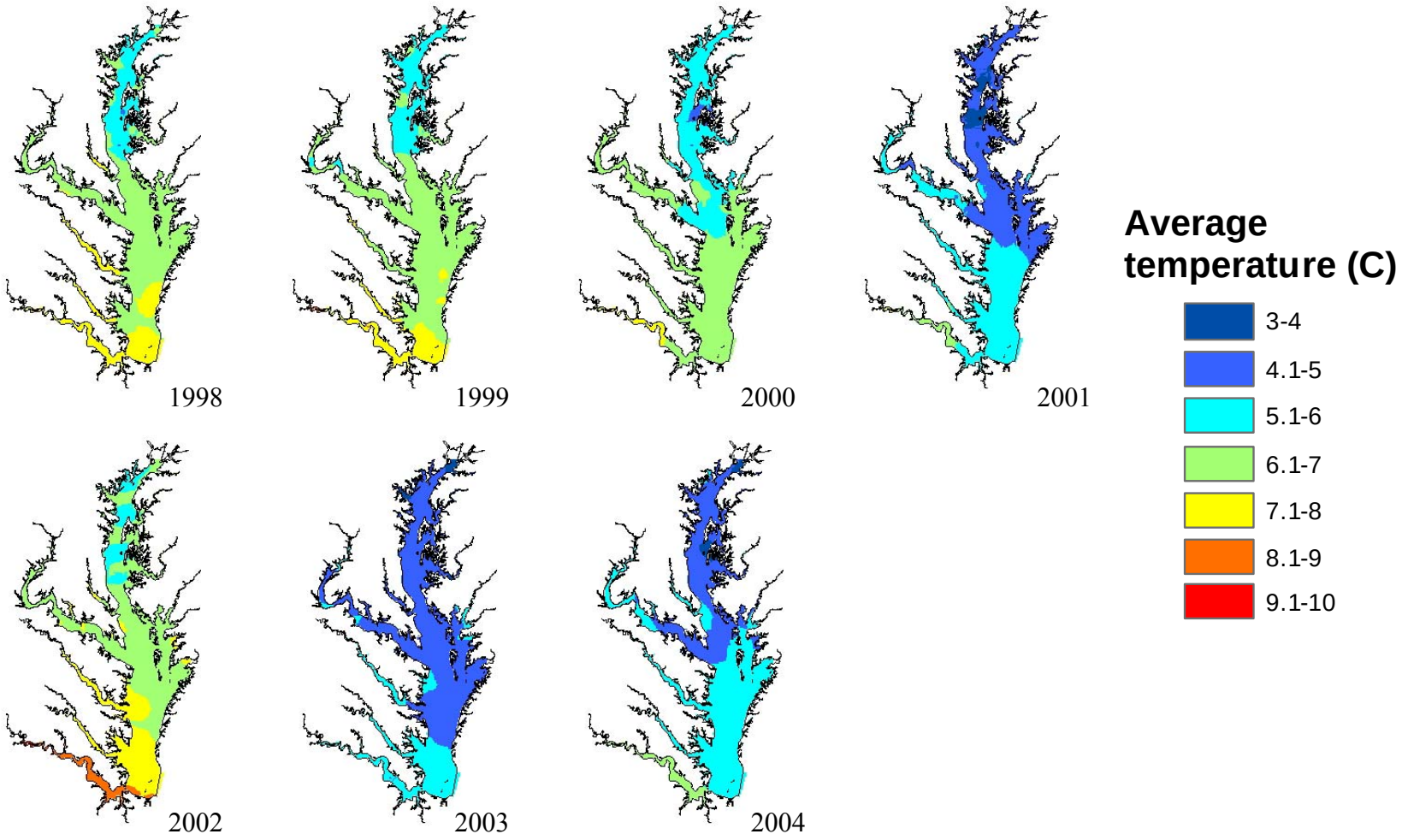


Figure 3.4b. Maps of average wintertime bottom water temperature (°C) in Chesapeake Bay for years 1998 to 2004 based on interpolated values from sinusoidal regression of Chesapeake Bay Program water quality data.

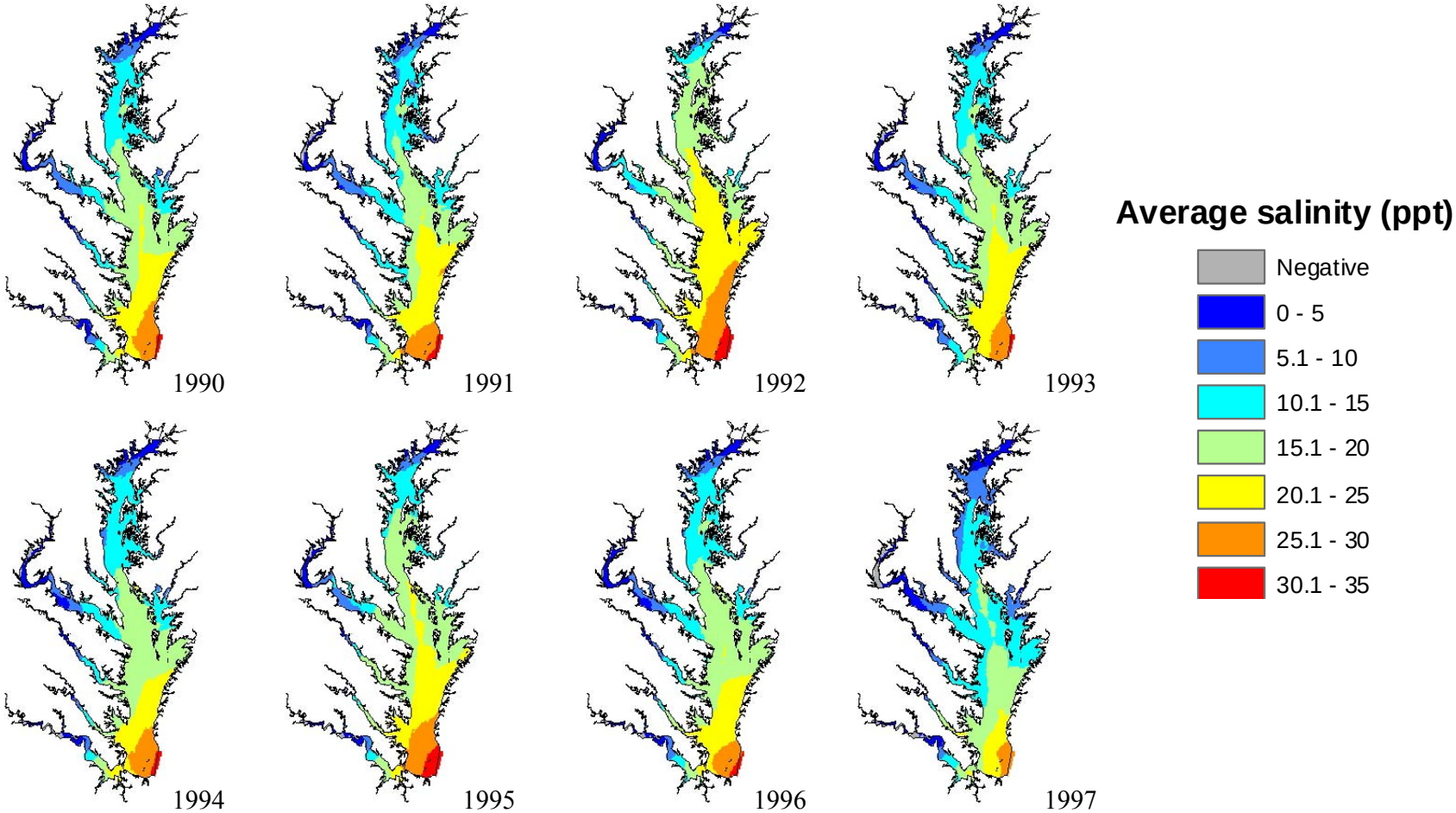


Figure 3.5a. Maps of average wintertime bottom salinity in Chesapeake Bay from 1990 to 1997 based on interpolated values from sinusoidal regression of Chesapeake Bay Program water quality data.

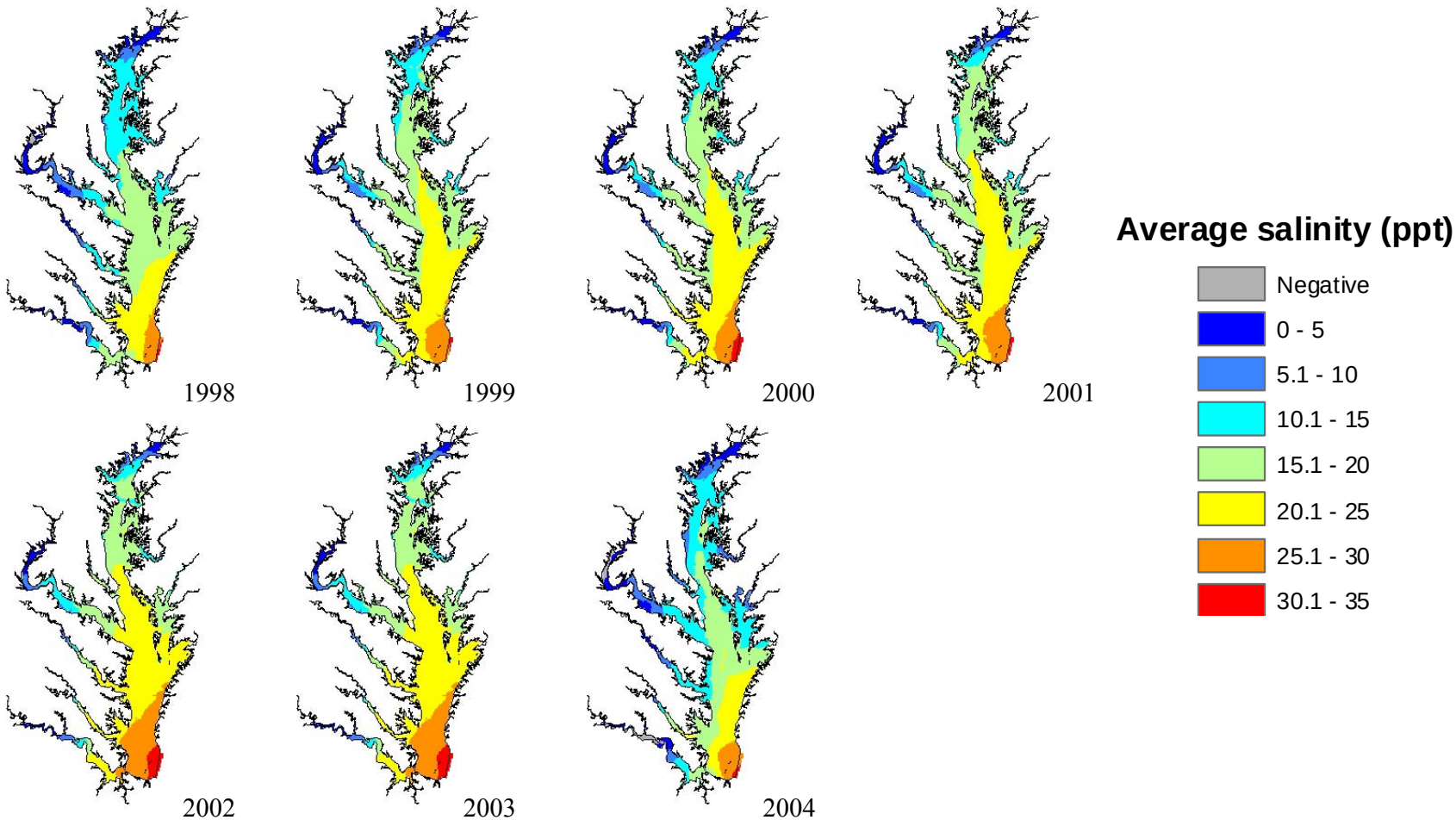


Figure 3.5b. Maps of average wintertime bottom salinity in Chesapeake Bay from 1998 to 2004 based on interpolated values from sinusoidal regression of Chesapeake Bay Program water quality data.

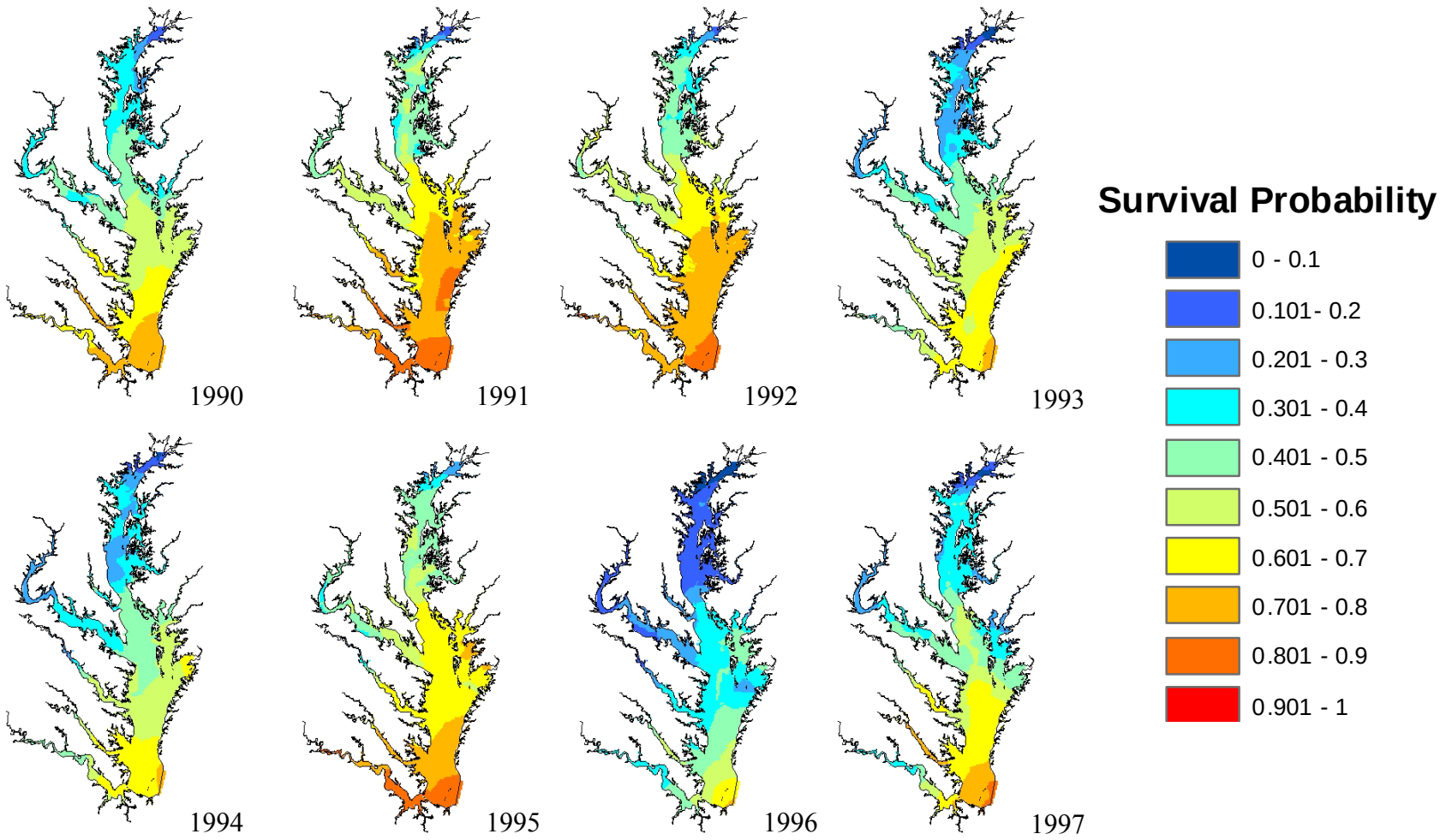


Figure 3.6a. Maps of blue crab winter survival probability in Chesapeake Bay from 1990 to 1997.

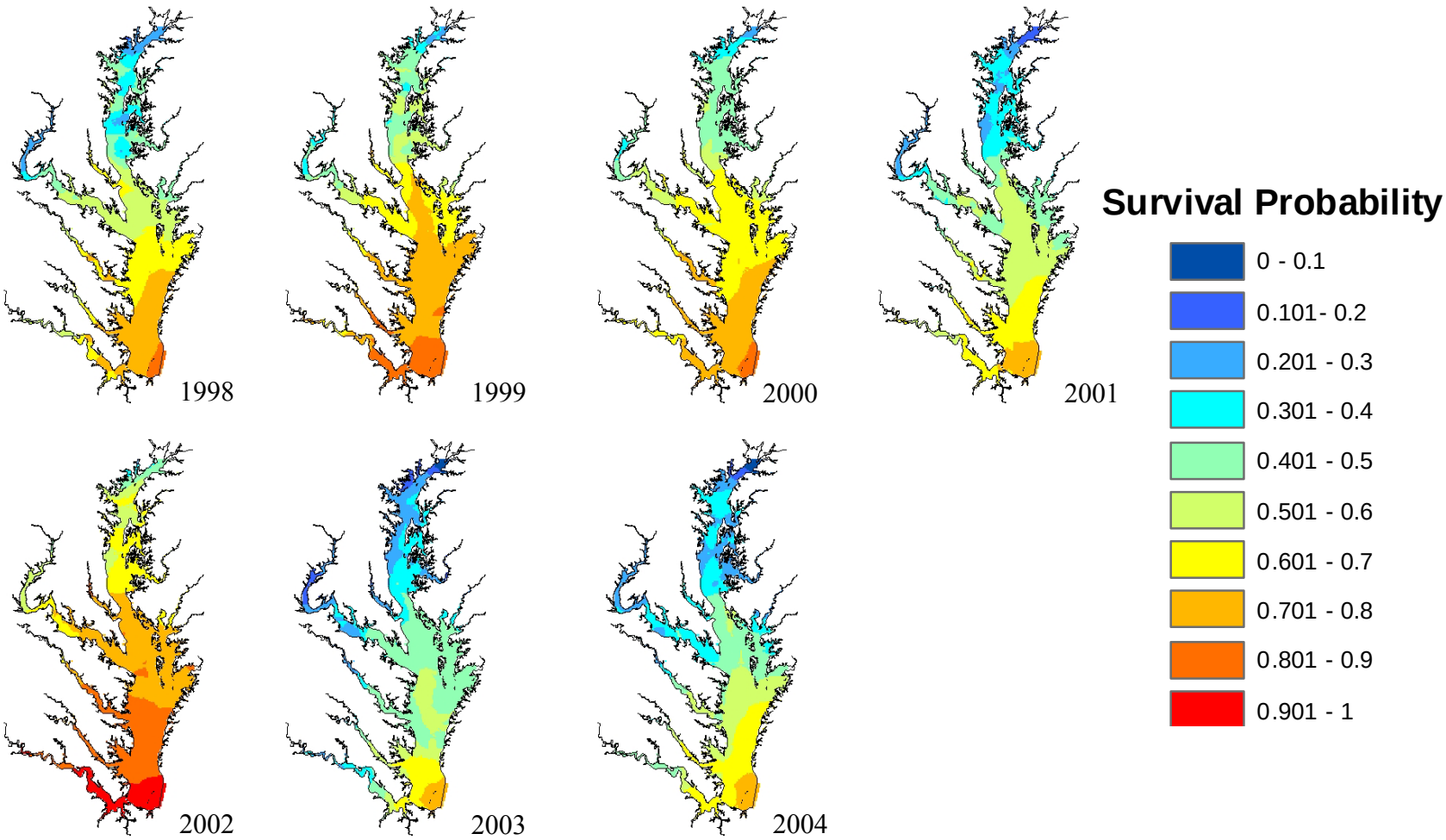


Figure 3.6b. Maps of blue crab winter survival probability in Chesapeake Bay from 1998 to 2004.

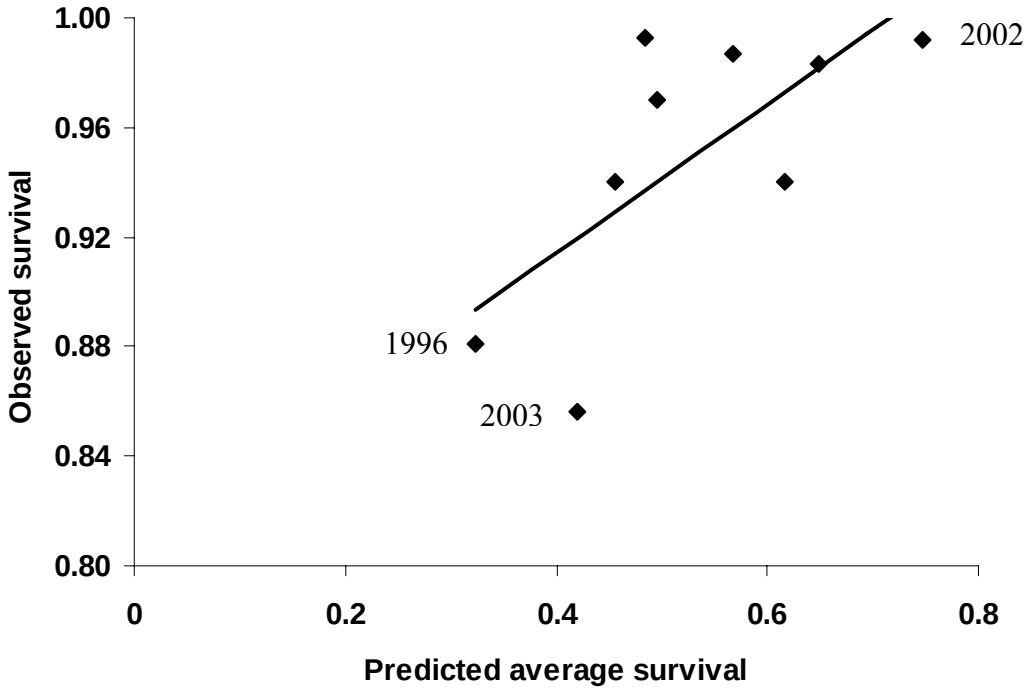


Figure 3.7. Relationship between Bay-wide average experimentally derived survival predictions and average survival observed in March by Maryland Department of Natural Resources (source: G.Davis, MDDNR). Each data point represents one year and included data from 1996-2004.

Adjusting for predicted winter survival reduced original geostatistical abundance estimates on average by 45% from 1990 to 2002, ranging from a reduction of 22% in 2002 to 70% in 1996 (Figure 3.8a). In particular, the new time series predicts much lower abundance in the early-mid 1990s than the original estimates. New exploitation fraction estimates, calculated using the adjusted abundance, exceeded original estimates based on geostatistical abundance every year (Figure 3.8b). The new estimates also exceeded original exploitation fraction using design based abundance each year with the exception of 1991. This likely occurred because,

in 1991, the geostatistical abundance estimate was much higher than the traditional design-based approach (Jensen and Miller 2005).

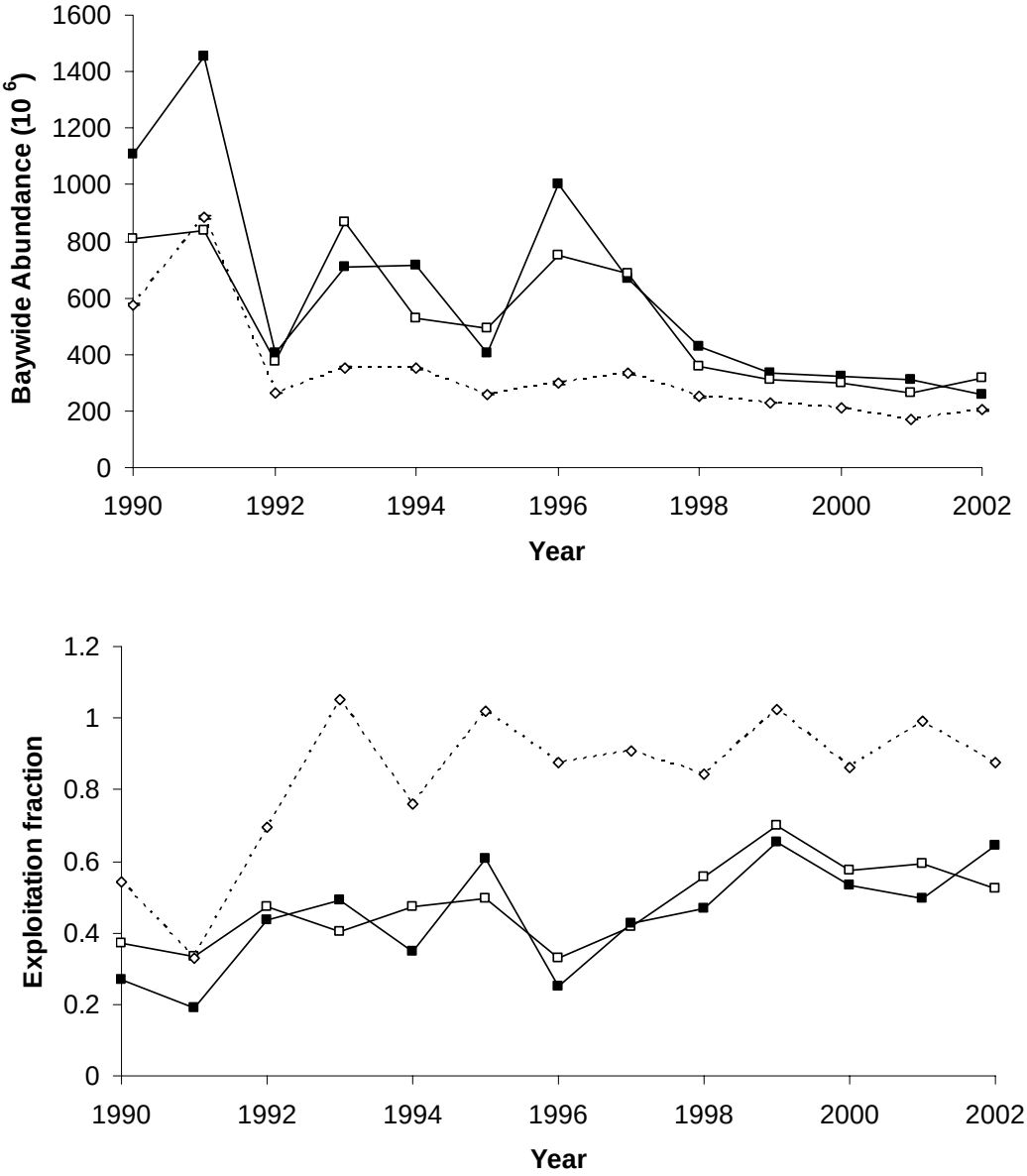


Figure 3.8. a) Time series of original design-based abundance (solid line, open squares, source: L. Fegley and G. Davis, MDDNR), original geostatistical blue crab abundance (solid line, closed squares, source: Jensen and Miller 2005) and adjusted abundance accounting for predicted winter survival (dashed line) from 1990-2002. b) Time series of original calculated exploitation fraction using designed-based abundance (solid line, open squares, source for catch data: Miller et al. 2005), geostatistical abundance (solid line, closed squares), and new exploitation fraction values using adjusted abundance estimates (dashed line).

Discussion

Previous work demonstrated that juvenile blue crabs are sensitive to low temperature and salinity combinations (Chapter 2). Here, I demonstrate that winter severity and corresponding levels of winter mortality vary spatially and interannually. Indices of winter severity should consider duration in addition to magnitude as blue crab mortality increases over time (Chapter 2). The survival function incorporates both measures to estimate survival probability.

Incorporating GIS derived maps of wintertime environmental conditions into survivorship functions is a powerful tool to assess spatial variability in winter habitat suitability and survival probability within individual years. In all years, the highest survival probabilities occurred in the warmer, more saline waters of the lower Bay. The lowest survival probabilities occurred in the upper mainstem and tributaries, approaching zero in some years. Blue crab winter distribution may be influenced by this difference in habitat suitability. In a generalized additive model, temperature and salinity were frequently found to be significant factors in determining the wintertime distribution of mature female crabs in the Chesapeake Bay (Jensen et al. 2005). Accordingly, the northernmost section of the Bay and upper tributaries are often characterized by low abundance or high variability in abundance (Jensen and Miller 2005). Further, observed mortality also tends to be highest in the colder, fresher areas of the bay (Rome et al. 2005). In contrast, highest average density occurs in the lower tributaries and eastern shore embayments, where environmental conditions are more favorable and corresponding survival probability is higher. Indeed, the winter distribution of blue crabs, as measured by the latitude of the centroid, has generally

moved southward as the population abundance has declined since the early 1990s (Jensen and Miller 2005). Thus, more crabs would appear to be overwintering in more preferable habitat during years of low abundance, and to be more dispersed throughout the Bay when abundance is high. Consequently, two years with similar temperature, salinity, and survival probability patterns may still differ in the level of absolute mortality depending on crab distribution that year. This finding demonstrates the importance of incorporating spatially explicit information into models of blue crab winter mortality.

In addition to spatial variability, the model predicted considerable interannual variation in blue crab winter survival. The two most severe winters, 1996 and 2003, resulted in the lowest predicted Bay-wide survival, while survival was highest in the two mildest winters, 1991 and 2002. Often, survival conditions were unfavorable for two consecutive years. For example, a long winter in 1993 was followed by a subsequent winter that was shorter but colder. Consequently, predicted blue crab survival was similar between years. Two consecutive years of unfavorable conditions occurred again in 1996, the coldest year in the time series, and 1997, which was characterized by the lowest salinity. Successive severe winters in 2003 and 2004 similarly resulted in low survival probability in both years.

An estimate of the number of crabs available to the fishery is one of the most sought after pieces of information in blue crab management (Miller et al. 2005). Winter mortality can reduce the brood stock and decouple recruitment of juveniles into the fishery from the spawning stock abundances. Most crabs that settle in year i will become vulnerable to the fishery at some point during year i+1 (Miller et al.

2005, Puckett et al. 2005). Crabs that overwinter as large juveniles may recruit to the fishery the following summer, while smaller juveniles may not recruit to the fishery until the following fall (B. Puckett, CBL, personal communication.). However, below-normal temperatures may disrupt the timing of recruitment to the fishery (Brylawski and Miller in press). Specifically, when comparing a cold (1996) vs. warm year (1998), Brylawski and Miller (in press) estimated a 10% shift in timing of entrance of crabs to the fishery. Therefore, a severe winter has the potential to influence exploitable crab abundance for the entire following year. There is often a disconnect between age-0 abundance in one year and age-1+ abundance the following winter. For example, a high number of recruits were observed by the survey in 1996 and 1997, but this did not translate into high age 1+ crabs the following year (Sharov et al. 2003). My results suggest that overwintering mortality of juveniles, in coincidence with other factors, could contribute to this decoupling.

As predicted by our results, high numbers of dead crabs were observed by Maryland Department of Natural Resources during March resampling in 1996 and 2003 (Figure 3.7). However, compared to the field estimates, the experimentally-derived mortality estimates are considerably higher in all years. In particular, in mild years very little mortality is observed in the Winter Dredge Survey, while even in 2002 our model still predicted mortality greater than 50% in the region where sites were resampled. Several factors could explain the elevated mortality values in the laboratory. Although care was taken to simulate realistic conditions to the greatest extent possible, inherent differences between captivity and the wild environment may have contributed to the differences we observed. Sediment was provided to a portion

of the crabs tested, but use of the substrate declined over the course of the experiment, and the presence of sediment did not significantly improve survival (Chapter 2). Crabs of both wild and hatchery origin were included in the experiment, and hatchery raised crabs experienced significantly lower survival than wild crabs (Chapter 2). Hence, the model reflects patterns that were largely driven by crabs of hatchery origin, which likely contributed to the lower survival probabilities.

Size-dependent patterns in mortality may also contribute to the lack of accord between predicted and observed survival rates. One potential caveat is the application of our model based upon juvenile crabs to the entire range of size classes. Although size was found to be a significant predictor of survival among juveniles (Chapter 2), size was essentially ignored in estimating survival probabilities in Chesapeake Bay and adjusted abundance. While this falsely assumes similar survival patterns across size classes, reliable survival functions are not available for adult crabs. In addition, the age-0 size class dominates the crab stock during the winter. The average size crab in the experiment (31mm) was similar to the average size of age-0 crabs observed in the Winter Dredge Survey (Sharov et al. 2003).

The Winter Dredge Survey survival data exhibit a size-dependent pattern that is opposite what we observed in the laboratory experiment. In the field, mortality increases with size and is most pronounced among adult crabs, while I found survival to improve with increasing size (Chapter 2). There may be several explanations for this contradictory pattern. First, the center of abundance for juveniles tends to be farther south than adults (G. Davis, MD Department of Natural Resources, personal communication), which would place them in a more favorable habitat. Second, as

crabs were not fed during the experiment, the elevated mortality levels I observed could partially be attributed to energy depletion. In the lab, feeding generally ceased during the acclimation period when water temperature fell below 10°C, but if crabs in fact continue to forage to a certain extent during the winter, starvation effects may not be as significant in the wild. Lankford and Targett (2001) suggest that while larger individuals may be less vulnerable to starvation-induced stress than small conspecifics, they may be more susceptible to acute thermal stress.

Even if larger crabs are more sensitive to low temperature, there may still be a size-dependent bias that accounts for differences between laboratory and field results among juveniles. Field estimates of survival are based on the number of dead specimens found and assume that live and dead crabs are equally vulnerable to the gear. However, the catchability of dead crabs compared to live crabs is unknown. Additionally, resampling in March may underestimate mortality as temperature data indicates that in some years winter conditions may persist into April. Thus additional crabs die after the resampling effort. Further, the abundance of the smallest recruits may be underestimated, as they are not fully vulnerable to the sampling gear and often inhabit shallow habitats where the survey cannot sample (Miller et al. 2005). It would be useful to expand the size range of crabs in the experiments and to validate laboratory results with field experiments at multiple locations and over several winters. Lastly, the field resampling effort is concentrated in the Maryland portion of the Chesapeake Bay (see Rome et al. 2005). While my results indicate that this is where crabs are most at risk of death, I also demonstrate the importance of a latitudinal gradient in temperature and salinity on winter survival. Mortality in one

section of the Bay may not necessarily be reflective of overall patterns. In the future, it would be useful to expand the field sampling to include additional stations in the lower Bay.

The adjusted abundance based on the experimentally derived survival estimates would suggest that abundance of crabs that survive winter has been overestimated in every year (Figure 3.8). In particular, several years with high numbers of recruits in the mid-1990s would have been particularly affected by low survival. This would result in an exploitation fraction of 1 or above in many years, which is unrealistic. Although it is desirable to adjust abundance based on estimated survival probabilities, several simplifications and assumptions limit the application of my model and interpretation of the results. First, the model is based on an experiment where crabs were exposed to limited levels of constant temperature and salinity. Temperature and salinity were treated as continuous variables in the survival model; however this assumption may not be valid if either factor exhibits a threshold response on survival. Due to the limited levels of temperature and salinity that I tested, in applying the model to the field I was required to extrapolate beyond the scope of the data in some instances. Testing for additional levels of temperature and salinity in future experiments would improve the precision and reliability of the model. In addition, in applying the model to observed environmental data, I assumed that average temperature is the important predictor of survival. However, we know that temperature varies on a seasonal, weekly, and diurnal pattern. There is evidence in other taxa that the rate of temperature change (Malloy and Targett 1994) impacts survival.

Seasonal salinity patterns were more difficult to model because it does not behave in a predictable sinusoidal pattern like temperature, but using average salinity ignores the effect of storm events and tidal cycles. While the experiment was conducted for four months, winter can last longer than this time period in northern sections of the Bay (Figure 3.3), so I was required to extrapolate beyond the scope of the data.

Wintertime conditions may have additional indirect effects on blue crab survival and population dynamics that are difficult to quantify under laboratory conditions. In addition to protracted stress, long winters may delay the start of growth, reproduction, and spawning in the subsequent season. Further, the degree of winter severity may influence biotic interactions. Our model predicts higher survival with increasing temperature; however, warmer temperatures generally require more active foraging to meet increased metabolic demands, which leads to a higher predation risk (Garvey et al. 2004). The potential significance of biotic factors on winter mortality is difficult to quantify and warrants further investigation.

Despite the limitations, I believe the model captures the general patterns of the effects of winter severity on blue crab survival. My results support field observations of higher mortality during severe winters and in regions of low temperature and salinity. In Chesapeake Bay, there has been increasing concern regarding a decline in catches, spawning stock biomass, and recruitment (Lipcius and Stockhausen 2002). Currently, the Chesapeake Bay blue crab stock is at low abundance and has experienced overfishing in the late 1990's and early 2000's (Miller et al. 2005). In a recent stock assessment, Miller et al. (2005) revised biological reference points

designed to manage and conserve the stock. Assuming a natural mortality (M) of 0.9, the recommended threshold ($\mu_{10\%}$) and target ($\mu_{20\%}$) exploitation reference points are 0.53 and 0.45 respectively. The exploitation fraction is dependent upon reported commercial catch and abundance estimates from the Winter Dredge Survey. Thus, to avoid overfishing, it is important to accurately estimate stock size to ensure that the threshold limit is not exceeded. During years of high winter mortality it may be necessary to adjust abundance estimates or target exploitation rates downward in order to protect a larger portion of the stock. Ignoring the proportion of crabs that do not survive the winter may result in overestimates of crab abundance and underestimates of the exploitation rate, which would lead to misconceptions as to how the fishery is impacting the blue crab stock.

The sensitivity of blue crabs to low temperature and salinity indicates that winter mortality may in part limit the current distribution of blue crab populations. Although blue crabs are occasionally found as far north as Nova Scotia, year-round populations only exist from Massachusetts southward (Williams 1984). The predicted warming of water temperatures stemming from global climate change has several potential impacts on the ecology and distribution of aquatic organisms (Kennedy 1990, Scavia et al. 2002). Generally, it is expected that the range of cool and warmwater species will shift northward as winters conditions become more favorable and the growing season lengthens in these habitats (Shuter and Post 1990, Najjar et al. 2000). Thus, in addition to a longer growing and reproductive season and milder winters in the Chesapeake Bay, these changes may allow *C. sapidus* to establish resident populations in northern latitudes.

Chapter 4: Summary

Due to the ecological and economic importance of the blue crab in the Chesapeake Bay, it is necessary to understand the processes that control their population dynamics. Over its range, the blue crab (*Callinectes sapidus*) is exposed to a wide range of environmental conditions that can potentially cause latitudinal variations in their life history. For example, in mid-Atlantic regions, growth ceases during the wintertime and crabs assume a semi-dormant state. In both Chesapeake and Delaware Bays, there have been numerous reports by watermen and scientists of high crab mortality during harsh winters. Thus, overwintering mortality may play an important role in regulating species abundance and distribution. The goal of my thesis was to determine what factors control blue crab winter mortality and then to incorporate these findings into a predictive framework to infer what types of mortality patterns we would expect to see in the Chesapeake Bay.

In Chapter 2, I constructed a temperature, salinity, and size-dependent survival model of juvenile blue crabs. The 121-day experiment was designed as a 2x2x2 factorial to test for the effects of temperature (3°C, 5°C), salinity (10, 25) and sediment (sediment, no sediment) on crab survival. The risk of mortality significantly increased with decreasing temperature, salinity, and crab size. Additionally, in designing the experiment I incorporated lessons learned from a preliminary trial to provide crabs with a more realistic overwintering environment, including a longer acclimation time and reduced light levels. The improved survival in the 2005 experiment compared to 2004 (Appendix 1) appears to verify the importance of simulating natural conditions to the greatest extent possible.

Contrary to my expectations, sediment did not significantly improve survival. It would be useful to explore this further to determine why habitat utilization declined and experiment with a range of sediment types. Another intriguing finding was the poor survival of crabs of hatchery origin compared to wild crabs. Whether this was a result of a low genetic diversity among the hatchery crabs or a sign that hatchery raised crabs may not be as hardy as their wild counterparts is unknown. Throughout the experimental work, I came to appreciate the complexity of the factors that regulate mortality processes. Although temperature and salinity may be the primary determinants of winter mortality, the results are not always straightforward. Additional factors that potentially affect winter mortality in the field are depth, storm events, disease, and biotic interactions.

Understanding the mechanisms behind the patterns we observed in the lab is an avenue for future research. In particular, due to the delayed onset of mortality and size dependent mortality patterns, I question the role of starvation in regulating winter mortality. Feeding and activity decreases with temperature and was negligible by the time the experiment began; however it cannot be ruled out that crabs forage occasionally over the winter to make up for depleted energy reserves as has been observed in other taxa. Even so, it is unknown whether the extent at which crabs feed in the wild would compensate for energy loss over unusually long winters. There is also a need to understand the physiological processes that break down as the lethal temperature limit is approached and for what length of time sub-optimal conditions can be tolerated. These may include osmoregulation, metabolic respiration, and magnesium regulation, among others. Frederich and Portner (2000) highlighted the

importance of upper and lower "pejus" temperature zones that are within critical temperatures for survival but outside the optimal range of performance. They suggested that the temperature range allowing the maximum performance and aerobic scope for activity may be more important in determining a species range than critical temperatures themselves. Regardless of which temperature is more important, the expected warming of seawater temperatures from global climate change is likely to result in milder winters for blue crabs Chesapeake Bay and other estuaries along the eastern seaboard. Although it is expected that the range of cool and warmwater species will shift northward in response to warmer conditions (Shuter and Post 1990), the interactions of changes in temperature, salinity and production in marine and estuarine systems are complex (Kennedy 1990). A shift in temperature is likely to uncouple the relationship between temperature and photoperiod (Lawrence and Soame 2004), and if food availability does not increase to the same extent as temperature to meet increased physiological demands, winter survival could become impaired (Sogard and Olla 2000).

In Chapter 3, I applied the survival model to wintertime bottom temperature and salinity data in the Chesapeake Bay to predict how blue crab survival varies over space and from year to year. Specifically, I created maps of winter duration and average wintertime temperature and salinity, which were incorporated into the survival equation. The lower Bay experiences a shorter, less severe winter than the upper Bay, as average temperature and salinity decrease and duration of winter increases with increasing latitude. The corresponding predicted survival patterns reflect this variation in habitat suitability, as predicted survival was lowest in the

upper mainstem and tributaries. The ultimate goal was to then determine how these predicted losses from winter mortality would adjust estimates of abundance from the Winter Dredge Survey.

Although the magnitude of my experimentally derived mortality estimates are likely too high, our findings demonstrate the need to account for spatially-explicit and interannual differences in winter severity into blue crab management efforts. Further, our methods provide a framework that can be built upon and modified in the future. Refined survival models can be constructed by testing a wider array of size classes at additional levels of temperature, and salinity. Our methods for modeling wintertime environmental conditions can be applied to water quality data in any given winter as it is regularly collected in the Chesapeake Bay. This approach could also be applied to other mid-Atlantic systems. Naturally, such simplistic modeling is not without its caveats. We ignore the importance of temperature and salinity variability and possible biotic interactions such as predation and disease. Future research questions could explore the importance of these factors. Lastly, it is important to continue to collect field data on crab mortality each winter, and to perhaps expand such efforts to include field experiments to verify the patterns we see in the lab.

Appendix A: Details of 2004 Experiment

Introduction

In 2004, a preliminary laboratory experiment was conducted to test temperature-dependent effects on crab survival.

Methods

Experimental Design

Juvenile crabs ranging from 11 to 42 mm in size were obtained from the blue crab hatchery at the Center of Marine Biotechnology (COMB) in January 2004. At the hatchery, crabs were reared at 20 degrees C and salinity 25. Once brought to CBL, crabs were acclimated to ambient salinity from the Patuxent River gradually by decreasing the salinity by 3-4 /day. Following the period of adjustment to salinity, the crabs were separated into individual containers in flow through tanks and randomly assigned to one of three temperature treatments: 1 C, 5 C, and ambient temperature. Temperature was lowered approximately 1-2 degrees per day to the treatment level. Crabs were fed trout pellets ad lib during the acclimation period, although they generally stopped feeding when the water temperature dropped below 13 degrees.

Initially, treatment water temperature was maintained through the use of chillers that cooled incoming water, however this was not sufficient to sustain the treatment temperatures. Therefore, the remainder of the 1 and 5 degree trials were completed in controlled temperature rooms. Crabs were checked daily for mortality to obtain survival times for each individual. Temperature and salinity were measured and fifty percent of the water was changed every day to ensure water quality.

The duration of the trials varied among treatments. The 1-degree trial was terminated at 58 days, at which time only one crab remained alive. The 5-degree trial was terminated at 96 days due to technical problems with the temperature control room that prevented constant temperature from being maintained. However, at this time only 5 crabs remained alive, and I determined that prolonging the duration of the experiment would not significantly change the survival analysis parameters. The variable treatment, which lasted for 76 days, was suspended when the incoming water temperature rose above 10 degrees.

Analysis

Survival analysis was used to quantify the pattern of mortality during the experiment. Details and background on survival analysis can be found in Chapter 2. An accelerated failure model was fit to the survival data with temperature as a continuous covariate. The model was fit with all supported distributions (exponential, Weibull, lognormal, gamma, log-logistic). The appropriate model was selected using the Akaike's information criterion statistic, log-likelihood ratio tests, and graphical diagnostics.

Results indicated that the Weibull function was the most appropriate baseline mortality function. Although the generalized gamma had a slightly lower AIC statistic, the likelihood ratio test between the Weibull and the generalized gamma indicated that the Weibull model could not be rejected ($0.10<p<0.05$). Additionally, the Weibull model is computationally simpler to use and examination of the graphical diagnostics indicated that the Weibull model best fit the data.

The Weibull distribution is a power function, indicating that the risk of death increases over time:

(7) $$h(t, x, \beta, \lambda) = \lambda t^{\lambda-1} \exp[-\lambda(\beta_0 + \beta_1 x_1 + ... + \beta_i x_i)]$$

where λ is the Weibull shape parameter, β_0 is the intercept parameter, and β_1 to β_i are the covariate parameter estimates (Hosmer and Lemeshow 1999). The corresponding survivorship function for the accelerated failure form of the Weibull model is

(8) $$S(t, x, \beta, \lambda) = \exp\left\{- t^{\lambda} \exp\left[- \lambda(\beta_0 + \beta_1 x_1 + ... + \beta_i x_i)\right]\right\}.$$

Results

Salinity averaged 10.4 (±1.1 SD) over the duration of the experiment. Temperature in the variable temperature treatment ranged from 6.4-10.3°C and averaged 8.0°C. Temperature significantly affected hazard and survival rates (Table 1). Parameter estimates were used to estimate the hazard and survival functions at different levels of the covariates.

The overall model was simulated over a 121-day period, which would be equivalent to the duration of winter from December 1 to March 31, for the three treatment temperature levels (1, 5, and 8° C). For all temperature levels, the probability of mortality increases steadily over time, but the magnitude of the hazard is greater at lower temperatures (Figure 1a). Similarly, predicted survival is lowest at low temperatures (Figure 1b). At one degree, 50% mortality would occur at day 22, and virtually all crabs would be dead by day 75. Survival is higher at 5 degrees, but the model still predicts that almost all crabs would be dead by the end of winter. At an average exposure of eight degrees, about 40% of crabs would remain at the end of

winter. Observed survival closely follows predicted survival at 1 degree, but the model overestimates survival at 5 degrees and underestimates survival at 8 degrees (Figure 1b).

Discussion

A fundamental issue was highlighted by the results obtained from this experiment. Although survival significantly improved with temperature, the level of mortality observed in the laboratory was far higher than that observed in the field (Sharov et al. 2003, Rome et al. 2005). For reasonable levels of winter temperature, my laboratory results indicate that the majority of crabs would not survive the winter period – contrary to field results. Thus the results from this laboratory experiment will not provide a reliable foundation for predicting winter mortality in the field. Several hypotheses to account for the discrepancy between lab and field results have been suggested.

First, I believe that the acclimation period in the experiments conducted thus far may not have been sufficient. When crabs were adapted to the lab the rate of temperature decrease was one degree per day, which is higher than what they would experience in the field. A longer acclimation period may help crabs adjust better to a laboratory setting. Evidence pertaining to the role of acclimation temperature in overwinter survival is available for estuarine fishes (Malloy and Targett 1994).

Other conditions in the lab differed from what blue crabs experience in their natural environment. Blue crabs bury in the sediment during the wintertime, but we did not have sediment in our tanks. Although temperature profiles of sediment in the bay are not well known, sediment may also provide a localized insulating effect on

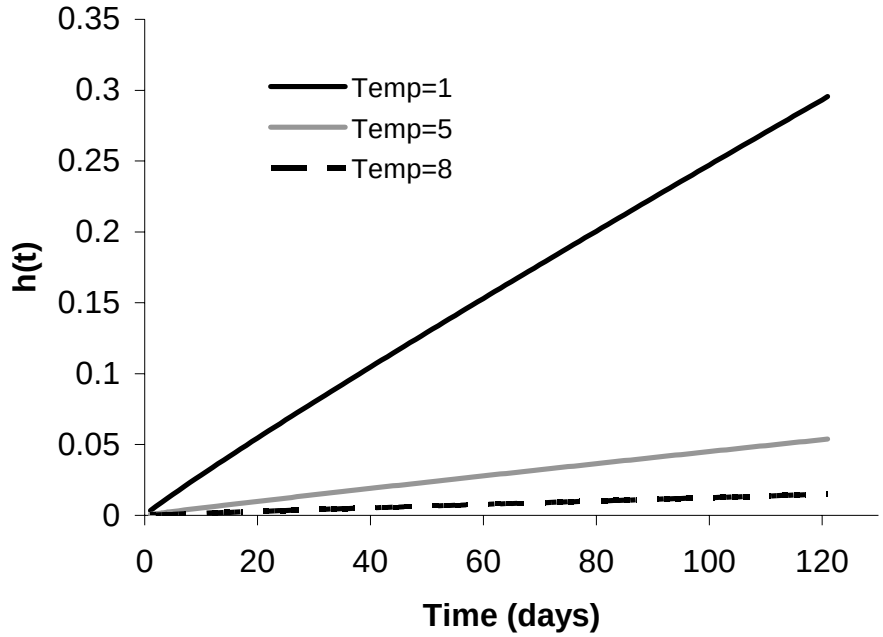
crabs and act as a buffer during swings in temperature. Crabs were not fed during the duration of the experiment, however they do not generally feed at cold temperature. If mortality was starvation induced, we would expect there to be an inverse effect of temperature since crabs would have higher metabolic rates at higher temperatures. In addition, during the experiment crabs were exposed to 12 hours light and 12 hours darkness. Blue crabs buried in sediment at the bottom of the Chesapeake Bay may be exposed to considerably longer periods of darkness. If light plays a role in regulating metabolism, crabs may have expended more energy in our experiment than necessary.

Several of these factors were taken into consideration when planning the 2005 experiment (Chapter 2). Specifically, crabs were brought into the lab earlier to ensure a more gradual acclimation to the lab. A sediment treatment was included to test for the effect of substrate on crab survival. Lastly, crabs were kept in the dark fulltime to mimic conditions likely experienced by crabs in the field during the winter. Thus, while the survival patterns from the 2004 experiment were unexpected, the questions that arose from analyzing the results allowed me to design a more effective experiment the following winter.

Table 1. Parameter estimates for the accelerated failure model with a Weibull distribution

Parameter	df	Estimate	SE	Chi-Square	Pr>ChiSq
Intercept, β_0	1	3.0716	0.0893	1184.13	<.0001
Temperature, β_1	1	0.2196	0.0206	113.09	<.0001
Scale, σ	1	0.5161	0.0416		
Weibull shape, λ	1	1.9376	0.1562		

a)



.

b)

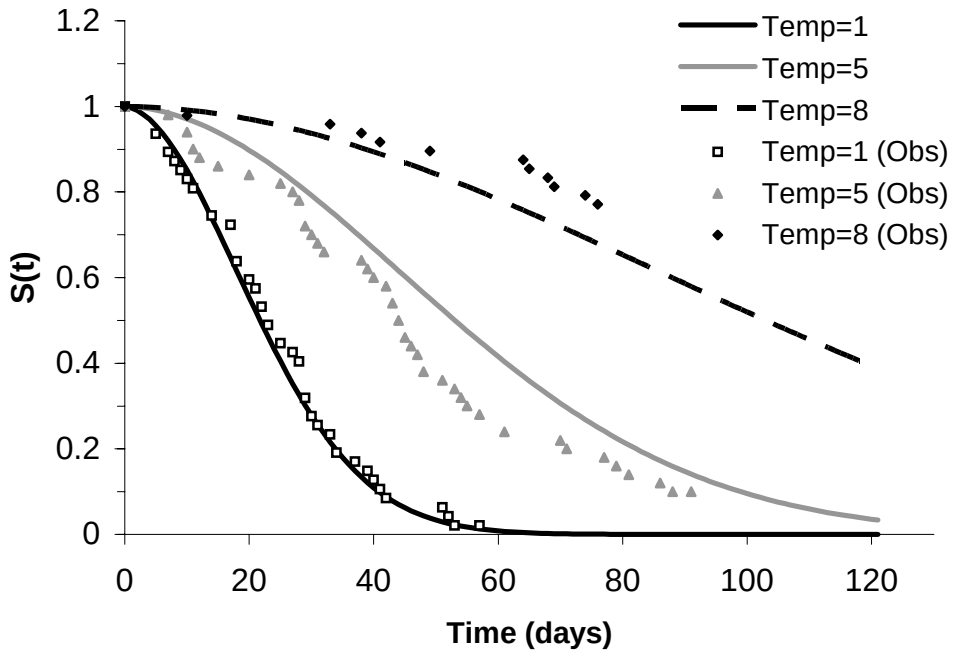


Figure 1. a) Mortality rate (hazard function) of blue crabs at experimental temperature levels. Parameter estimates for the fitted Weibull distribution are in Table 1. b) Observed (▪, reduced from event times using the KM product limit estimator) and estimated survival functions (-, for the Weibull hazard functions in a).

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