ABSTRACT

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Recent marked declines of the commercially and ecologically important blue crab in Chesapeake Bay have prompted requests for improved scientific information on blue crab population dynamics. I evaluated recruitment rates to blue crab fisheries using three independent approaches: direct observations, length-frequency analysis, and lipofuscin-based ageing. Three cohorts of known-age pond-reared blue crabs were sampled monthly, growth rates were modeled and compared to estimates from length-frequency analysis of field-collected crabs. Mean growth rates for juvenile pond-reared and field-collected cohorts ranged from 0.4 to 1.4 mm d^{-1}. A temperature-dependent growth model back-calculated settlement dates and predicted partial recruitments of juvenile winter size distributions. Predictions coincided with observations for wild blue crabs. Lipofuscin accumulated exponentially with age. The high growth rates, rapid recruitment rates, and lipofuscin-based age designations suggest that peeler/soft crab fisheries in the summer and hard crab fisheries in the fall/winter are predominately dependent on recruits less than 18 months of age.

GROWTH AND RECRUITMENT RATES OF JUVENILE BLUE CRABS (CALLINECTES SAPIDUS) IN CHESAPEAKE BAY

By

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Chapter 1: Introduction and Objectives

Accurate estimates of vital rates (i.e., growth, mortality, fecundity, etc.) are prerequisites for quantifying population dynamics, developing stock assessments, and managing exploited species. There are several approaches for estimating vital rates, most of which rely on accurate age estimates and knowledge of demographic structure (Hilborn and Walters 1992). For many fishes, age can be accurately estimated by visual interpretation of concentric rings in otoliths or other hard structures (DeVries and Frie 1996). However, ageing species such as crustacean, which periodically molt their exoskeleton and lack permanent hard structures, has proven more difficult. Nevertheless, the demand for accurate estimates of vital rates and information on the population dynamics of exploited crustacean species continues to escalate as commercial landings of crustaceans become increasingly important on a global scale (Smith and Addison 2003). Here, I utilize three principal approaches to estimate growth and recruitment rates of a commercially and ecologically important crustacean, the blue crab (*Callinectes sapidus*).

Blue Crab Fishery

In the United States, the blue crab supports substantial commercial and recreational fisheries from New England to Florida and along the Gulf of Mexico (Rugolo et al. 1998). During 1998-2003, average annual U.S. blue crab commercial landings approached 8.6×10^4 metric tons, valued at an average of $160.7 million (NMFS Commercial Landings Statistics: http://www.st.nmfs.gov/st1/commercial/). The largest commercial blue crab fishery in the U.S., both currently and historically

occurs in Chesapeake Bay (Sharov et al. 2003), although the Bay continues to support an ever smaller percentage of total U.S. harvests of the species (Stagg & Whilden 1997). The Chesapeake Bay blue crab commercial fishery is complex with several gear types utilized seasonally and geographically and differential exploitation based on size, shell status (e.g., hard or soft/peeler), maturity, and sex (Miller 2001a). During its 120 years of commercial existence, the blue crab fishery in Chesapeake Bay has been characterized by increased fishing intensity and rapid fluctuations in harvest (Van Engel 1958, Cronin 1998). Despite large fluctuations in harvest, the blue crab continues to support Chesapeake Bay's most valuable fishery (Miller and Swanson 2001).

Blue Crab Ecology

In addition to economic value, the blue crab is an important ecological member of estuarine communities throughout much of its range, which extends from Argentina to Nova Scotia (Norse 1977). As opportunistic benthic omnivores, blue crabs have highly variable diets that include bivalves, crustacean, fish, polychaetes, and amphipods (Baird & Ulanowicz 1989). In fact, the blue crab may be classified as an ecological dominant, a species that is important to ecosystem structure and function (Davic 2000), due to their ability to affect the diversity, abundance and structure of benthic species (Hines et al. 1990). In return, blue crabs are preyed upon by several fish species, providing a potentially important link between the benthic and pelagic food webs (Baird & Ulanowicz 1989).

Status of Chesapeake Bay Blue Crab Stock

Recent trends of decadal-long declines in abundance are noteworthy: from 1992 to 2000 estimated Chesapeake Bay spawning stock abundance and biomass declined by 81 and 84%, respectively (Lipcius and Stockhausen 2002). During that same time period, Lipcius and Stockhausen (2002) reported that larval abundance and post-larval recruitment decreased by an order of magnitude. Moreover, evidence of recent declines in commercial landings, total abundance, average size, and percentage of legal size crabs (Abbe and Stagg 1996), in addition to declines in spawning stocks and recruitment, have stimulated concern among stakeholders about the status of the stock and the sustainability of the fishery (Rugolo et al. 1998, BBCAC 2001).

The first Bay-wide blue crab stock assessment, completed in 1997, suggested an elevated stock abundance during the 1980's followed by a return to average abundance levels during the mid-90's (Rugolo et al. 1998, Miller et al. 2005). In 2001, the Bi-State Blue Crab Advisory Committee (BBCAC 2001) noted that the Chesapeake Bay blue crab stock was fully exploited and the fishery was overcapitalized in terms of fishing effort; increasing the risk of collapse if the fishing effort remained at current levels, with concurrent population decline. Accordingly, a new management framework was developed to ensure that target and threshold reference points were distinguishable (Miller and Swanson 2001, Miller et al. 2005). A threshold based on exploitation rate was established to preserve 10% of the unexploited spawning potential (Miller and Swanson 2001, BBCAC 2001). The corresponding target reference point was developed to preserve 20% of the unfished spawning potential, a measure that would effectively double the spawning stock

(Miller and Swanson 2001, Miller et al. 2005). The most recent Bay-wide stock assessment indicated that the stock is not currently overfished, but exploitation rates are above the target reference point (Miller et al. 2005). As managers continue to increase regulations (e.g., gear type, time of year, size limits, etc.) to achieve target reference points, there have been requests for improved scientific information on factors such as growth that affect blue crab population dynamics.

Estimating Growth

Blue crab growth is discontinuous in nature. Growth occurs at discrete intervals after a molting event with subsequent periods of growth stasis during the intermolt period (Hartnoll 2001). Of the two components included in the molting cycle, intermolt period appears to be the most variable and to have the most profound influence on growth rate (Smith 1997, Brylawski 2002). Temporal variability associated with the intermolt period is influenced by several external factors, such as temperature, salinity, and food supply (Hartnoll 2001). Temperature is likely the dominant factor, such that increases in temperature (within a tolerable range) result in a consistent and substantial shortening of the intermolt period (Hartnoll 2001).

Accordingly, estimating growth rates of blue crab are complicated by the complex molting cycle and the lack of accurate age estimates. Although there are several approaches for estimating growth rate, the three most common are: (1) direct observation of individuals, (2) length-frequency analysis, and (3) indirect analysis (e.g., analysis of hard parts) (DeVries & Frie 1996). Direct observation of known-aged individuals is the most direct and accurate method for quantifying growth, but also the most labor-intensive, time-consuming, and costly method of determining

growth (DeVries & Frie 1996). Direct observations are typically applied as a validation technique to evaluate accuracy of growth estimates determined by other methods.

Traditional length-based approaches, such as length frequency modal analysis, are often used to estimate growth of crustacean. Length frequency analysis is dependent upon the identification of modes in the length frequency distribution, which are often interpreted as year classes or cohorts (Hartnoll 2001). A series of length frequency samples can be used to follow the progression of modes through time. In instances where modes are clearly defined, analysis and interpretation are clear. However, when growth varies seasonally and spawning is protracted such as the blue crab in Chesapeake Bay, the effectiveness of length-based approaches can be reduced.

Direct observation and length frequency modal analysis have often provided the empirical data that is used in the two approaches to model blue crab growth: continuous growth modeling and molt-process modeling (Miller et al. 2005). Typically, the von Bertalanffy growth function (VBGF) has been applied to model growth as a continuous function (Rothschild et al. 1992, Rugolo et al. 1998, Ju et al. 2001). Continuous growth models, such as the VBGF, provide a statistical representation of change in size at age that is easily incorporated in stock assessment models (Miller and Smith 2003). However, continuous growth models fail to depict the incremental nature of growth and the seasonal variation in growth.

The deficiencies of continuous growth models prompted the use of a seasonalized von Bertalanffy growth function (Ju et al. 2001) and a molt-process

growth model (Smith 1997). Seasonalized VBGF, an elaboration of the VBGF, includes additional parameters that account for the seasonal oscillations in growth. With the exception of capturing the cessation of growth during winter, the seasonalized VBGF suffers from the same problems as the non-seasonalized growth function. Molt-process growth models (sensu Smith 1997) provide a more realistic depiction of the incremental nature of blue crab growth as both growth per molt and intermolt period are explicitly modeled. Recently, molt-process growth models developed for blue crabs have incorporated a temperature degree-day framework to capture the physiological effects of temperature (e.g., winter torpor) while simultaneously accounting for chronological time (Smith 1997, Bunnell and Miller 2005).

Finally, in attempts to improve the accuracy of age estimates, alternative indirect analyses have been developed in lieu of traditional morphometric approaches. Among the most successful are biochemical and histological approaches that measure the accumulation of fluorescent granular pigments that accumulate in post-mitotic cells, classically referred to as lipofuscin (Ju et al. 2001, Chowdhury et al. 2004). All organisms that breathe oxygen generate free radicals as a by-product of cellular metabolism (Brunk and Terman 2002). Typically, reactive oxygen species are cross-linked with proteins and other biomolecules to provide a scavenging mechanism that impedes these highly reactive radicals (Brunk & Terman 2002). Lipofuscin (LF) is believed to be the product of oxidative stress and the accumulation of non-degradable oxidized material in lysosomes (Hill and Womersley 1993, Brunk and Terman 2002, Chowdhury et al. 2004). Because the amount of LF increases with chronological age

it is often referred to as an age pigment (Brunk & Terman 2002). Accordingly, several studies have quantified LF to estimate age, growth, and longevity for several crustacean, including lobsters (Wahle et al. 1996, Sheehy et al. 1997), shrimp (Vila et al. 2000, Bluhm & Brey 2001), crayfish (Belchier et al. 1998), crabs (Ju et al. 2001, 2003), and amphipods (Bluhm et al. 2001).

Objectives

The goal of my research was to determine growth and recruitment rates of juvenile blue crabs in Chesapeake Bay by incorporating three principal independent approaches: direct observation, length-based analysis, and indirect analysis-lipofuscin. Specifically, I conducted continuous pond-rearing experiments with known-age juvenile blue crabs and monthly sampling of two Chesapeake Bay sub-estuaries in support of the following objectives: 1) develop a continuous growth model of known-age juvenile blue crabs and investigate the effects of temperature on growth, 2) estimate growth rates of field-collected blue crabs from length frequency modal analysis, 3) construct a temperature-dependent molt-process growth model to predict settlement dates and seasonal growth of blue crabs originating from the Chesapeake Bay Winter Dredge Survey, 4) calibrate lipofuscin (LF) accumulation rates with respect to chronological age in known-age crabs, 5) apply LF-based ageing to field-collected crabs, and 6) estimate age- and seasonal-specific partial recruitments to the peeler/soft and hard crab commercial fisheries.

I hypothesize that juvenile blue crab growth is rapid; therefore, peeler fisheries in the summer and hard crab fisheries in the fall/winter are predominately dependent on recruits younger than 18 months of age. The notion of an annual crop

dynamic was initially proposed by Van Engel (1958) and more recently supported by research that suggests crabs ≥ 2 years of age may be minor contributors to the harvestable stock (Ju et al. 2003, Sharov et al. 2003).

Chapter 2: Seasonal growth and recruitment rates of pond-reared and field-collected juvenile blue crabs in Chesapeake Bay

Abstract

Recent declines in Chesapeake Bay blue crab landings and total abundance have stimulated concern about the status of the blue crab stock and the sustainability of the fishery. Here, I estimate growth rates and predict seasonal patterns of partial recruitment through direct observation of pond-reared cohorts, length frequency modal analysis of field-collected cohorts, and modeling of winter size distributions. Three cohorts of known-age juvenile blue crabs (c. 70 days old), produced at a research hatchery, were released (June 2003, October 2003, and September 2004) into separate earthen brackish-water ponds and sampled monthly. To collect wild juveniles, monthly (June-October) sampling was conducted in two Chesapeake Bay sub-estuaries. A temperature-dependent molt-process growth model was used to predict seasonal size distributions of crabs originating from the Chesapeake Bay Winter Dredge Survey. The highest growth rates occurred at small sizes (c. 20 mm) and at temperatures greater than 20 °C, during which time mean instantaneous and absolute growth rates of up to 3.1% CW d^{-1} and 1.5 mm d^{-1} were observed. Mean absolute growth rates for pond-reared and field-collected cohorts ranged from 0.4 to 1.5 mm d^{-1} during their first year of life. These growth rates indicated that cohorts emerging from winter torpor at small sizes (< 30 mm) were capable of recruiting to the peeler/soft crab fishery by July and the hard crab fishery by October, both within one full growing season. Adult size distributions predicted by the molt-process growth model corresponded well with size distributions observed in the ensuing

year's Winter Dredge Survey. Further, predicted peaks in recruitment coincided with summer peaks in peeler/soft crab landings and fall peaks in hard crab landings. The high growth rates and rapid recruitment reported here indicate that peeler/soft crab fisheries in the summer and hard crab fisheries in the fall/winter are predominately dependent on recruits less than 18 months.

Introduction

The blue crab (*Callinectes sapidus* Rathbun) is an ecologically and economically important species in many western Atlantic coastal and estuarine habitats. As opportunistic benthic omnivores, blue crabs affect the diversity, abundance and structure of benthic communities (Hines et al. 1990, Silliman and Bertness 2002, Harding 2003). In return, blue crabs are preyed upon by several fish species, providing an important link between the benthic and pelagic food webs (Baird and Ulanowicz 1989). In the United States, blue crabs are fished commercially and recreationally from New England to Florida and along the Gulf coast (Stagg and Whilden 1997). The largest fishery occurs in Chesapeake Bay; the overall economic contribution of commercial and recreational crabbing in the Bay region is estimated in the hundreds of millions of dollars annually (Sharov et al. 2003).

In Chesapeake Bay, the commercial fishery is comprised of two primary sectors: peeler/soft and hard crab fisheries. Peeler (just prior to molt) and soft-shell (after molt) crabs are harvested from spring through summer, during which time, commercial size limits vary from 76.2 to 88.9 mm carapace width—CW. Peeler/soft crab landings are dominated by peeler crabs (> 95 %); combined landings typically peak in late May with a smaller secondary peak occurring in mid-summer (July or August) (Figure 2.1, NMFS Commercial Landing Statistics: http://www.st.nmfs.gov/st1/commercial). Annually, peeler/soft crab landings are typically less than 10% of total Chesapeake Bay blue crab harvest. Hard crabs comprise the majority of landings.

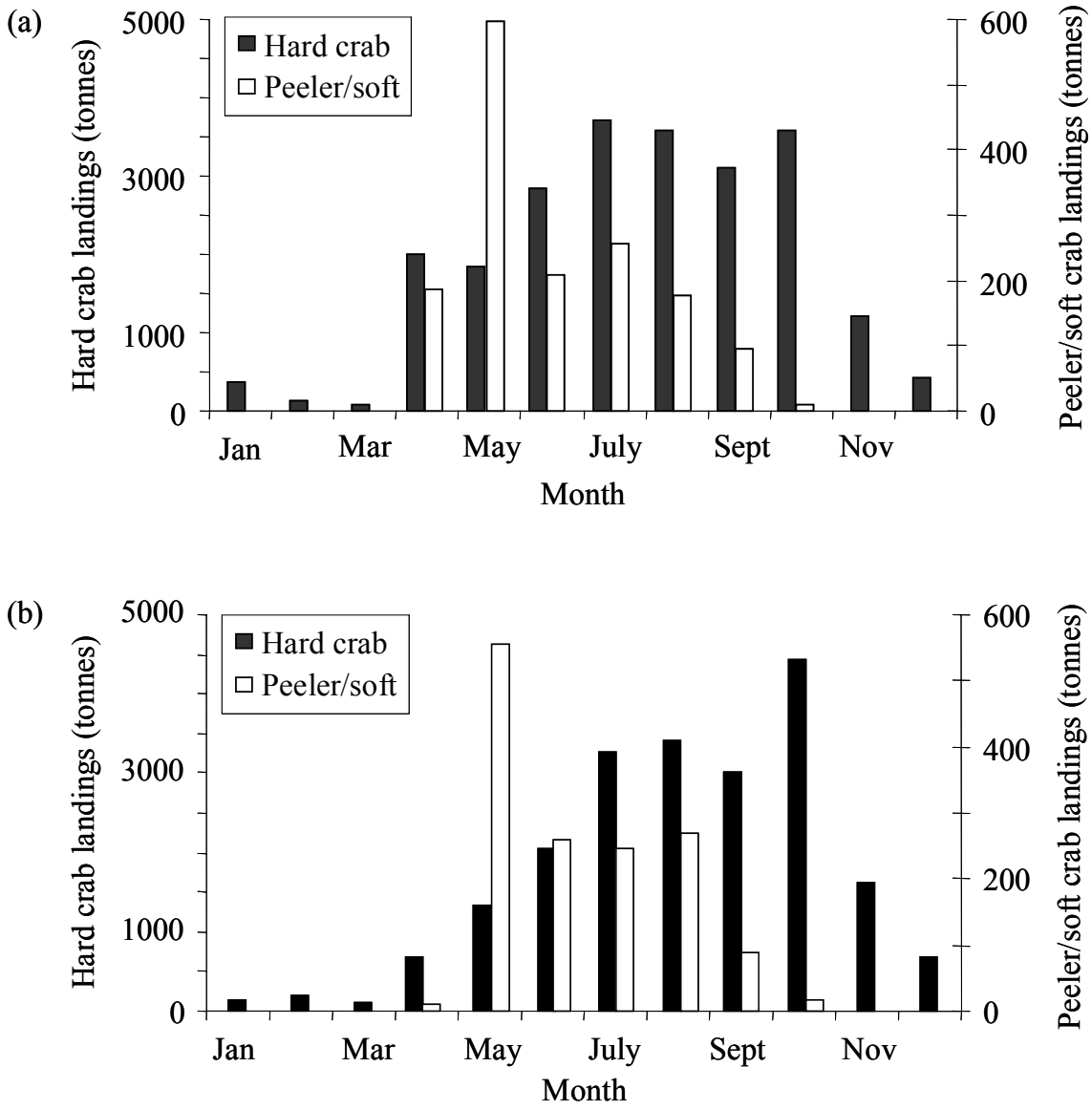

Figure 2.1. Chesapeake Bay commercial landings of hard crabs and peeler/soft crabs in a) 2002 and b) 2003. Source: NMFS Commercial Landing Statistics.

Commercial size limits for the hard crab fishery (127 to 133.3 mm CW) also vary by season. Annually, c. 70% of the hard crab harvest is landed from June to October, with a peak typically occurring in October (Figure 2.1, NMFS Commercial Landing Statistics).

The first Bay-wide stock assessment for the blue crab assigned age-classes 0+, 1+, and 2+ to crabs ≤ 59 mm, 60-119 mm, and >120 mm, respectively (Rugolo et al. 1998); implying that recruitment to the peeler fishery occurs at 1+ years of age and recruitment to the hard crab fishery occurs at 2+ years of age. Following the initial stock assessment, more recent studies (conducted in 1999) have documented rapid growth and indicated that blue crabs are capable of recruiting to the hard crab fishery (≥ 127 mm) after one full growing season (Ju et al. 2001, Sharov et al. 2003). The notion of an annual crop dynamic is supported by biochemical (lipofuscin)-based age estimates and absolute abundance estimates of age classes 0 and 1+ (based on CW during winter), which suggest that crabs ≥ 2 years of age may be minor contributors to the harvestable stock (Ju et al. 2003, Sharov et al. 2003; see Chapter 3).

Recently (1992 to 2000) documented declines in spawning stock biomass, larval abundance, and post-larval recruitment (Lipcius and Stockhausen 2002) have stimulated public concerns about the status of the Bay stock and the sustainability of the fishery. While Chesapeake Bay blue crab harvests are known to undergo strong fluctuations (Volstad et al. 1994, Cronin 1998), concurrent declines in commercial landings and total abundance (Figure 2.2) have led to increased management actions

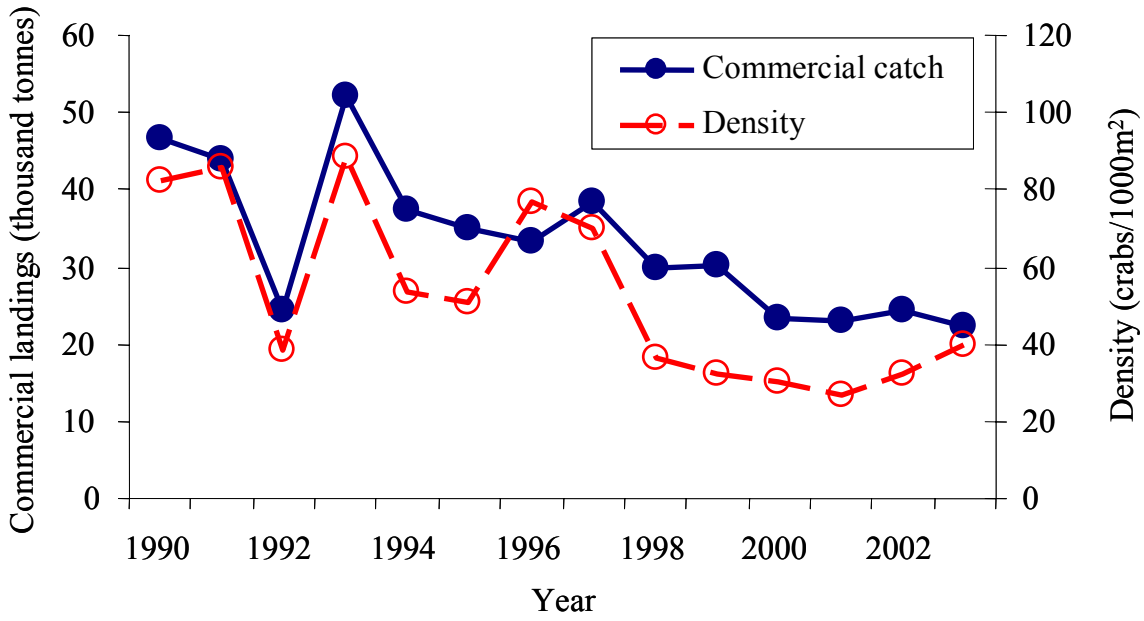

Figure 2.2. Commercial landings and density (estimated from the Winter Dredge Survey) of blue crabs in Chesapeake Bay from 1990 to 2003. Sources: NMFS Commercial Landing Statistics and MD Department of Natural Resources.

(BBCAC 2001, Miller and Swanson 2001). Such actions entail risk, uncertainty, and substantial short-term economic loss to commercial sectors (Lipton and Sullivan 2002). Thus, there is a need to develop an accurate stock assessment of the Chesapeake Bay stock.

Accurate estimates of growth are fundamental to understanding the population dynamics of blue crabs (Miller and Smith 2003, CBSAC 2004). Growth, the addition of biomass by individuals, and thus accumulation to the population, is an underlying mechanism in many ecological and fishery interactions, which are often size-dependent. Growth typically governs the interactions of predator and prey (particularly for the notoriously cannibalistic blue crab), influences the time required to reach sexual maturity, determines reproductive success, and translates recruitment to fishery production (Miller and Smith 2003).

Several aspects of blue crab biology have confounded accurate estimates of growth, most notably, the discontinuous growth pattern, lack of accurate age estimates, and protracted spawning season (May to September in Chesapeake Bay). Blue crab growth in CW is saltatory in nature, and occurs following ecdysis. Hence, the rate of growth is determined by the increase in size at each molt (growth per molt—GPM) and the time interval between successive molts (intermolt period—IMP) (Mauchline 1976). Based on literature values, GPM appears to be relatively invariant, thus the variability in growth rates likely originates from differences in IMP (Leffler 1972, Fitz and Weigert 1991, Smith 1997, Brylawski 2002). In addition, blue crab growth rates are temperature-dependent; growth variability increases in shallow temperate estuaries (e.g., Chesapeake Bay) where large temperature fluctuations

occur seasonally (Leffler 1972). In Chesapeake Bay, growth typically occurs from April to October, followed by winter torpor at temperatures less than 10 °C (Smith 1997, Ju 2000, Brylawski 2002).

For many temperate fishes, age estimates can be derived from hard part analysis (i.e. otoliths, scales, fin spines) by counting opaque and translucent rings that form annually (DeVries and Frie 1996). Such age determinations are not possible for crustaceans, which periodically molt, thereby removing any evidence of age or previous size (Ju et al. 1999). Further complicating matters is the protracted spawning season, which results in a wide distribution of sizes in a single year class (Prager et al. 1990). Spawning near the mouth of Chesapeake Bay occurs from May to September, but peaks from July through September (Prager 1996) when temperatures are optimal for embryonic development and larval growth (Sulkin et al. 1976). Larvae are subsequently transported to the continental shelf where they develop through six to eight larval stages. The final larval stage, the megalopa, invades and settles (45-155 days post hatch based on lab rearing) in estuarine waters from early July to November, with settlement peaks occurring in September and October (van Montfrans et al. 1990).

To overcome difficulties encountered with discontinuous growth patterns, lack of accurate age estimates, and protracted spawning, growth has been measured by conducting mark-recapture studies (Fitz and Wiegert 1991), laboratory studies (Leffler 1972, Cadman and Weinstein 1988, Ju et al. 2001, Brylawski 2002), and field caging experiments (Tagatz 1968, Brylawski 2002). The success of previous studies has been hampered by poor tag retention (Fitz and Wiegert 1991) and potentially

unnatural rearing conditions (i.e. food and space, Brylawski 2002). Here, I have attempted to model juvenile (0+) blue crab growth through direct observations and empirical measurements of known-age cohorts reared in earthen ponds.

Hypothesis/Objectives

I hypothesized that juvenile blue crab growth is rapid during summer and fall (> 0.5 mm d^{-1}), and therefore peeler fisheries in the summer, and hard crab fisheries in the fall/winter, are predominately dependent on recruits less than 18 months of age.

I conducted continuous pond-rearing experiments with known-age cohorts of juvenile blue crabs to model juvenile growth rates and investigate the effect of temperature on cohort growth rates. I compared pond-derived growth rates to growth rates estimated using length-frequency modal analysis of crabs sampled from two sub-estuaries of Chesapeake Bay. Growth rates estimated from direct observations of known-age cohorts and length frequency modal analysis were used to evaluate age- and season-specific partial recruitments to the two principal commercial fisheries. Finally, a temperature-dependent molt-process growth model was developed to back-calculate settlement dates and predict partial recruitments of juvenile winter size distributions in Chesapeake Bay.

Methods

Pond Rearing Study

Known-age juvenile blue crabs were provided by a blue crab hatchery program at the Center of Marine Biotechnology (University of Maryland Biotechnology Institute, Zmora et al. 2005). Three cohorts were released into

separate earthen, brackish water ponds (salinity = 7-11, area = 360 m^2, max. depth = 1.2 m) at Horn Point Laboratory (Cambridge, MD) in June 2003, October 2003, and September 2004 to simulate summer and fall settling cohorts. Cohorts were released at 63, 66, and 83 days of age and c. 20 mm CW (crab instar 6 (C6), O. Zmora, Center of Marine Biotechnology, pers. comm., Table 2.1). Initial stocking densities approached 0.84 crabs m^{-2}, within the range, but on average an order of magnitude lower than those reported for seagrass beds in Chesapeake Bay (Orth and van Montfrans 1987). Ponds received constant flow of ambient water from the Choptank River. Temperature was monitored continuously (1hr intervals) at a depth of 1 m with a HOBO ® data logger. Natural refuge was provided by submerged aquatic vegetation (principally *Ruppia maritima*). The forage base consisted of abundant small fishes (*Fundulus heteroclitus* and *F. majalis*, *Cyprinodon variegates, Gobiasoma bosc*), brackish water clam (*Rangia cuneata*), polychaetes, and amphipods.

Crabs in each cohort were sampled monthly from March to October with a seine (1 by 3.1 m with 6 mm mesh) and/or baited traps (wrapped with 6 mm wire mesh); prior to release, individual CW and sex were recorded. Carapace width was measured (to nearest mm) from the tips of the lateral spines for all analyses.

Table 2.1. Birth date, brood size, mean carapace width (mm) ± 1 standard deviation (s.d.), age, date, and number at release for three cohorts of known-age blue crabs released into separate earthen brackish water ponds at Horn Point Laboratory.

Cohort	Birth date	Brood/ Parentage	Mean CW (mm) ± s.d. at release	Age (days) at release	Date of release	# released
1	28 March 2003	1.2 million, ♀ from York River	16.8 ± 3.8	66	4 June 2003	272
2	17 July 2003	200,000, ♀ from York River, 42,000 ♀ from Rhode River	21.3 ± 5.9	83	8 October 2003	302
3	6 July 2004	3 million, ♀ from Rhode River	20.5 ± 4.2	63	7 September 2003	293

At bimonthly intervals, a subset from each cohort was sacrificed for lipofuscin analysis (Chapter 3); weight (g) was additionally recorded for these samples.

Individual length-at-age data and least squares regression were used to obtain initial parameter estimates for a seasonalized von Bertalanffy growth function (VBGF) (Pitcher and MacDonald 1973):

$$CW(t) = CW_{\infty}[1 - e^{-[C\sin(2\pi(t-t_s)+K(t-t_0)]}] \qquad (2.1)$$

where *CW(t)* is carapace width (mm) at age *t*, CW_{∞} is the asymptotic maximum carapace width (mm), *K* is the annual growth coefficient (yr^{-1}), t_0 is the theoretical age (yr) when length is zero, C is related to the magnitude of the seasonal oscillation, and t_s is the starting age (yr) for the sine curve. Initial estimates were incorporated into a nonlinear regression model; the iterative Gauss-Newton algorithm (Kutner et al. 2004) was used to estimate parameters. The nonlinearity of the VBGF can lead to biases and nonnormality in parameter estimates, reducing the validity of linear statistical theory (Cerrato 1990, Hernandez-Llamas and Ratkowsky 2004). As a result, Cerrato (1990) concluded that likelihood ratio tests should be used when comparing VBGF. Here, likelihood ratio tests were conducted to compare inter- and intra-cohort specific seasonalized growth models. When VBGF were significantly different, likelihood ratios were calculated to statistically compare parameter estimates (in particular CW_{∞} and K).

Carapace width estimates from seasonalized VBGF were used to calculate absolute growth rate (mm d^{-1}):

$$g = \frac{CW_{t2} - CW_{t1}}{t_2 - t_1} \qquad (2.2)$$

where CW_{t2} and CW_{t1} are expected carapace width (mm) at time t_2 and t_1, respectively. To account for the non-linear nature of growth, mean instantaneous growth rate was calculated as:

$$G = \frac{\log_e CW_{t+1} - \log_e CW_t}{\Delta t} \quad (2.3)$$

and converted to %CW d^{-1}:

$$G' = (1 - e^{-G}) \times 100 \quad (2.4)$$

where CW_t and CW_{t+1} are the expected initial and final carapace width (mm), respectively and Δt is the time interval in days.

Fulton condition factor (Anderson and Neumann 1996), the allometric relationship between wet weight (g) and CW^3, was calculated for individuals in each cohort. Regression coefficients for the relationship between condition and CW were compared among cohorts. Logistic regression was used to determine mean age and temperature degree-day (TD day, see below) at which 50% of the cohort obtained minimum commercial size (82.5-88.9 mm peeler/soft; 127-133.3 mm hard crab), my operational definition of recruitment.

Field Study

Blue crabs were collected from two separate Chesapeake Bay sub-estuaries (Figure 2.3) during 2003 and 2004 to (1) compare growth rates obtained from length frequency modal analysis with estimates derived from pond rearing experiments and (2) test spatial and annual variation in growth rates and age composition (Chapter 3) of field-collected blue crabs. In 2003, bottom trawls in the lower Choptank River and

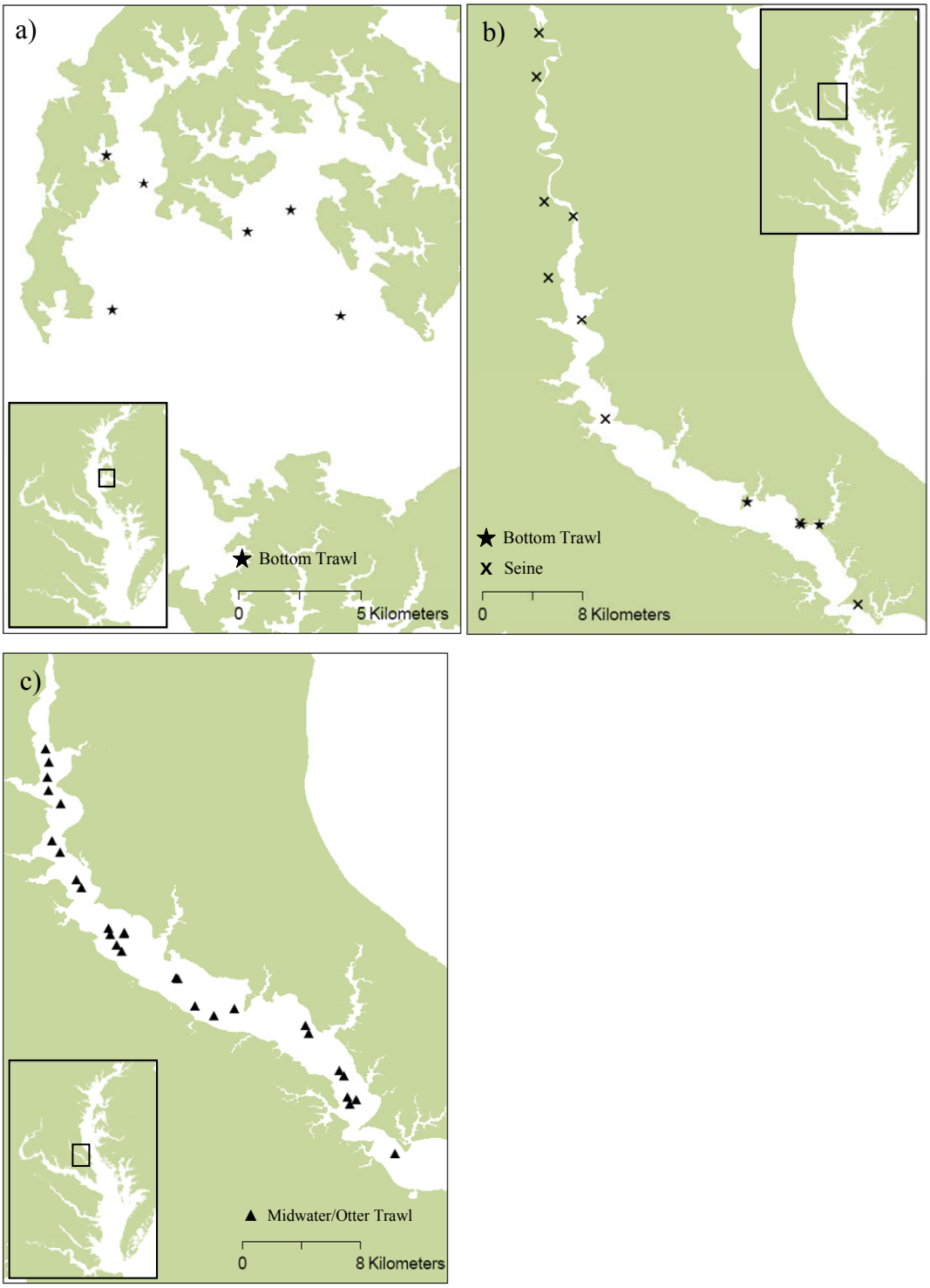

Figure 2.3. A) Bottom trawl sampling sites in the Choptank River and Broad Creek (June-October 2003). B) Seine (June-July 2004) and bottom trawl (July 2004) sampling sites in the Patuxent River. C) Midwater/otter trawl sampling sites in the Patuxent River (August-October 2004). The midwater/otter trawl sites sampled in August are shown here.

adjoining Broad Creek were conducted, sampling from six fixed stations (Figure 2.3a). Station depths ranged from 1 to 9 m. Surface temperature and salinity were determined at each station (with YSI ® meter). Crabs were collected aboard the 26' RV Parker deploying a 4.9 m semi-balloon otter trawl with a tickler chain and 12 mm cod-end mesh, towed at 4.6-5.6 km hr^{-1} for 6 minutes (Table 2.2). On 12 July, the majority of individuals ranging from 11-30 mm CW were collected in floating vegetation with a dip net. Choptank River sampling sites were those designated as part of a MD Department of Natural Resource (DNR) sampling program; MD DNR collected blue crabs with the same gear and provided supplemental samples (< 30% of each monthly sample) during June, July, and August (Table 2.2). The mean date of sampling events was used when samples were collected less than seven days apart.

In 2004, blue crabs were collected from the Patuxent River with four different gear types (Table 2.2; Figure 2.3b, c). Crabs were collected with a 1.5 by 30.5 m beach seine with 3.2 mm bag mesh in June and aboard the 23' RV Aires deploying a 4.9 m bottom trawl (as in 2003) in July. June and July samples were collected at depths ≤ 1.5 m. June seining was conducted at nine stations distributed throughout 72 river km; three sites were sampled in July, ranging river km 16 to 20 (Figure 2.3b). Surface water temperatures and salinities were recorded at each sampling station. From August through October blue crabs were collected as part of a fishery-independent multispecies survey (PAXFIMS, PI: Dr. Miller) on the 64' RV Aquarius. Collections were conducted on consecutive days with one of two gear types, an obliquely towed 18 m^2 midwater trawl with 6mm cod-end mesh and a 9 m otter trawl with 6 mm cod- end mesh. Midwater trawl tow durations were ten minutes at

Table 2.2. Gear type, number and size range of crabs collected, and temperature-salinity ranges on sampling dates in the Choptank River (2003) and the Patuxent River (2004). River kilometer was included for Patuxent River collections to indicate variations in spatial coverage.

Choptank River (2003)					
Date	Gear type	# collected ($\Sigma n = 635$)	CW (mm) range	Temp. (°C) range	Salinity range
6-14*	4.9 m bottom trawl	37	80-155	-	-
6-18	4.9 m bottom trawl	99	20-175	21.9-24.0	8.9-9.2
7-12	4.9 m bottom trawl	126	11-160	27.3-28.6	8.4-9.1
7-18*	4.9 m bottom trawl	52	51-154	-	-
8-22*	4.9 m bottom trawl	21	88-170	-	-
8-27	4.9 m bottom trawl	96	60-177	27.2-27.6	8.4-8.5
9-26	4.9 m bottom trawl	161	54-178	23.0-23.3	10.7-11.8
10-24	4.9 m bottom trawl	43	86-178	12.9-15.1	11.5-11.6

*MD Department of Natural Resources collection

Patuxent River (2004)						
Date	Gear type	# collected ($\Sigma n = 464$)	CW (mm) range	River km range	Temp. (°C) range	Salinity range
6-10	30.5 m beach seine	29	23-121	5-72	21.4-26.9	0.1-10.0
6-24	30.5 m beach seine	27	27-74	5-72	23.8-27.9	0.1-10.6
7-12	4.9 m bottom trawl	93	22-171	16-20	28.2-29.0	9.8-10.2
8-3	9 m otter trawl	58	53-174	5-45	25.1-26.3	4.0-11.3
8-4[+]	9 m midwater trawl	48	84-174	5-45	-	-
9-9	9 m midwater trawl	49	21-175	5-45	21.2-26.3	5.2-9.0
9-10	9 m otter trawl	27	23-175	5-45	21.8-22.8	5.0-9.0
10-5	9 m otter trawl	81	18-197	5-45	16.4-18.2	7.2-10.6
10-6	9 m midwater trawl	52	16-187	5-45	16.3-17.8	5.6-10.6

[+]Temperature and salinity range not reported for 8-4 due to instrument failure

5.6 km hr^{-1}; step durations were two minutes. Duration of bottom trawl tows was five minutes. Monthly sampling sites (16 for otter trawl, 10 for midwater trawl) were randomly selected and distributed throughout c. 45 river km (Figure 2.3c). Station depths ranged from 4 to 20 m. All crabs collected were wrapped within wet burlap and transported live back to the laboratory for lipofuscin analysis (Chapter 3); CW (mm), weight (g), and sex were recorded for each individual.

A length frequency modal analysis was conducted to estimate growth rates of field-collected crabs. Size classes were specified at 10 mm intervals. Modes in each monthly size distribution were identified using NORMSEP (FiSAT ©, Gulland and Rosenberg 1992), which applies a maximum likelihood procedure to separate a length-frequency distribution into its normal components. From the NORMSEP procedure, I obtained modal means, standard deviation, and expected number of observations within each mode. A chi-square goodness-of-fit test was conducted to test the assumption that modal frequencies were normally distributed. Modes were assumed to represent distinct cohorts and considered separate if the difference between modal means was greater than twice the smaller of the modal standard deviations (Rosenberg and Beddington 1988 cited in Jennings et al. 2001).

Modal distributions for a particular date were used to identify cohorts. I assumed that the modal mean of cohorts increased in size at each monthly time step (with the exception of October), but restricted the modal progression of each cohort to the nearest larger modal mean the following month. Mean instantaneous growth rates were then calculated by determining the progression of a cohort's modal mean through time. Modal mean CW was regressed against date, the slope of which was

interpreted as linear growth in mm d^{-1}. Analysis of covariance, with date as the covariate, was used to compare linear growth rates among cohorts. Difference of least squares means (Tukey-Kramer adjusted) was used to compare intercepts among cohorts. Fulton condition factors were calculated for field-collected individuals and regressed on CW. Regression coefficients were then compared between sub-estuaries and between estuarine and pond systems.

Mean date at recruitment to the two fisheries was determined from cumulative size frequencies using logistic regression for each cohort identified. Because crabs were collected in Maryland waters, Maryland commercial size limits (82.5-88.9 mm peeler/soft; 127-133.3 mm hard crab) were applied in analyses. From 1 April to 14 July, minimum commercial size limits for peeler/soft crabs and hard crabs were 82.5 and 127 mm, respectively. From 15 July to 15 December, the minimum commercial size limit was increased to 88.9 mm for peeler/soft crabs and 133.3 mm for hard crabs. Note that the size limit for soft shell crabs is 82.5 mm throughout the year, but due to the predominance of peeler landings, size limits for peeler crabs were used.

Temperature-dependent growth model

To incorporate the two components of growth, GPM and IMP, I constructed a molt-process growth model (sensu Smith 1997) derived from observations of pond-reared cohorts. Growth was predicted using the growth function:

$$CW_n = CW_{initial} \times GPM^n \qquad (2.5)$$

where CW_n is the carapace width of an individual after n number of molts, $CW_{initial}$ is the pre-molt carapace width of an individual, and GPM is the proportional increase in pre-molt CW following each molt. Because I did not explicitly measure GPM for

pond-reared cohorts, a constant proportion of pre-molt CW (20%, Brylawski 2002) was applied. There were no literature values on GPM for blue crabs less than 20 mm; therefore, GPM was assumed to be 50% of pre-molt CW from settlement (C1-2.5 mm) to c. 20 mm (C6), which has been observed in hatchery settings under food replete conditions and temperatures near 20 °C (O. Zmora, Center of Marine Biotechnology, pers. comm.).

Intermolt period was not explicitly modeled; rather I modeled a temperature-dependent molt-frequency function to predict the number of molts after settlement (C1) and the corresponding crab instar (Table 2.3, see Figure 2.17a). Temperature was incorporated into the model as temperature-degree (TD) day, calculated for each pond-reared cohort as:

$$TDdays = \sum_{i} (t_a - t_b) \qquad (2.6)$$

where i is the number of days, t_a is the mean daily temperature (°C) and t_b is the base temperature (10 °C, Ju 2000) below which growth (molting) was assumed to cease. In order to construct the molt-frequency function, I needed an estimate of the number of molts from settlement experienced by each pond-reared individual. Observed CW (CW_n) for pond-reared individuals, mean initial CW ($CW_{initial}$ = 2.5mm when CW_n < 24 mm, else $CW_{initial}$ = 18.9 mm, Table 2.3), and the appropriate proportional GPM (20 or 50%, depending on crab instar) were input into equation 2.5 to solve for n, number of molts (rounded to the nearest integer). When $CW_{initial}$ was equal to 18.9

Table 2.3. Number of molts after settlement, crab instar and corresponding mean carapace width (CW), minimum temperature degree-days (TD days) accumulated at each instar, and proportional growth per molt (GPM) used in developing the molt-process model and applied to project seasonal growth of blue crabs originating from the Winter Dredge Survey.

# of molts	Instar	Mean CW (mm)	TD days	GPM (%)
0	1	2.5	0	50
1	2	3.8	161.0	50
2	3	5.6	233.2	50
3	4	8.4	305.3	50
4	5	12.7	377.5	50
5	6	18.9	449.7	20
6	7	22.8	521.9	20
7	8	27.3	594.0	20
8	9	32.8	666.2	20
9	10	39.4	738.4	20
10	11	47.2	810.6	20
11	12	56.7	882.7	20
12	13	68.0	955.0	20
13	14	81.6	1027.1	20
14	15	98.0	1190.9	20
15	16	117.5	2283.8	20
16	17	141.1	3376.7	20
17	18	169.3	4469.6	20

mm, the mean CW at C6, the predicted number of molts was increased by five (i.e., five molts from C1 to C6).

Mean number of molts was estimated for pond-reared cohorts and then plotted against the corresponding TD days calculated from settlement. Settlement was assumed to have occurred 33 days post hatch for hatchery crabs (Zmora et al. 2005). Join point regression (Joinpoint Regression Program v. 3.0, National Cancer Institute) was applied to describe the relationship between number of molts and TD days for pond-reared cohorts. The program fits the simplest join point model given the user defined minimum and maximum number of join points, which I set at zero and three, respectively. Beginning with the minimum number of join points, a Monte Carlo permutation method was used to determine whether more join points are statistically significant, up to the maximum number specified.

Predicted seasonal growth and settlement

I applied the temperature-dependent molt-process model and annual temperature records to predict seasonal growth and settlement dates for juvenile blue crabs collected in the 2001/2002 and 2002/2003 Winter Dredge Surveys (WDS) (Figure 2.4). The WDS is a stratified random dredge survey conducted annually, but over two calendar years (December to March). Samples were collected from c. 1200 stations in waters deeper than 1.5 m using a 1.8 m wide Virginia crab dredge with a 13 mm liner (for more details, see Sharov et al. 2003). For convenience, size distributions for the entire WDS were assigned to 1 January of the second calendar year. Sizes were truncated at 70 mm to incorporate only those individuals in the

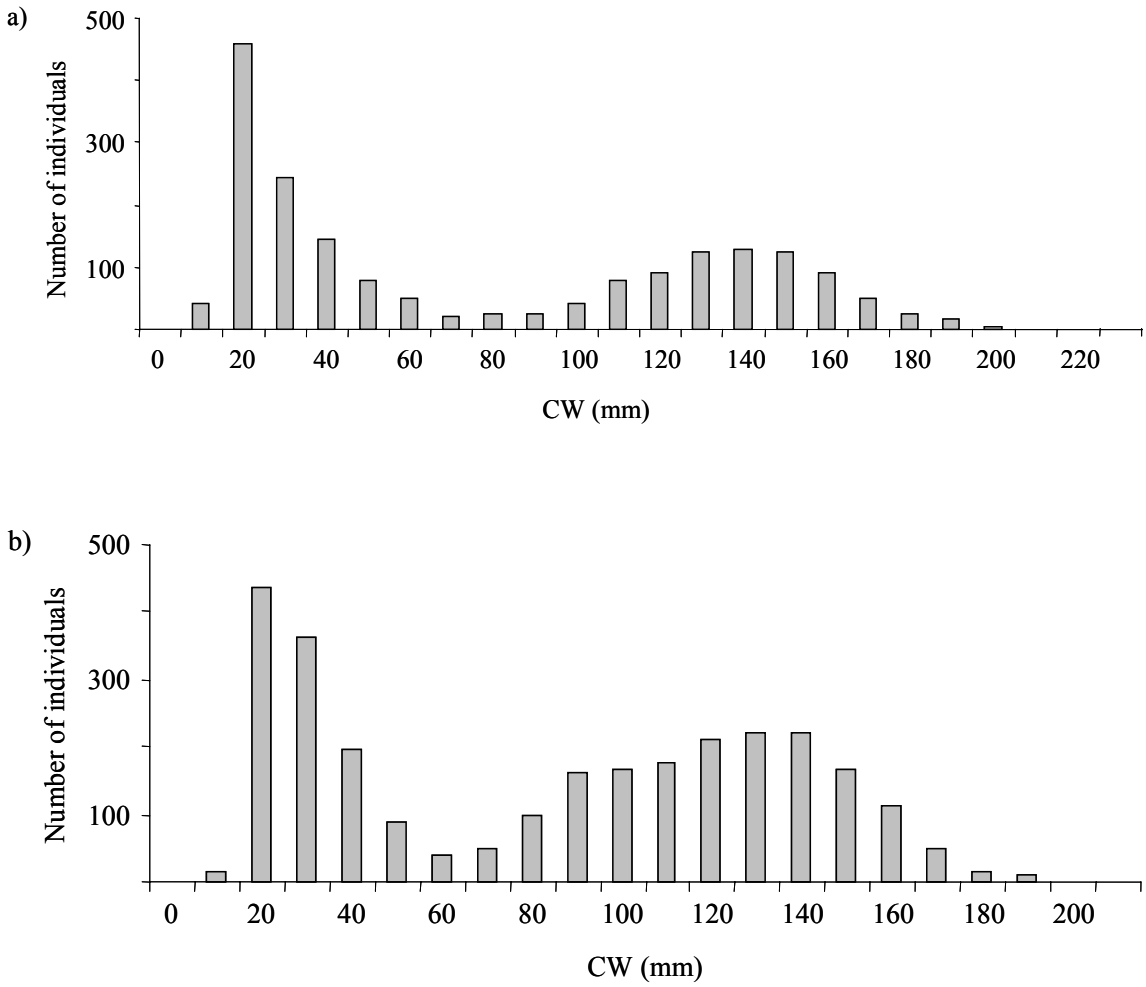

Figure 2.4. Length frequency distribution of blue crabs collected in the a) 2001/2002 and b) 2002/2003 Winter Dredge Survey. Frequencies of 1 (210 and 230 mm size classes in 2001/2002) are not apparent in the figure. Data from MD Department of Natural Resources, Virginia Institute of Marine Science, and NOAA Chesapeake Bay Office.

smaller mode of the bimodal winter size distribution (Figure 2.4). Consequently, initial sizes in model simulations ranged from 5 mm (C3) to 69 mm (C13).

Observed CW (CW_n) for each individual (< 70 mm) originating from the WDS was used to calculate number of molts from settlement and corresponding crab instar by solving for *n* rounded to nearest integer (eq. 2.5, Figure 2.5, for MATLAB code see Appendix 2a). By inverse prediction of the molt-frequency function, number of molts was used to estimate accumulated TD days from settlement (TD day_{WDS}, Figure 2.5, for MATLAB code see Appendix 2b). Thus, predicted TD day_{WDS} was identical for individuals of the same crab instar. Predicted TD day_{WDS} was used to back-calculate date of settlement, which was determined when back-calculated TD days (TD day_t, back-calculated from 1 January on a daily (t) time step) equaled or exceeded predicted TD day_{WDS} (Figure 2.5, for MATLAB code see Appendix 2c). Daily temperature data (2.1 m depth) obtained from Gloucester Point, York River (Virginia Institute of Marine Science 2005; http://www.vims.edu/data_archive) was applied to back-calculate date of settlement due to the prominence of newly settled and early juvenile crabs in the lower Bay. Interpolation was used to estimate gaps in the data record, which were typically less than three consecutive days. Predicted settlement dates were binned by two week intervals.

To project seasonal growth, TD day_{WDS} (calculated as before, Figure 2.6) was increased incrementally by TD day_t, calculated on a daily (t) time step (for MATLAB code see Appendix 2d). The resulting TD days (Σ TD day_t) were input into the molt-process function to predict the number of molts from settlement (n_t) and ensuing

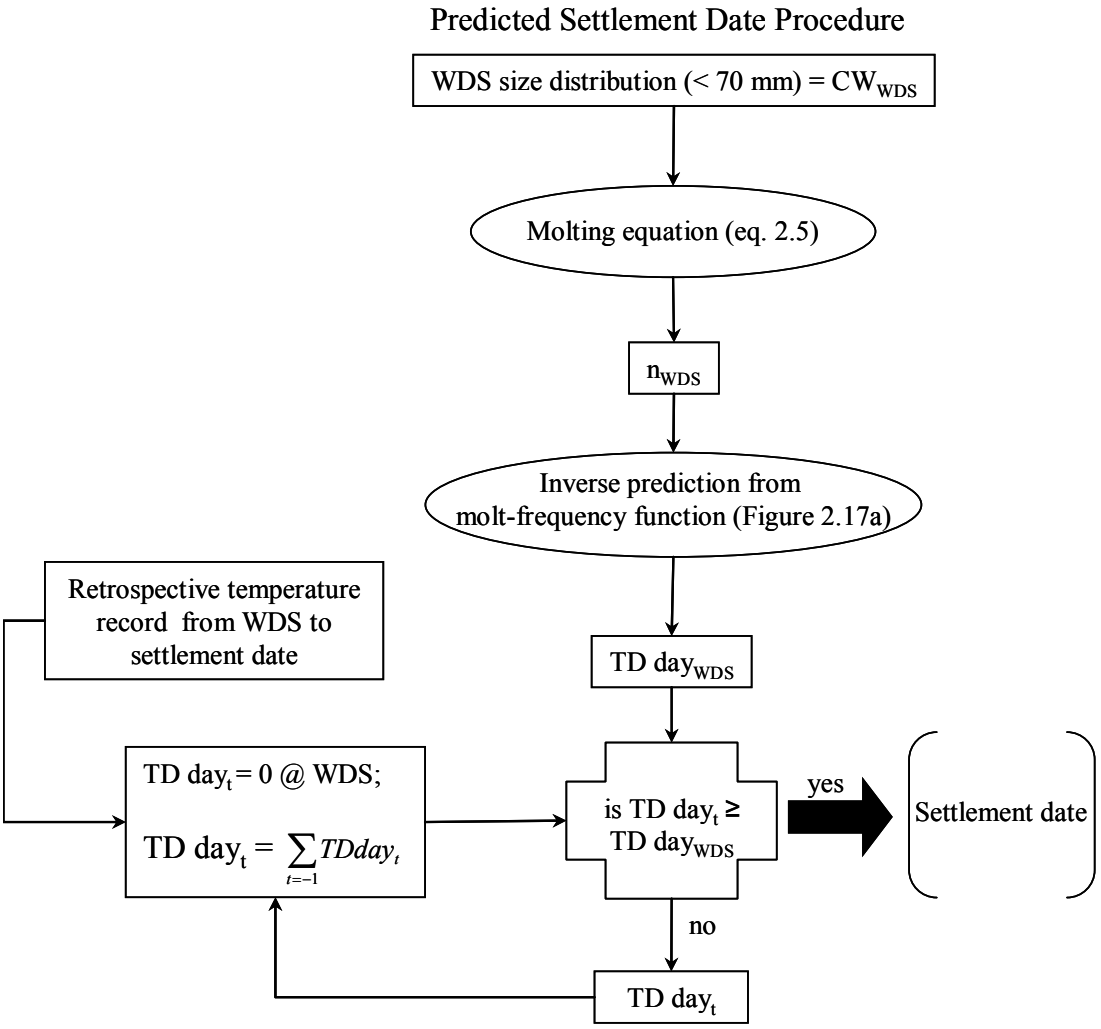

Figure 2.5. Schematic of the back-calculated settlement date procedure. Simulations began with individuals originating from the Winter Dredge Survey; individual carapace width (CW_{WDS}) was converted to number of molts after settlement (n_{WDS}) by solving for n in equation 2.5. The resulting number of molts was input into the molt-process model (derived from pond-rearing experiments), which by inverse prediction estimated TD days from settlement (TD day_{WDS}) for each individual. When back-calculated TD day (TD day_t) either equaled or exceeded TD day_{WDS}, settlement was assumed to have occurred. Note, rectangles represent data input and model parameters, ovals represent formulas, plus-blocks represent decision rules, and solid block arrows and brackets indicate model output.

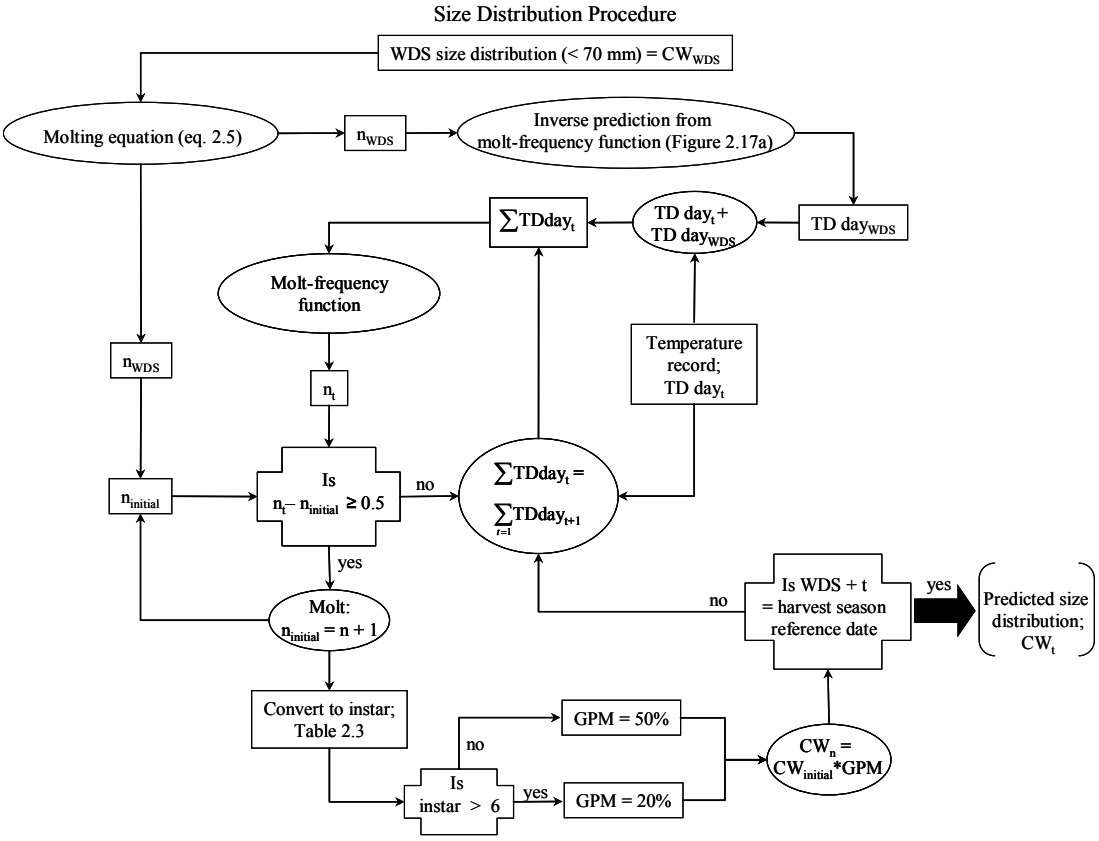

Figure 2.6. Schematic of the projected size distribution procedure. Simulations began with individuals originating from the Winter Dredge Survey. Individual carapace width (CW_{WDS}) was converted to number of molts after settlement by solving for n in equation 2.5. The resulting number of molts provided two components: the initial number of molts ($n_{WDS} = n_{initial}$ at t = 0) and input into the molt-frequency function, which by inverse prediction output TD days from settlement (TD day_{WDS}) for each individual. Cumulative temperature degree-days (ΣTD day_t) were input into the molt-process model to predict the ensuing number of molts (n_t), when the difference between n_t and $n_{initial}$ was greater than 0.5 (i.e. I rounded to nearest integer) a molt was predicted and n was increased by 1. The new number of molts was converted to instar (Table 2.3) to determine the appropriate proportional growth per molt, which was multiplied by the carapace width prior to the molt ($CW_{initial}$). When day, t, was equal to the harvest season reference date, the procedure stopped, yielding a predicted size distribution. Note, rectangles represent data input and model parameters, ovals represent formulas, plus-blocks represent decision rules, and solid block arrows and brackets indicate model output.

crab instar (Figure 2.6, for MATLAB code see Appendix 2e). The number of molts during a particular time period was calculated by simply subtracting the predicted cumulative number of molts (integer) at the beginning of the time interval from cumulative number of molts (integer) at the end of the time interval (Figure 2.6). The corresponding number of molts, n, during an interval and CW at the beginning of the interval ($CW_{initial}$) were substituted into the growth function (eq. 2.5) to solve for CW_n. Daily temperature data (3 m depth), collected from the Horn Point Laboratory research pier (Cambridge, MD; S. Tobash-Alexander unpubl. data), was used to project seasonal size distributions. A mid-Bay location was assumed to be more representative of spring and summer distributions following juvenile dispersal throughout the Bay.

Predicted size distributions were used to determine partial recruitment to the two primary commercial fisheries. Daily growth was modeled beginning on January 1. July was partitioned to account for the changing commercial size limits on July 15 (82.5 to 88.9 mm for peeler/soft crab and 127 to 133.3 mm for hard crab fishery). Model simulations were terminated on December 15, the end of the commercial fishing season in MD. Model simulations were conducted for two years; each year was modeled with and without a mortality component (Figure 2.7).

Mortality was incorporated into the model using the exponential decay equation:

$$N_t = N_0 \times e^{-(F+M)t} \qquad (2.7)$$

where N_t is the population size at time t, N_0 is the initial population size, F is instantaneous fishing mortality rate and M is instantaneous natural mortality rate. At

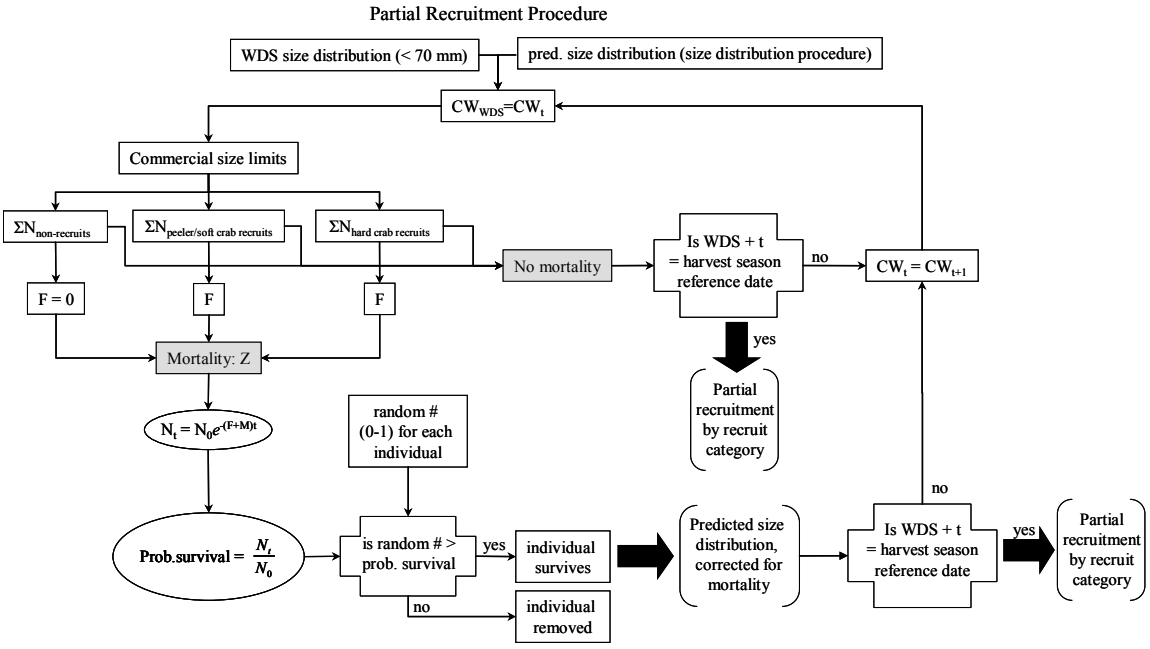

Figure 2.7. Schematic of the partial recruitment procedure. Simulations began with individuals originating from the Winter Dredge Survey. Sizes were partitioned into three categories (non-recruits, peeler/soft crab recruits, and hard crab recruits) based on commercial size limits and predicted carapace width (CW_t). There were two model scenarios: with and without mortality (shaded rectangles). Scenarios without mortality were identical to output obtained from the size distribution procedure. In the mortality scenario, non-recruits were only subjected to natural mortality, and peeler/soft crab and hard crab recruits were subjected to fishing and natural mortality. Each individual (within recruit category) was assigned a random number between 0 and 1. When the random number exceeded the probability of survival, that particular individual was removed. The remaining survivors represented the predicted size distribution (corrected for mortality) and were used to determine partial recruitment to the two primary commercial fisheries when day, t, was equal to the harvest season reference date. The predicted size distribution at the end of one simulation provided the size distribution and initial number of individuals for the next simulation. Size limits for peeler fishery (82.5mm: April 1 to July 14; 88.9mm: July 15-Dec. 15) and hard crab fishery (127mm: April 1 to July 14; 133.4mm: July 15-Dec. 15) vary by season. Note, rectangles represent data input and model parameters, ovals represent formulas, plus- blocks represent decision rules, and solid block arrows and brackets indicate model output.

daily intervals, mortality was calculated for three broad categories (when applicable): non-recruits, peeler/soft crab recruits, and hard crab recruits (Figure 2.7, for MATLAB code see Appendix 2f). Because the peeler/soft crab fishery is dependent upon shell status, recruits were assumed to be susceptible to the fishery 7 days for each predicted molt (Bunnell and Miller 2005). Crabs were not vulnerable to both fisheries on the same day. A random number (between 0 and 1), selected from a uniform distribution, was generated for each individual crab within a respective category. When the random number exceeded the proportion surviving ($\frac{N_t}{N_0}$), the individual was removed from model simulations (Figure 2.7). Annual instantaneous fishing mortalities were assumed to equal 1.3 yr^{-1} in 2002 and 1.0 yr^{-1} in 2003 (Miller et al. 2005). Estimates of F were derived from direct enumeration in which annual catch in numbers (from all sources) were divided by the absolute abundance at the beginning of the year (derived from WDS) resulting in an exploitation rate; fishing mortality was determined using Baranov's catch equation, assuming a constant M (T. Miller, Chesapeake Biological Laboratory, pers. comm.). Total fishing mortality was partitioned among peeler/soft and hard crab recruits based on the number of crabs harvested by each fishery. Peeler/soft crab landings, in numbers, comprised 10% and 6% of the total blue crab landings in the Bay in 2002 and 2003, respectively (Miller et al. 2005). In model simulations, I applied a natural mortality of 0.9 yr^{-1}, which was used in calculating the aforementioned total fishing mortalities (Miller et al. 2005). Annual mortality rates were converted to daily instantaneous rates during model simulations; *F* was divided by 259, the length in days of the MD commercial fishing season, and *M* was divided by 365. I assumed that M was constant across all size

classes and that all recruits to the respective fishery were equally susceptible to fishing mortality (i.e. no size selectivity within the fishery). NORMSEP was used for modal analysis of predicted size distributions. A Chi-square goodness-of-fit test was conducted to determine the effects of mortality scenario on predicted final size distributions and to provide an indication of overall model fit to the subsequent year's observed WDS distribution (converted to relative frequencies). To investigate the potential for pulses of predicted recruitment to co-occur with seasonal changes in commercial landings, 2002 and 2003 monthly peeler/soft and hard crab commercial landings (NMFS Commercial Landing Statistics, Figure 2.1) were compared using a chi-square test of independence. Monthly commercial landings were compared over months in which predicted partial recruitment was different between years.

Results

Pond-reared known-age crabs

Male and female seasonalized VBGF were not significantly different for pond cohorts 1 ($\chi^2 = 10.0$, P = 0.07) and 2 ($\chi^2 = 5.2$, P = 0.4); therefore, data for males and females were combined for further analyses. Regression coefficients for the trend between condition and CW were similar among pond-reared cohorts (F=3.0, P = 0.06) (Figure 2.8a). Seasonalized growth models indicated that cohort 1 (June release) and cohort 2 (October release) were capable of approaching c. 140 mm after one full growth season (March to October, Figure 2.9). Likelihood ratio tests of seasonalized VBGF parameter estimates (Table 2.4), indicated that CW_∞ was slightly higher for cohort 2 ($\chi^2 = 4.2$, P = 0.04) and K was significantly higher for cohort 1

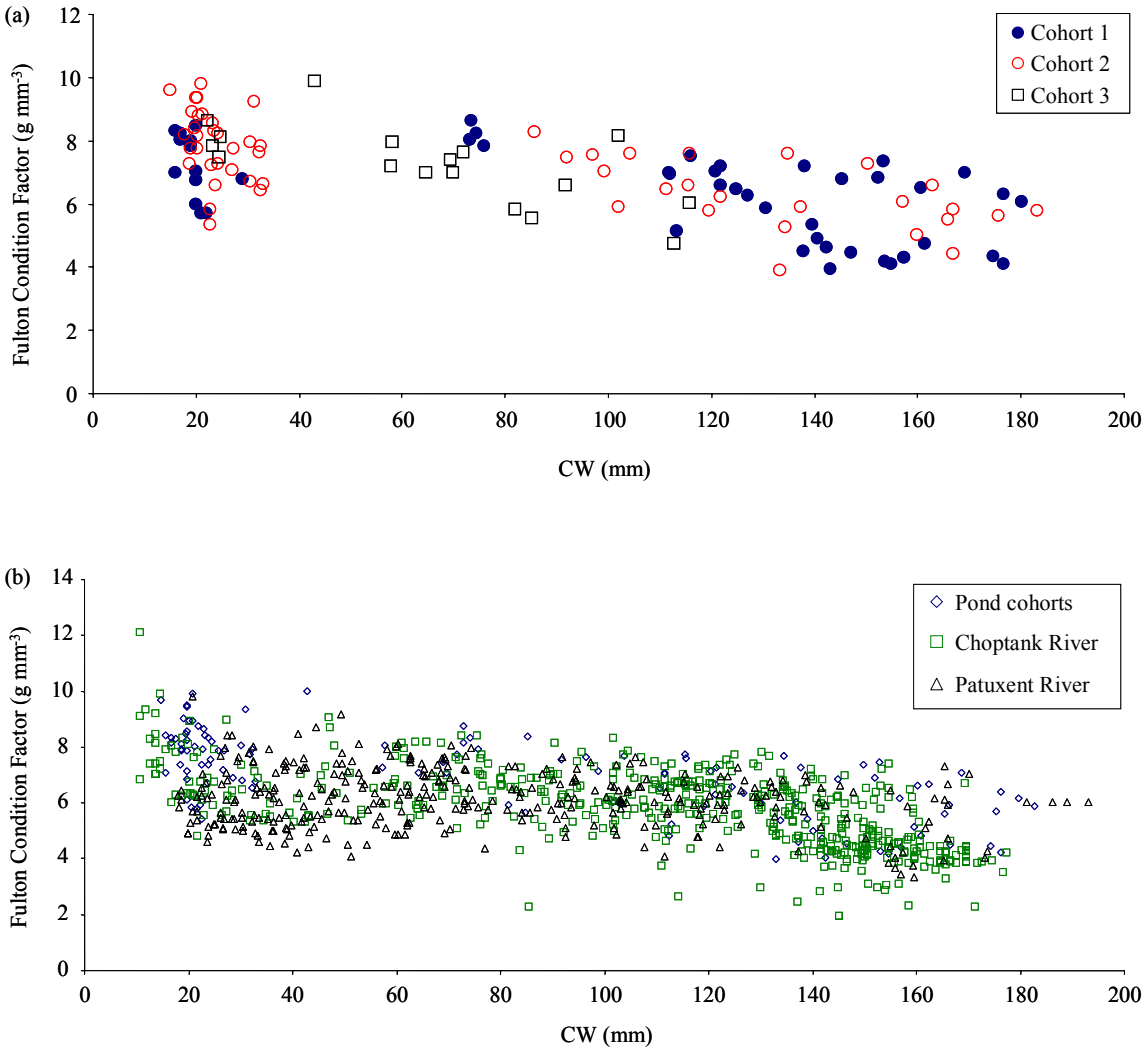

Figure 2.8. Fulton condition factor (g mm^{-3}) at carapace width (mm) for a) three pond-reared cohorts and b) pond-reared, Choptank River, and Patuxent River blue crabs.

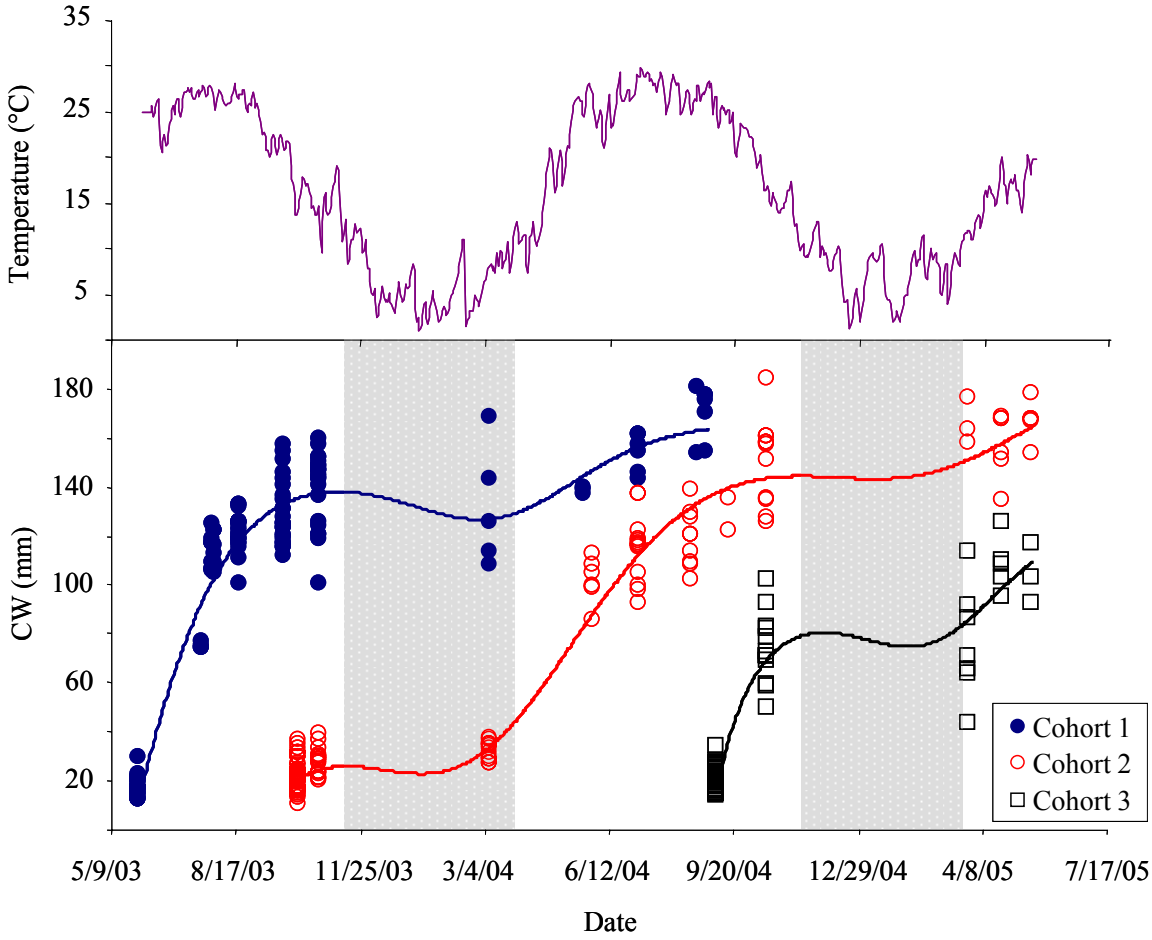

Figure 2.9. Temperature history (upper panel) and observed carapace width (mm) for three pond-reared cohorts released in June 2003 (cohort 1), October 2003 (cohort 2), and September 2004 (cohort 3). Observed carapace width fitted with seasonalized von Bertalanffy growth models (solid lines); von Bertalanffy parameter estimates for each cohort are provided in Table 2.4. Shaded regions represent dates during which water temperatures were below 10 °C.

Table 2.4. Parameter estimates and 95% confidence intervals derived for a seasonalized von Bertalanffy growth model fitted to three known-age pond-reared cohorts. Cohorts were released in June 2003 (cohort 1), October 2003 (cohort 2), and September 2004 (cohort 3). Parameters are defined as follows: CW_∞ is the asymptotic maximum carapace width (mm), K is the annual growth coefficient (yr^{-1}), t_0 is the theoretical age (yr) when length is zero, C is related to the magnitude of the seasonal oscillation, and t_s is the starting age (yr) for the sine curve. Parameter estimates and confidence intervals derived from non-linear regression.

	Seasonalized				
Cohort	CW_∞ (mm) ± CI	K (yr^{-1}) ± CI	t_0 (yr) ± CI	C ± CI	t_s ± CI
1	170.7 ± 10.8	1.9 ± 0.5	-0.03 ± 0.09	0.5 ± 0.08	0.3 ± 0.04
2	216 ± 42	1.0 ± 0.4	0.3 ± 0.04	0.2 ± 0.07	0.9 ± 0.05
3	133.3 ± 14.2	4.7 ± 0.4	0.3 ± 0.02	1.0*	0*

*Parameter estimates constrained to reported values

(χ^2 = 5.0, P = 0.02). Seasonalized VBGF parameter estimates derived from pond cohorts 1 and 2 were similar to those reported in previous studies (Table 2.5, Figure 2.10). Estimates of K were 1.3-3.8 times higher than those estimated from laboratory and length-frequency analysis in Chesapeake Bay. Because cohort 3 had not yet experienced a full growing season by the end of the study, parameter estimates (Table 2.4) were not biologically meaningful. Nevertheless, parameter estimates and length at age data implied rapid growth from release (September) to late fall (Figure 2.9).

As expected, the highest growth rates occurred at small sizes (c. 20 mm) and temperatures greater than 20 °C, during which time mean instantaneous and absolute growth rates of up to 3.1% CW d^{-1} and 1.5 mm d^{-1} (cohort 1) were observed (Figure 2.10 and Figure 2.11). Cohort 3 showed a similar rate of growth after release. No growth was observed from November to March at temperatures less than 10 °C (Figure 2.9 and 2.11). Following initial winter torpor, spring growth rate estimates for cohort 2 approached 1.0% CW d^{-1} and 0.7 mm d^{-1} (Figure 2.11). Despite favorable temperatures, at sizes greater than c. 115 mm, observed growth rates were typically less than 0.3% CW d^{-1} (0.4 mm d^{-1}). Hence, the rapid growth at small sizes and relatively slow growth at larger sizes lead to convergence in observed CW for cohorts 1 and 2 after one full growing season (Figure 2.10).

The high observed growth rates resulted in rapid recruitment to commercial size limits. Cohort 1 (June release), were it a wild cohort, would have recruited to the peeler fishery on average in late July at c. 4 months of age and to the hard crab fishery in early October at c. 7 months of age (Table 2.6, Figure 2.12). Cohort 2

Table 2.5. Von Bertalanffy parameter estimates reported here and in previous studies for blue crabs in Chesapeake and Delaware Bay. Parameters are defined as follows CW_∞ is the asymptotic maximum carapace width (mm), K is the annual growth coefficient (yr^{-1}), and t_0 is the theoretical age (yr) when length is zero. Table adapted from Ju et al. 2001.

Study site	CW_∞ (mm)	K (yr^{-1})	t_0 (yr)	Source
Chesapeake Bay Exp. Pond*	170.7-216	1.0-1.9	-0.03-0.3	This study
Chesapeake Bay Lab and Field	178	0.51	0	Rothschild et al. 1992
Chesapeake Bay Field	262.5	0.59	0.01	Rugolo et al. 1998
Chesapeake Bay Exp. Pond*	207.5	1.2-1.7	0.2-0.3	Ju et al. 2001
Lab	180.9	0.49	0.08	Ju et al. 2001
Delaware Bay Field	200.6-234.7	0.62-0.75	-0.2	Helser and Kahn 1999
Meta-analysis	202.9	0.82	0	Miller et al. 2005

*Parameter estimates derived from a seasonalized von Bertalanffy growth model

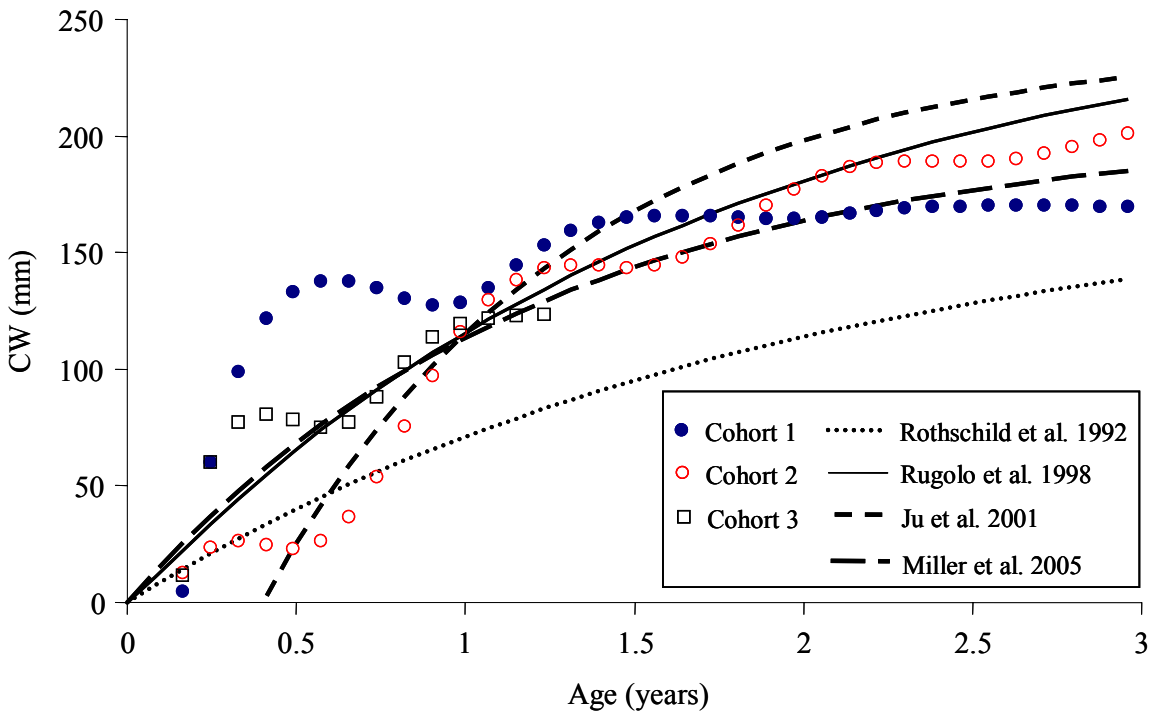

Figure 2.10. Seasonalized von Bertalanffy growth models constructed for three pond-reared cohorts of blue crabs released in June 2003 (cohort 1), October 2003 (cohort 2), and September 2003 (cohort 3). Overlaid are von Bertalanffy growth models reported in previous studies.

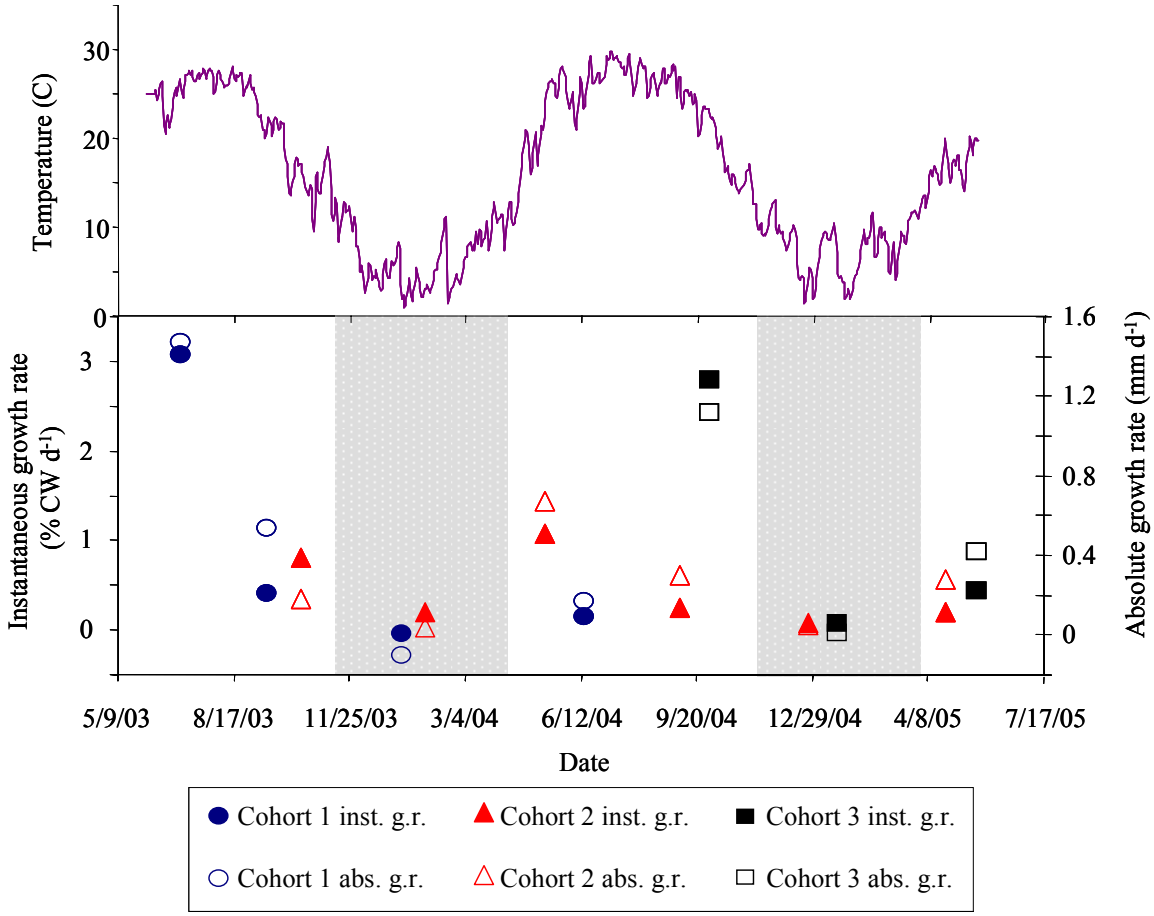

Figure 2.11. Temperature history (upper panel) and mean daily instantaneous (inst. g.r.) and absolute growth rates (abs. g. r.) for three pond-reared cohorts released in June 2003 (cohort 1), October 2003 (cohort 2), and September 2004 (cohort 3). Growth rates were estimated from seasonalized von Bertalanffy growth models constructed for each cohort (Figure 2.9). Dates are presented as mean date of the time interval over which growth rate was estimated. Shaded regions represent dates during which water temperatures were below 10 °C.

Table 2.6. Mean age (days), date, and temperature degree-day (TD day) at recruitment for three pond-reared cohorts released in June 2003 (cohort 1), October 2003 (cohort 2), and September 2004 (cohort 3). Mean recruitment determined when 50% of the cohort obtained legal commercial size. Size limits for peeler/soft crab fishery (82.5 mm: 1 April to 14 July; 88.9 mm: 15 July to 15 December) and hard crab fishery (127 mm: 1 April to 14 July; 133.3 mm: 15 July to 15 December) vary by season.

	Mean age (days) and date at recruitment		Mean TD day at recruitment	
Cohort	Peeler/soft crab fishery	Hard crab fishery	Peeler/soft crab fishery	Hard crab fishery
1	123, 29 July 2003	194, 8 Oct. 2003	1227	2228
2	314, 26 May 2003	430, 20 Sept. 2004	1060	3050
3	268, 31 March 2005	N/A	949	N/A

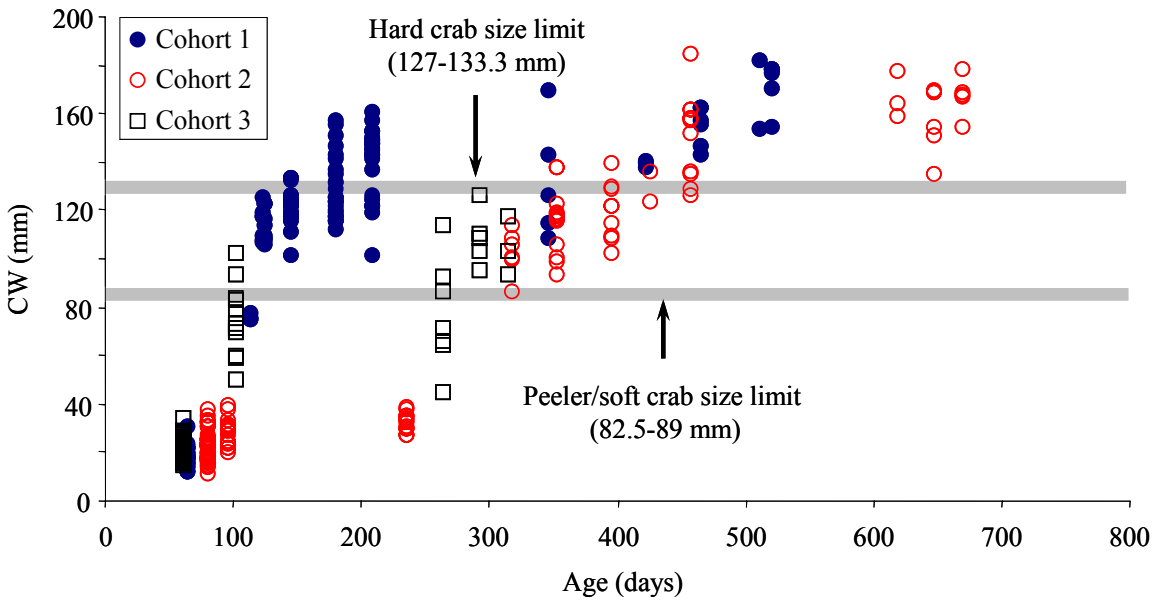

Figure 2.12. Observed carapace width (mm) at age for three known-age pond-reared cohorts. Horizontal bars represent legal commercial size limits for the peeler/soft crab and hard crab fisheries. Size limits for peeler/soft crabs (82.5 mm: 1 April to 14 July; 88.9 mm: 15 July to 15 December) and hard crabs (127 mm: 1 April to 14 July; 133.3 mm: 15 July to 15 December) vary by season.

(October release) overwintered soon after release, delaying its predicted recruitment. Mean recruitment of cohort 2, to the peeler and hard crab fisheries, would have occurred in May and September at c. 10 and 14 months of age, respectively (Table 2.6, Figure 2.12). Cohort 3 would have recruited on average to the peeler fishery in late March at c. 9 months of age (Table 2.6, Figure 2.12). The duration of rearing for cohort 3 remained insufficient to determine recruitment to the hard crab fishery. Despite the different temperature histories experienced by pond cohorts (i.e. June 03, October 03, and September 04 release dates), mean TD day at predicted recruitment was far less variable than either mean age or date at recruitment (Table 2.6).

Length frequency modal analysis

During 2003 and 2004, 635 crabs from Choptank River and 464 crabs from Patuxent River were measured for length frequency analysis. Assuming (1) early production was pulsed, (2) no net influx or efflux into or out of the system, and (3) collection methods did not bias size distributions, modal progression of cohorts was estimated from length frequency analysis. There was lack of significant modal fits to length frequency data in many months (Table 2.7), despite the overall appearance of normality among modes. Two sub-annual cohorts were identified from Choptank River and Patuxent River samples (Table 2.8, Figure 2.13 and 2.14).

Modal mean CW at date for the first Choptank River (2003) cohort occurred at 49 mm in June, 78 mm in July, 113 mm in August, and 128 mm in September (Table 2.8, Figure 2.13). Modal mean at date for the second cohort occurred at 87 mm, 138 mm, 161 mm, and 157 mm in June, July, August, and September,

Table 2.7. Results of chi-square goodness-of-fit test conducted for Choptank River (2003) and Patuxent River (2004) length frequency modal analyses. Significant P values (*, $\alpha = 0.05$) indicate that monthly modes were not normally distributed.

Choptank River (2003)			
Month	Total χ^2	df	P
June	31.6	19	0.04*
July	33.2	16	0.007*
August	18.3	13	0.15
September	26.0	9	0.002*
October	29.3	8	0.0003*

Patuxent River (2004)			
Month	Total χ^2	df	P
June	14.4	6	0.03*
July	22.0	15	0.1
August	13.0	12	0.4
September	6.2	16	0.9
October	30.3	18	0.03*

Table 2.8. Modal means (± 1 standard deviation) obtained from Choptank River (2003) and Patuxent River (2004) length frequency modal analysis. Modes were assumed to represent distinct cohorts. Choptank River cohort designations are those from Figure 2.13 and mean modal carapace widths (CW) presented here are plotted in Figure 2.15a. Patuxent River cohort designations are those from Figure 2.14 and mean modal CW presented here are plotted in Figure 2.15b.

Choptank River (2003): Mean monthly CW (mm) ± s.d.					
Cohort	16 June	15 July	25 August	26 September	24 October
1	49.4 ± 9.8	78.2 ± 21.8	113.3 ± 20.9	128.6 ± 25.2	121.1 ± 14.8
2	87.0 ± 4.8	137.7 ± 14.5	160.8 ± 9.9	157.0 ± 11.2	161.5 ± 10.6

Patuxent River (2004): Mean monthly CW (mm) ± s.d.						
Cohort	10 June	24 June	12 July	4 Aug.	10 Sept.	6 Oct.
1	33.3 ± 4.8	62.3 ± 20.5	61.3 ± 13.1	108.8 ± 11.1	138.8 ± 18.8	162.0 ± 17.5
2	-	-	111.6 ± 18.8	140.1 ± 20.1	170.4 ± 10.3	162.0 ± 17.5

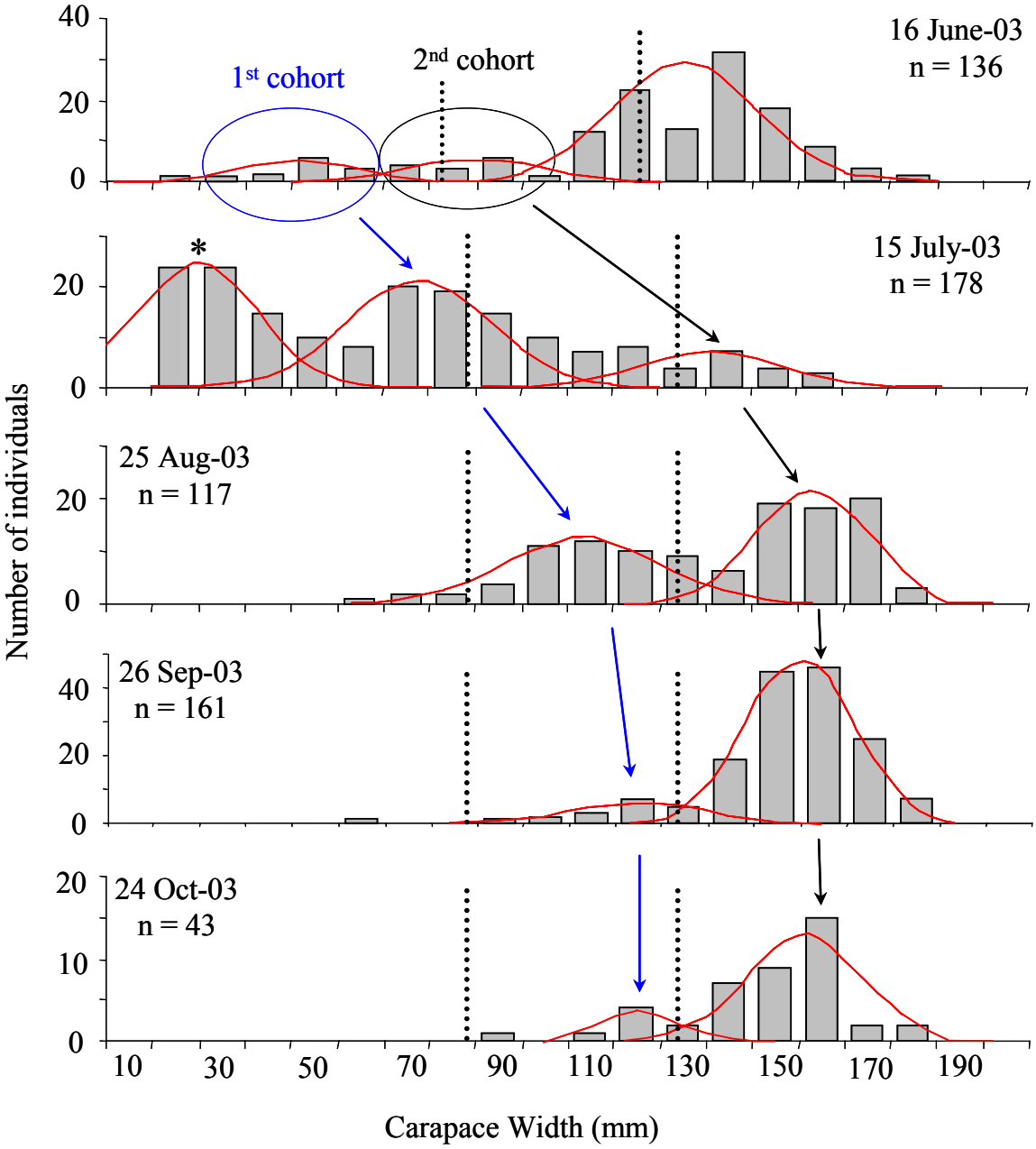

Figure 2.13. Length frequency histogram showing monthly modal progressions (arrows) of two sub-annual cohorts of blue crabs collected from the Choptank River (June to October 2003). Vertical dashed lines represent commercial size limits for the peeler/soft crab and hard crab fisheries. On 15 July, size limits change from 82.5 to 88.9 mm (peeler/soft crabs) and 127 to 133.3 mm (hard crab). Note, the smallest mode on 15 July (*) was not included in modal progression because those crabs were collected with a dip net from floating vegetation.

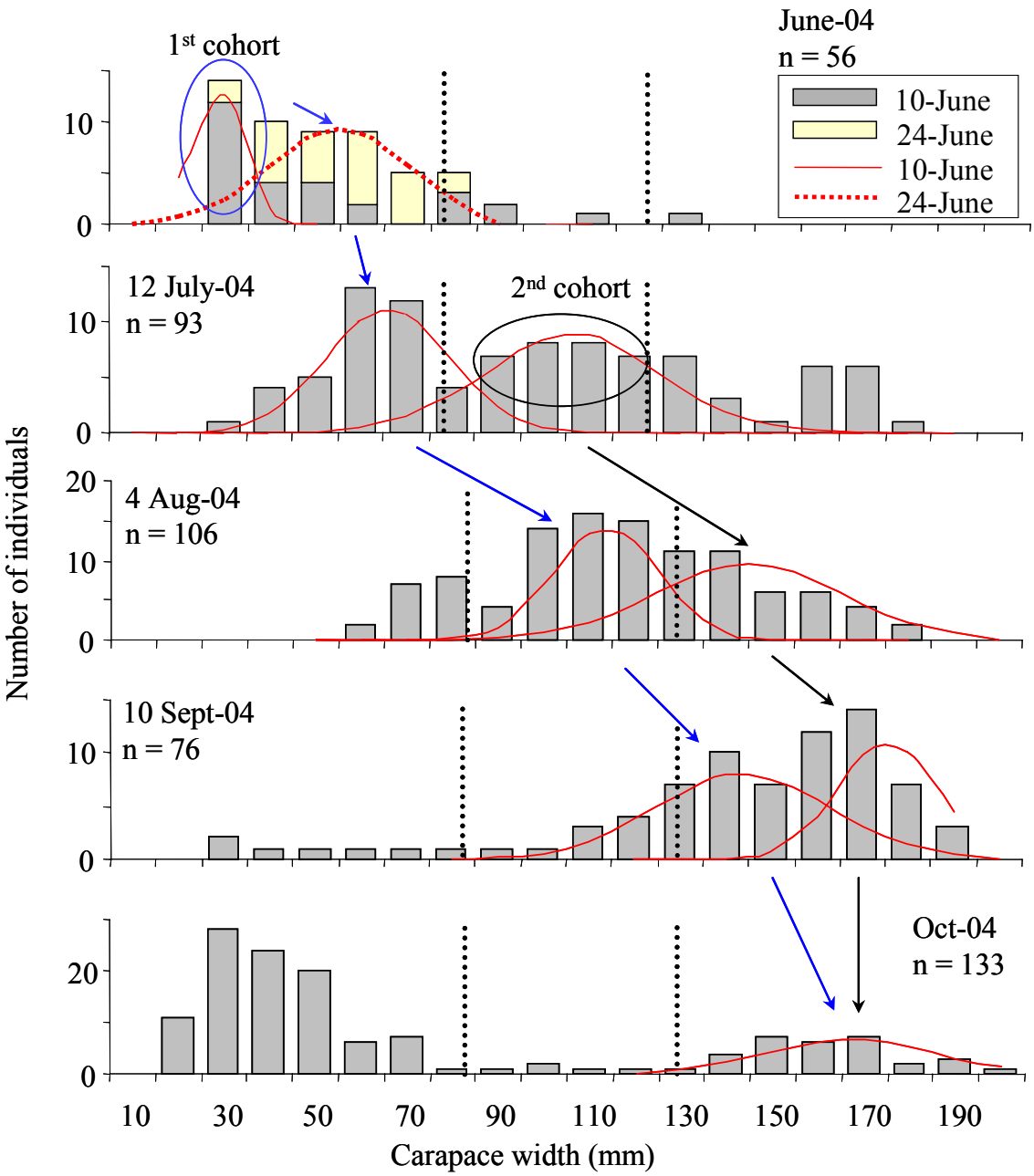

Figure 2.14. Length frequency histogram showing monthly modal progressions (arrows) of two sub-annual cohorts of blue crabs collected from the Patuxent River (June to October 2004). Two seine collections, separated by two weeks, were conducted in June. Vertical dashed lines represent commercial size limits for the peeler/soft crab and hard crab fisheries. On 15 July, size limits change from 82.5 to 88.9 mm (peeler/soft crabs) and 127 to 133.3 mm (hard crab).

respectively (Table 2.8, Figure 2.13). The first Patuxent River (2004) cohort progressed from 33 mm in early June, to 60 mm in late June, 109 mm in August, and 139 mm in September (Table 2.8, Figure 2.14). The second cohort progressed from 112 mm in July to 170 mm in September (Table 2.8, Figure 2.14). In October, modal progressions slowed for all cohorts excluding Patuxent River cohort 1. Choptank and Patuxent River cohorts exhibited seasonal growth variability similar to that observed for corresponding pond-reared cohorts (Table 2.9, Figure 2.15).

Mean instantaneous growth rates (June to October) for Choptank River cohorts 1 and 2 were 0.7% and 0.5% CW d^{-1}, respectively (Table 2.9). Linear growth rates over the same time period (Table 2.9) were not significantly different ($F = 0.1$, $P = 0.7$) among Choptank River cohorts, but the intercept for cohort 1 was significantly smaller than cohort 2 ($t = -4.3$, adj. $P = 0.004$). Mean instantaneous growth rates for Patuxent River cohorts 1 and 2 (June to October) were 1.4% and 0.5% CW d^{-1}, respectively (Table 2.9). Linear growth rates ($F = 2.9$, $P = 0.2$) and intercepts ($t = -2.4$, adj. $P = 0.06$) were not significantly different, although intercepts were nearly different. Linear growth rate for Patuxent River cohort 1 was significantly higher ($F = 11.5$, $P = 0.01$) than estimates for Choptank River cohort 1; growth rates for cohort 2 across systems were not significantly different ($F = 2.7$, $P = 0.2$). Intercepts among same ordered cohorts across systems were not significantly different (cohort 1: $t = -1.0$, adj. $P = 0.4$; cohort 2: $t = -2.4$, adj. $P = 0.06$). Condition of Patuxent River samples was significantly higher than Choptank River samples ($F = 68.1$, $P < 0.0001$) and pond-reared cohorts ($F = 28.7$, $P = <0.0001$, Figure 2.8b). Condition of Choptank River samples and pond-reared cohorts was similar ($F = 2.2$, $P = 0.1$).

Table 2.9. Estimates of absolute and instantaneous growth rates for pond-reared and field-collected cohorts. Growth rates were determined from June to October, unless otherwise noted.

Cohort	Abs. growth rate (mm d^{-1})	Inst. growth rate (% CWd^{-1})
Pond cohort 1	0.8	1.4
Pond cohort 2	0.4	0.3
Pond cohort 3*	1.1	2.6
Choptank cohort 1	0.6	0.7
Choptank cohort 2	0.5	0.5
Patuxent cohort 1	1.1	1.4
Patuxent cohort 2^{+}	0.6	0.5

*Growth rates determined from September to October

$^{+}$Growth rates determined from July to October

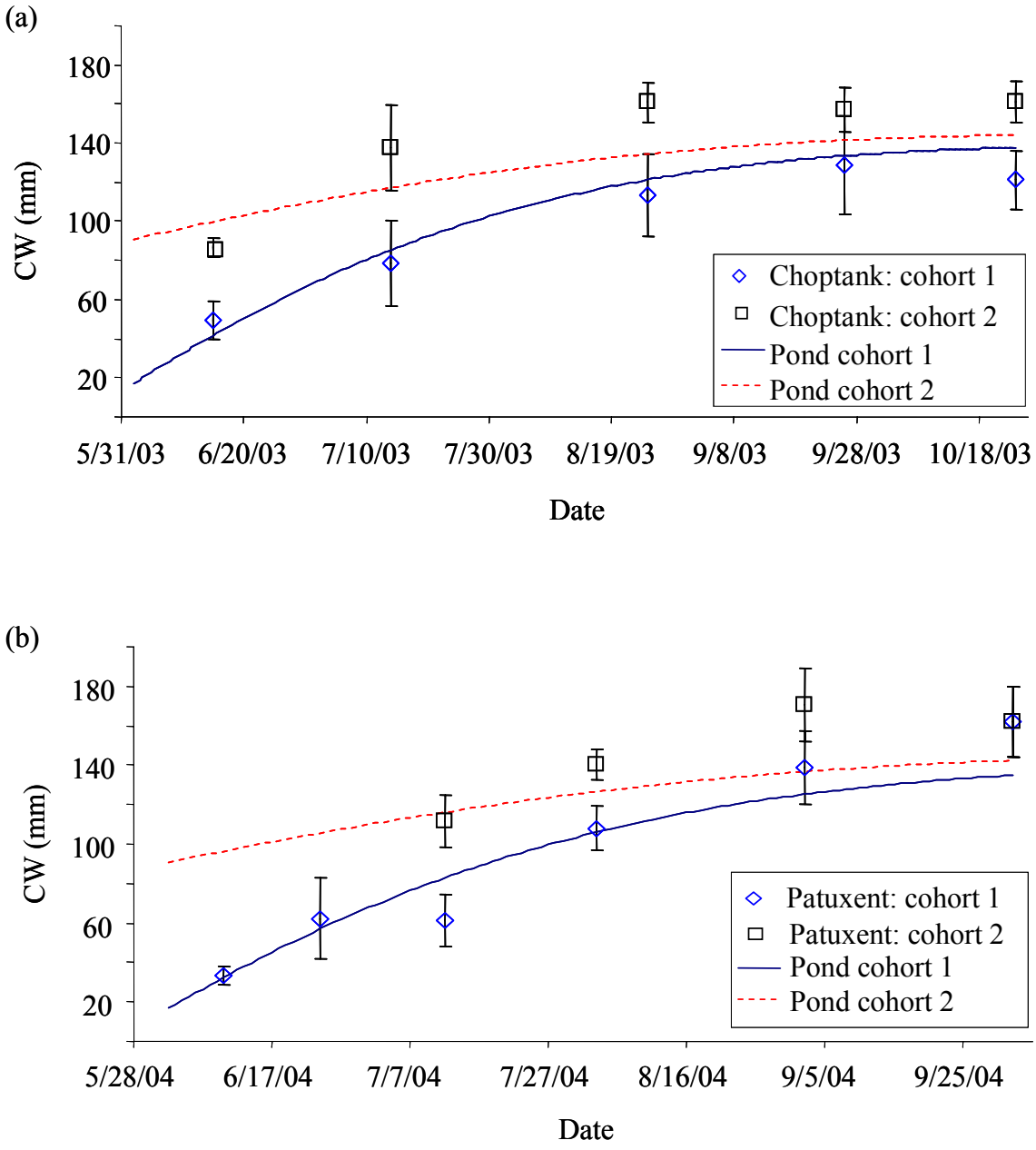

Figure 2.15. Modal means (± 1 standard deviation) obtained from length frequency modal analysis of a) Choptank River samples and b) Patuxent River samples. Cohort designations are those in Figure 2.13 (Choptank R.) and Figure 2.14 (Patuxent R.) and Table 2.8. The solid and dashed lines represent seasonalized von Bertalanffy growth curves derived for pond cohorts 1 and 2, respectively.

Differences in growth rates for cohort 1 across systems did not affect mean date of recruitment to the peeler fishery, which was estimated at July 19 (2003) and July 17 (2004) in the Choptank and Patuxent Rivers, respectively (Table 2.10, Figure 2.16 a, c). The higher growth rates for Patuxent River cohort 1 had a more pronounced influence on mean recruitment to the hard crab fishery. Patuxent River cohort 1 recruited on average to the hard crab fishery in late August, whereas mean recruitment for Choptank River cohort 1 occurred after our last sampling event (October 24), but likely the following spring (Table 2.10, Figure 2.16 b, d). Recruitment to the hard crab fishery was similar across systems for cohort 2, with mean recruitment occurring during July in both systems.

Temperature-dependent growth

Join point regression analysis of the molt-frequency function (Figure 2.17a) yielded a single join point at 1093 TD days (95% CI: 938—1678 TD days). Therefore, two distinct linear regressions, with significantly different slopes ($p = 0.0002$), were used to estimate mean number of molts < 1093 TD days ($y = -1.2 + 0.01x$) and ≥ 1093 TD days ($y = 12.9 + 0.0009x$). The resultant molt-process growth model appeared quite robust over the duration of pond rearing, although, observed CW was slightly under-estimated from c. 70 to c. 100 mm (Figure 2.17b).

Model simulations began with an initial population size of 1041 (2002) and 1180 (2003) crabs, representing the first mode of the bimodal WDS size distribution. For both years of model simulations, mean modal size of the winter-time "juvenile" (< 70 mm) cohort occurred at c. 20 mm CW (C6). Predicted dates of settlement

Table 2.10. Mean date at recruitment for Choptank and Patuxent River cohorts. Mean date at recruitment determined when 50% of the cohort obtained legal commercial size. Size limits for peeler/soft crab fishery (82.5 mm: 1 April to 14 July; 88.9 mm: 15 July to 15 December) and hard crab fishery (127 mm: 1 April to 14 July; 133.3 mm: 15 July to 15 December) vary by season.

	Mean date @ recruitment	
Cohort	Peeler/soft crab fishery	Hard crab fishery
Choptank 1	19 July 2003	After 24 October 2003 (last sampling event)
Choptank 2	Prior to 16 June 2003 (first sampling event)	9 July 2003
Patuxent 1	17 July 2004	29 August 2004
Patuxent 2	Prior to 12 July 2004 (first detection)	28 July 2004

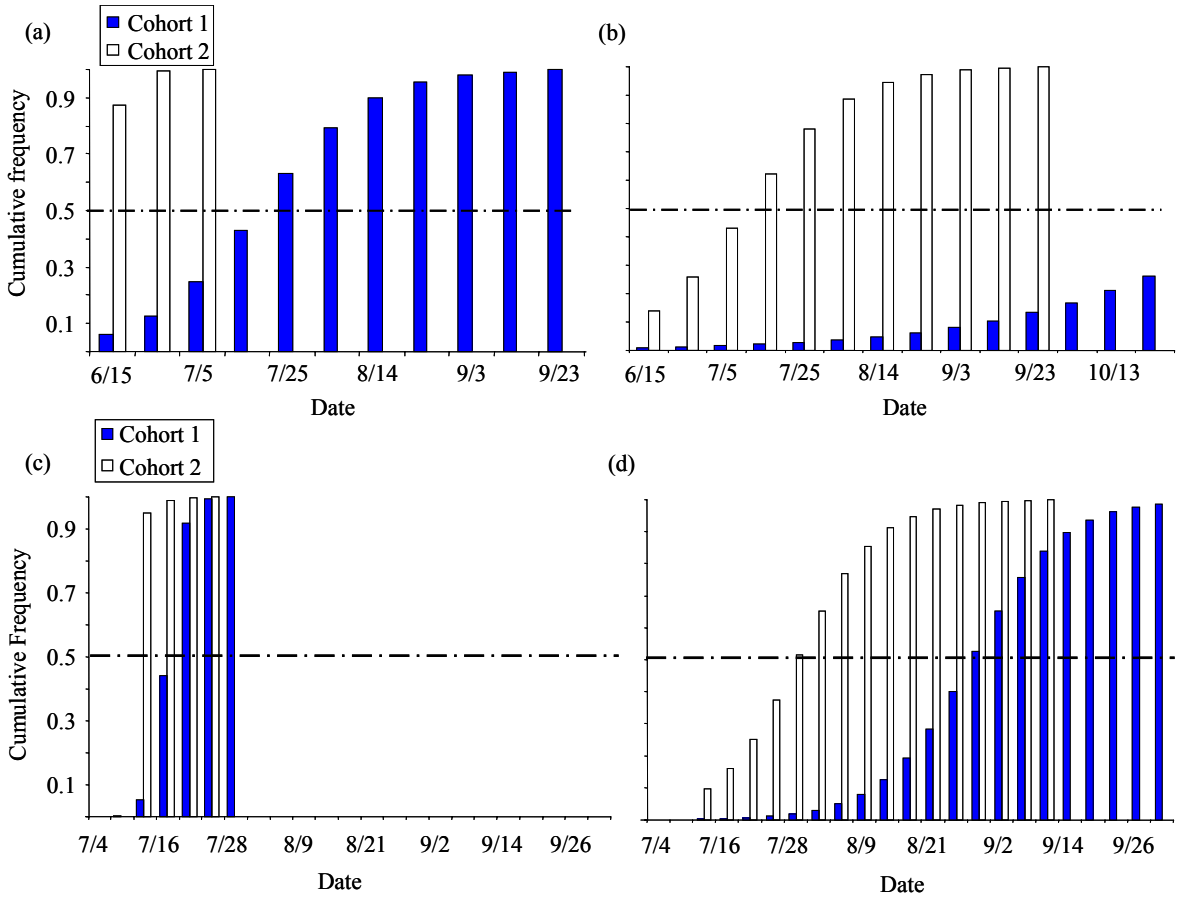

Figure 2.16. Cumulative probability plot of partial recruitments for Choptank River cohorts to a) the peeler/soft crab fishery and b) the hard crab fishery, and Patuxent River cohorts to c) the peeler/soft crab fishery and d) the hard crab fishery. Cohort designations are those in Figures 2.13 (Choptank R.) and 2.14 (Patuxent R.) and Table 2.8. Dotted and dashed horizontal lines represent mean (50%) recruitment.

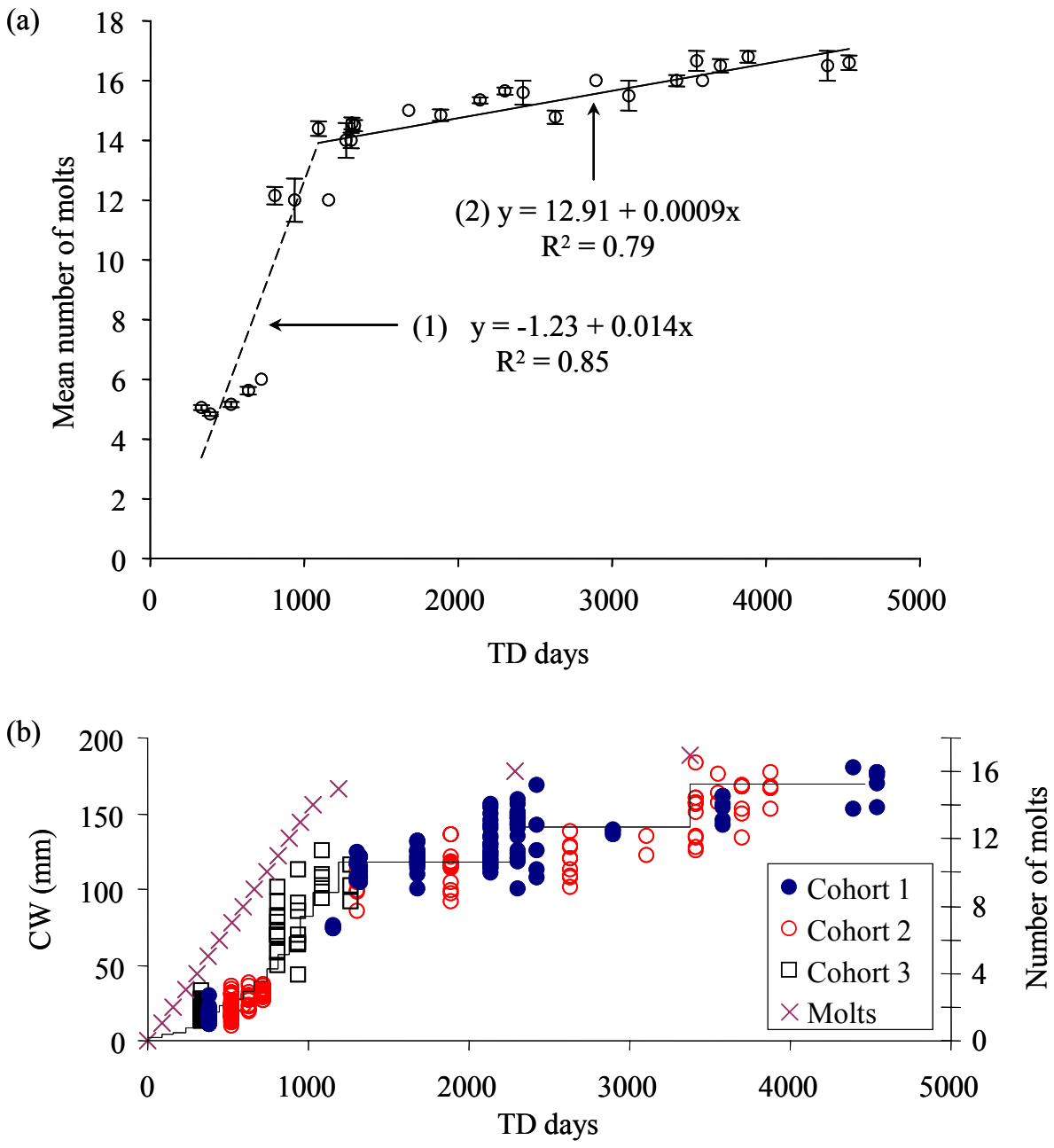

Figure 2.17. (a) Join point regression molt-frequency function derived from three pond-reared cohorts and used to predict number of molts from settlement as a function of temperature degree-days (TD days). A single join point was designated at 1093 TD days. Therefore, linear regression 1 and 2 were used to estimate mean number of molts < 1093 TD days and ≥ 1093 TD days, respectively. Standard errors are represented by the error bars. (b) Predicted carapace width (solid line) and number of molts (x) predicted from the molt-process model. Growth per molt was assumed to be 50% from C1 (2.5 mm) to C6 (c. 20 mm) and 20% thereafter.

ranged from 31 August (C13) to 30 October (C3) in 2001 and from 26 August (C13) to 7 October (C3) in 2002 (Figure 2.18). Despite estimating settlement dates for similar size-distributions in both years of model simulations, ranges of settlement dates differed between years due to discrepancies in temperature. Temperatures experienced by early juveniles near the mouth of the Bay from August to December were 10% warmer (i.e., 10% more TD days accumulated) in 2001 than 2002, hence fewer days in 2001 were required to surpass TD day_{WDS}.

Mortality did not affect seasonal growth projections (Figure 2.19 and 2.20) or final size distributions in 2003 ($\chi^2 = 5.1$, $P = 0.5$), but had a significant affect on the final size distributions in 2002 ($\chi^2 = 14.9$, $P = 0.02$). In both years, juveniles began recruiting to the peeler/soft crab fishery prior to 1 June (Table 2.11, Figure 2.19 and 2.20). The majority of recruitment to the peeler/soft crab fishery occurred during June in both years. Complete recruitment (i.e., 100%) to the peeler/soft crab fishery was predicted to occur by 1 September. Recruitment to the hard crab fishery began prior to 1 August in 2002 and prior to 1 September in 2003 (Table 2.11, Figure 2.19 and 2.20). Mean recruitment to the hard crab fishery occurred prior to 1 November in 2002 and during November in 2003. Trends of earlier recruitment in 2002 (Table 2.11) were associated with temperatures, which were c. 10% warmer from 1 January to 31 December**.** Despite earlier predicted recruitment to both fisheries in 2002 due to temperature differences, monthly peeler/soft crab landings from May to July and hard crab landings from July to

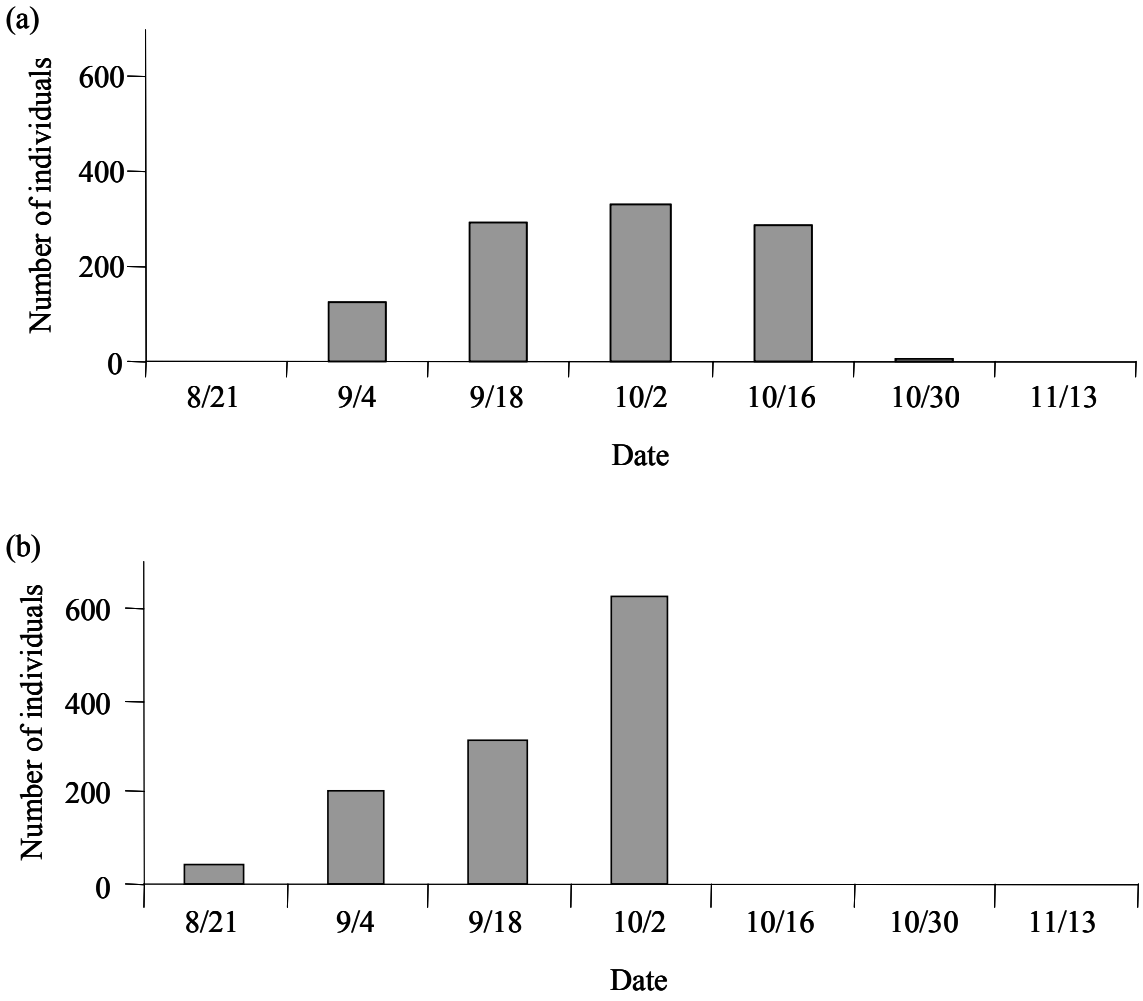

Figure 2.18. Back-calculated settlement date for crabs (< 70 mm) originating from a) the 2001/2002 and b) the 2002/2003 Winter Dredge Surveys. Settlement date represents the mean date of two week bin intervals.

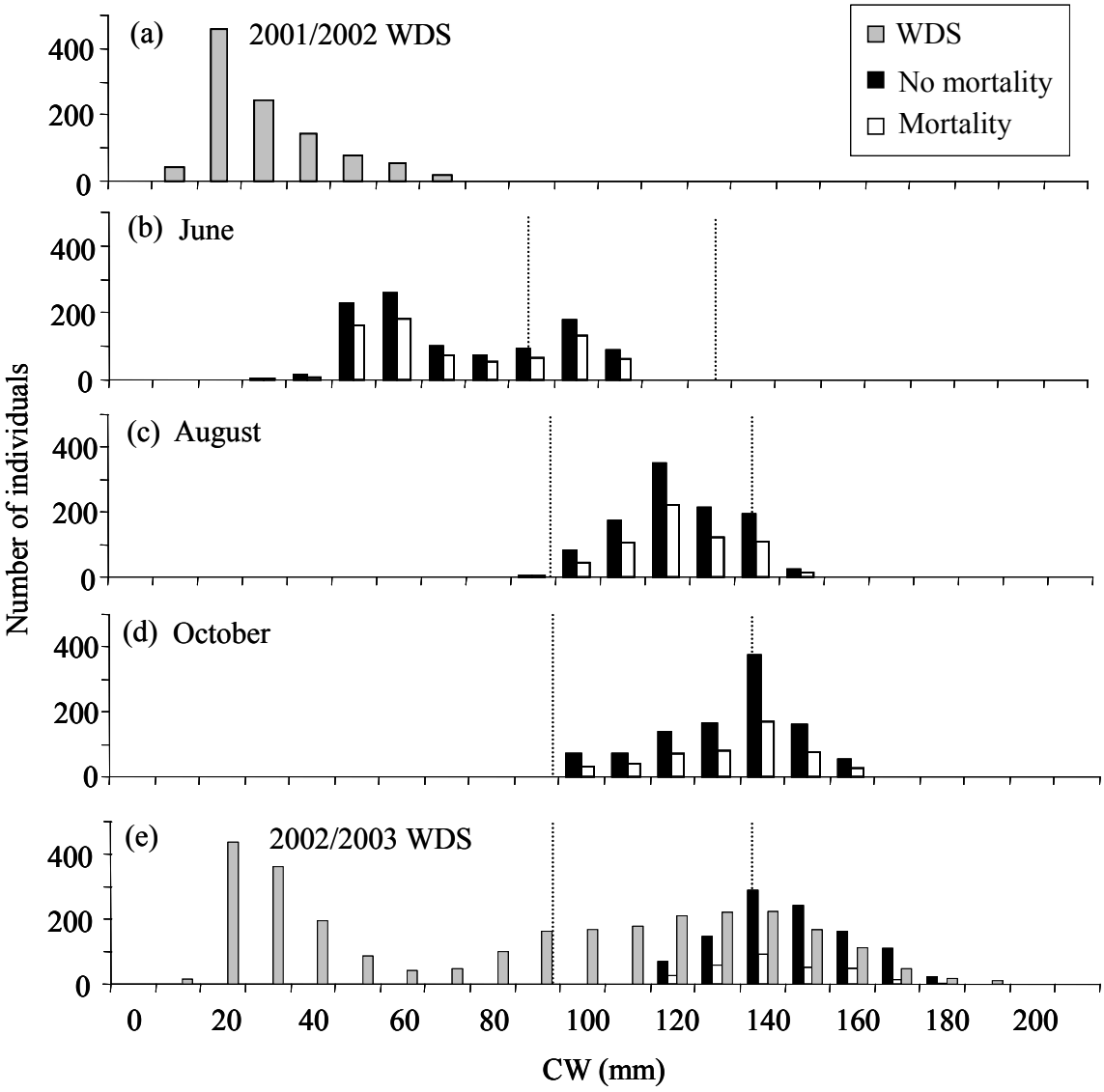

Figure 2.19. Predicted seasonal size distributions with (open bars) and without (closed bars) mortality. (a) Size distribution of crabs < 70 mm originating from the 2001/2002 Winter Dredge Survey (n =1041, shaded bars) and predicted size distribution in b) June, c) August, d) October, e) 15 December and corresponding 2002/2003 Winter Dredge Survey (WDS, shaded bars). Vertical dotted lines represent the legal commercial size limits. Size limits for peeler/soft crabs (82.5 mm: 1 April to 14 July; 88.9 mm: 15 July to 15 December) and hard crabs (127 mm: 1 April to 14 July; 133.3 mm: 15 July to 15 December) vary by season.

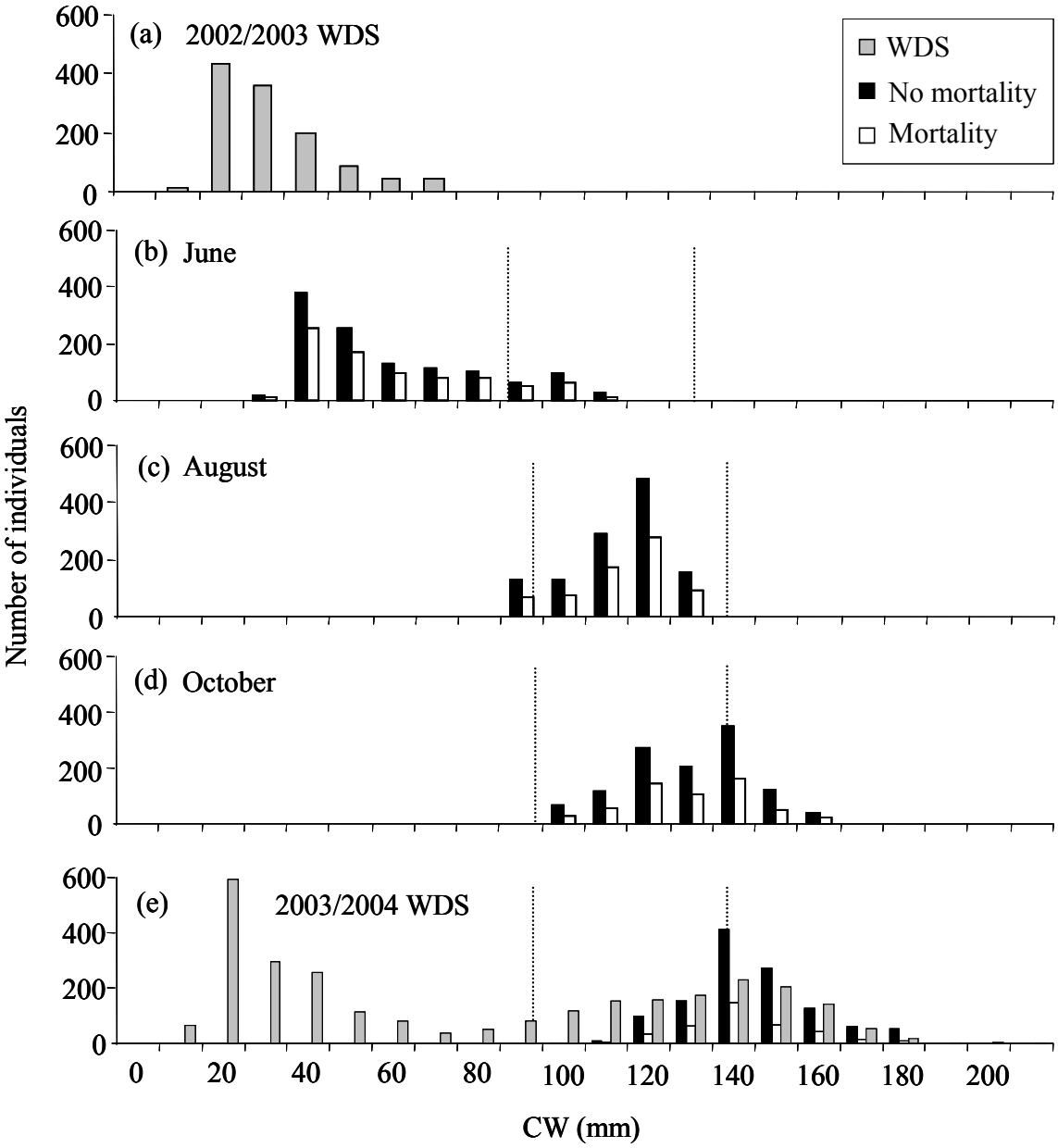

Figure 2.20. Predicted seasonal size distributions with (open bars) and without (closed bars) mortality. (a) Size distribution of crabs < 70 mm originating from the 2002/2003 Winter Dredge Survey (n = 1180, shaded bars) and predicted size distribution in b) June, c) August, d) October, e) 15 December (closed bars) and corresponding 2003/2004 Winter Dredge Survey (WDS, shaded bars). Vertical dotted lines represent the legal commercial size limits. Size limits for peeler/soft crabs (82.5 mm: 1 April to 14 July; 88.9 mm: 15 July to 15 December) and hard crabs (127 mm: 1 April to 14 July; 133.3 mm: 15 July to 15 December) vary by season.

Table 2.11. Estimated number of blue crab recruits and cumulative percentage (in parentheses) for two years of model simulations determined from predicted monthly size distributions of blue crabs (< 70 mm CW) originating from the 2001/2002 and 2002/2003 Winter Dredge Survey. Size limits for peeler/soft crab fishery (82.5 mm: 1 April to 14 July; 88.9 mm: 15 July to 15 December) and hard crab fishery (127 mm: 1 April to 14 July; 133.3 mm: 15 July to 15 December) vary by season.

	2001/2002 WDS				2002/2003 WDS			
	No mortality		Mortality		No mortality		Mortality	
Date	Peeler/soft crab	Hard crab	Peeler/soft crab	Hard crab	Peeler/soft crab	Hard crab	Peeler/soft crab	Hard crab
1 Jan.	0	0	0	0	0	0	0	0
1 April	0	0	0	0	0	0	0	0
1 May	0	0	0	0	0	0	0	0
1 June	340 (0.33)	0	241 (0.33)	0	178 (0.15)	0	119 (0.15)	0
1 July	1039 (0.99)	2 (0.002)	676 (0.99)	0	1130 (0.96)	0	726 (0.96)	0
1 Aug.	1037 (0.99)	121 (0.12)	615 (0.99)	66 (0.11)	1052 (0.89)	0	621 (0.90)	0
1 Sept.	1041 (1)	176 (0.17)	556 (1)	79 (0.14)	1180 (1)	186 (0.16)	622 (1)	87 (0.14)
1 Oct.	1041 (1)	447 (0.43)	495 (1)	193 (0.39)	1180 (1)	373 (0.32)	574 (1)	168 (0.29)
1 Nov.	1041 (1)	774 (0.74)	400 (1)	278 (0.70)	1180 (1)	597 (0.51)	511 (1)	237 (0.46)
15 Dec.	1041 (1)	774 (0.74)	295 (1)	182 (0.62)	1180 (1)	812 (0.69)	376 (1)	226 (0.60)

November were not significantly different among years ($\chi^2 = 0.4$, P = 0.8; $\chi^2 = 6.1$, P = 0.1).

In 2002, crabs accumulated 2621 TD days during model simulations. All simulated crabs were predicted to reach instar 17 (Table 2.3, Figure 2.17a) by October 29, after which growth ceased. In contrast, simulated crabs accumulated 2398 TD days during 2003. Therefore, only 55% of crabs reached instar 17 by October 29 and when growth ceased (November 17), 85% of crabs reached instar 17. Despite having the same instar at the end of model simulations, there was considerable variation in CW (117-171 mm, Figure 2.19 and 2.20). Interestingly, the smallest crabs (< 20 mm) at the beginning of model simulations were not necessarily the smallest crabs at the end of model simulations (Figure 2.21). Predicted size distribution at the end of model simulations (15 December) were similar to observed size distributions of crabs ≥ 120 mm CW collected from the 2002/2003 and 2003/2004 WDS (Table 2.12, Figure 2.19 and 2.20). Modal means of final predicted size distributions (Table 2.12) for both years (and mortality scenarios) were within ± 2 standard deviations of the corresponding WDS modal mean. According to the chi-square goodness-of-fit test, none of the predicted size distributions provided adequate fit to the observed distribution (Table 2.12). Still, the magnitude of χ^2 values indicated that the mortality scenarios provided a better fit than scenarios without mortality.

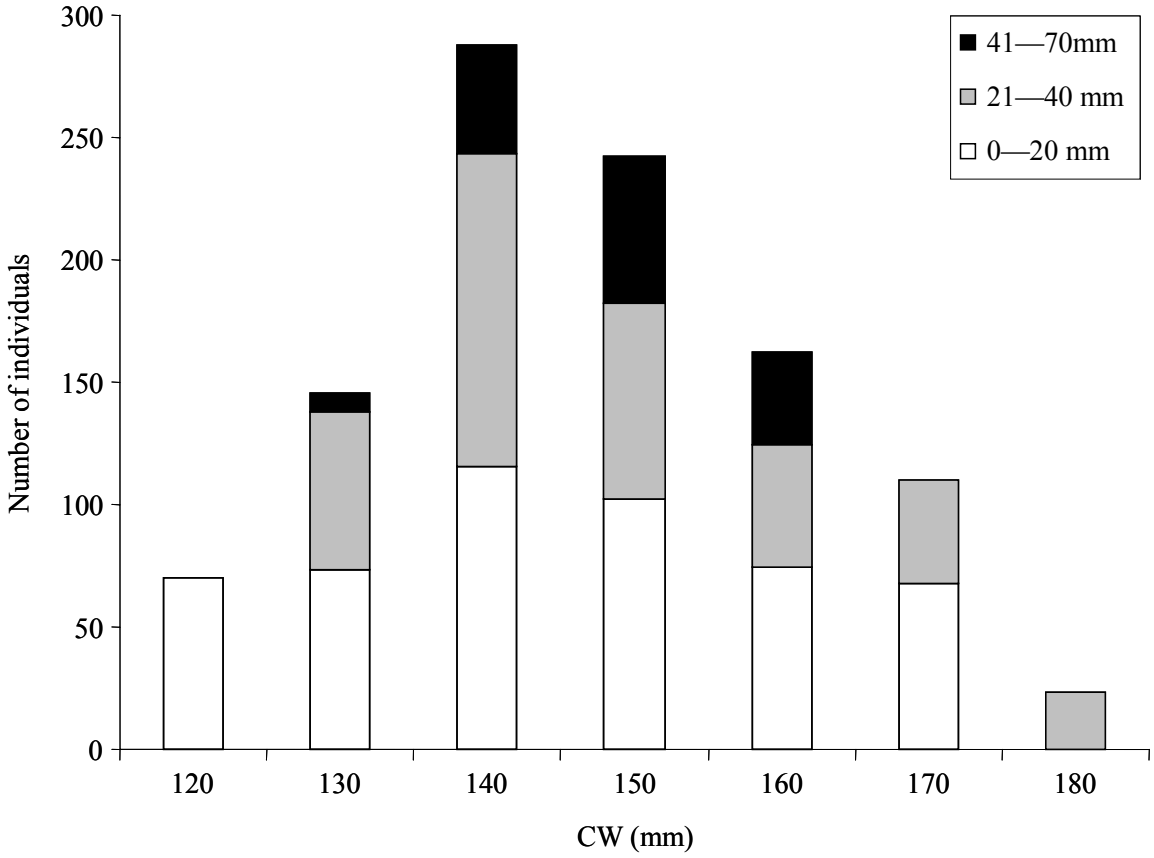

Figure 2.21. Final predicted size distribution (no mortality scenario) for juvenile blue crabs (< 70 mm) originating from the 2002/2003 Winter Dredge Survey; final distributions (by bins) were partitioned into categories of initial size at the beginning of the model simulation.

Table 2.12. Predicted (15 December) and observed (Winter Dredge Survey) modal mean ± 1 standard deviation for two years of model simulations. Chi-square goodness-of-fit test ($\alpha = 0.05$) used to determine the appropriateness of model fit to Winter Dredge Survey distributions. Chi-square values calculated over equal size ranges (120-180 mm).

Year	Simulation	Modal mean ± s.d.	χ^2	df	P
2002/2003	Mortality model	143.1 ± 13.6	23.4	6	0.0001
	No mortality model	146.7 ± 14.5	49.1	6	<0.00001
	WDS-observed	129.3 ± 29.7	-	-	-
2003/2004	Mortality	142.3 ± 13.8	13.7	6	0.03
	No mortality	144.5 ± 14.7	16.1	6	0.01
	WDS-observed	130.5 ± 24.3	-	-	-

Discussion

I estimated growth rates and predicted seasonal patterns of partial recruitment for juvenile blue crabs through direct observation of pond-reared cohorts, length frequency modal analysis of field-collected cohorts, and modeling of winter size distributions. The observed growth rates followed expected seasonal patterns, with growth occurring from March to November, followed by growth stasis at temperatures below 10 °C. During the growth season, growth rates peaked (c. 1.5 mm d^{-1}) at small sizes coincident with favorable temperatures (c. 20 °C), and steadily declined to c. 0.5 mm d^{-1} at sizes greater than 115 mm. Nevertheless, my results indicated that cohorts settling in summer or fall and emerging from winter torpor at small sizes (< 30 mm) were capable of recruiting to the peeler/soft crab fishery by July and the hard crab fishery by October, both within one full growing season (March-October). In fact, peaks in summer peeler/soft crab landings and fall hard crab landings coincided with peaks in predicted recruitment of juveniles (< 70 mm) emerging from winter torpor. The high growth rates and rapid recruitment rates observed in this study support the hypothesis that peeler/soft crab fisheries in the summer and hard crab fisheries in the fall/winter are predominately dependent on new recruits younger than 18 months of age.

Growth and recruitment rates similar to those estimated here have been reported for blue crabs in Delaware Bay; Helser and Kahn (1999) noted that summer settling cohorts (overwintering at c. 60 mm) entered the peeler and hard crab fisheries by June and October, respectively. More rapid recruitment rates have been reported at lower latitudes, where seasonal temperatures permit growth year round. Tagatz

(1968) indicated that juvenile blue crabs in Florida were capable of recruiting to the commercial fishery (120 mm) within one year post-hatch. This parallels growth and recruitment rates for two other commercially dominant Portunidae species, *Portunus pelagicus* and *P. trituberculatus*. In Australian estuaries, *P. pelagicus* reaches a size suitable for exploitation (127 mm) early in their second calendar year of life (Potter et al. 1983). Likewise, wild and hatchery-released *P. trituberculatus* recruit to the Japanese commercial fishery (120mm) within 0.5 to 1 year of age (Secor et al. 2002).

Still, uncertainties remain in applying these results to the assessment of blue crab in Chesapeake Bay. For instance, hatchery and pond reared crabs may have growth advantages due to improved condition from nurturing in artificial hatchery settings, increased densities of prey, or proximity to resources in ponds (i.e., reduced migration and energy expenditures). Concerns over sampling across size classes and cohorts for modal analysis and model simulations remain an issue. Small juveniles < 15 mm were considerably under sampled in the WDS due to gear selectivity (Sharov et al. 2003). Incorporating a large mode of individuals < 15 mm in model simulations would delay predicted settlement dates and partial recruitments. Moreover, samples and temperature records used in my study did not cover the entire Chesapeake Bay system. Size distributions and growth rates of blue crabs are likely to vary locally, due to spatial and temporal heterogeneity in water quality and habitat (Ju et al. 2003).

Pond Rearing

Despite unknown density effects and potential sampling and hatchery-induced biases, empirical estimates derived from earthen pond systems represent direct and

potentially robust estimates of seasonal and annual growth rates (Ju et al. 2001). In Chesapeake Bay seagrass beds, reported densities of juvenile blue crabs (< 25 mm) range from 0.2 m^{-2} to 89.2 m^{-2}, but were an order of magnitude lower (0.2-6.9 m^{-2}) for crabs larger than 25 mm (Orth and van Montfrans 1987). Seagrass beds are assumed to provide refuge from predators and increased foraging efficiency, leading to enhanced juvenile growth (Perkins-Visser et al. 1996), particularly at low densities (e.g. < 0.84 m^{-2} as in this study) where the scope for growth is likely to be greater (Wahle et al. 2001). A number of studies across several crustacean species have shown that increased stocking density negatively affected growth rates or a surrogate such as weight (Naranjo-Páramo et al. 2004, Arnold et al. 2005, Nga et al. 2005). Yet, there is evidence to the contrary: Triño et al. (1999) demonstrated that stocking densities (0.5-3.0 m^{-2}) of pond cultured *Scylla* spp. (Portunidae) did not significantly affect growth rates. Further, Perkins-Visser et al. (1996) documented an increase in juvenile blue crab growth at higher stocking densities (50 vs. 10 m^{-2}), potentially due to increased cannibalism. Intra-cohort competitive interactions are well documented in blue crabs (Hines and Ruiz 1995, Moksnes et al. 1997) and may lead to growth depensation, where dominant individuals are capable of sequestering more resources than their subordinates (Wootton 1998). In this study, there was no discernable trend within cohorts of an increase in coefficient of variation of mean length-at-age with increasing age, indicating that resources such as food and space may not be limiting factors in pond-rearing experiments.

I assumed that samples obtained from each pond cohort were representative and applicable to describe (from models, see below) growth dynamics of wild

populations. Ponds were sampled with a seine in March and October when vegetation was reduced and seines could be efficiently deployed. From April to September, baited traps were used because SAV was more substantial and crabs were actively foraging. Seines are often assumed to provide a representative sample of motile organisms in littoral habitats and are used in studies estimating indices of abundance (Hayes et al. 1996). Nevertheless, biases result from size selectivity, related to mesh size (selecting against smaller individuals) and the speed of deployment (selecting against larger more mobile individuals). Baited traps are also selective, perhaps to a greater extent than seines, due to many factors (e.g., water temperature, size, sex, condition, molt stage, and behavior) (Miller 1990, Hubert 1996). Pot selectivity has been documented in crustaceans where antagonistic behavior may result in larger individuals, assumed to be more aggressive, comprising the majority of entrants (Smith et al. 2004). Due to behavioral interactions or alternate forage preferences, crabs (< 70 mm) were not typically captured in pots, despite the small mesh size (6 mm) capable of retaining them. Therefore, the rapid growth observed from release to c. 100 mm in pond-reared cohorts may be in part due to selection by baited pots for larger, in this case faster growing, crabs.

Estimates of growth may also be biased when utilizing hatchery-reared individuals that may have conferred growth advantages from unnatural feeding and nurturing in hatcheries (Davis et al. 2004). Similarities in Fulton condition factors among pond cohorts and Choptank River samples indicated that hatchery and pond-rearing conditions did not necessarily confer a growth advantage when compared to wild populations. Indeed, Davis et al. (2004) indicated that growth rates were not

significantly different among hatchery-reared and wild juveniles (> 20 mm). Also inherent with artificial propagation are potential biases introduced by year-round production, which may result in atypical spawning episodes and reduced mean length of larval duration (e.g., mean: 33 days for hatchery development, Zmora et al. 2005 vs. 75 days in natural systems, Orth and van Montfrans 1987), ultimately leading to ages that are unrealistic when applied to natural systems. Pond cohort 1 was spawned on 28 March, which is substantially outside the expected spawning period from June to September in Chesapeake Bay. To correspond with natural production cycles, pond cohort 1 (c. 17 mm in June) would have settled in late fall of the previous year, increasing the age of the cohort by c. 180 days. The resulting increase in age would certainly affect recruitment estimates and dampen the growth coefficient (K) in VBGF. Still, the remaining two pond cohorts, which emulated natural production cycles, were capable of recruiting to both fisheries prior to 18 months of age. Moreover, similarities in seasonal growth dynamics derived from two independent approaches in different systems suggests that 1) biases in pond-rearing experiments may be minimal and 2) growth rates observed in pond systems may be applicable to wild populations.

Through continuous pond-rearing experiments, we were able to mitigate several difficulties traditionally encountered when estimating blue crab growth rates. The availability of sub-annual known-age cohorts through large scale hatchery production was vital in obtaining accurate length-at-age data required for modeling growth. Earthen ponds may be more robust than traditional laboratory/caging experiments and mark-recapture studies that potentially introduce additional

constraints and biases. Laboratory/caging experiments may adversely impact growth rates through spatial limitations (i.e. a container effect) (Brylawski 2002). Cheng and Chang (1993) indicated that container size significantly affected IMP and GPM in *Homarus americanus*. Indeed, Ju et al. (2001) reported a K (von Bertalanffy growth coefficient) of 0.49 and 1.09 yr^{-1} for laboratory- and pond-reared blue crabs, respectively. While mark-recapture studies are advantageous in that individuals are subjected to natural conditions, success has been hindered by the difficulty in tagging small crabs (< 70 mm CW), poor tag retention (van Montfrans et al. 1986, Fitz and Wiegert 1991, Hines et al. 1995), molting inhibition, and mortality (Potter et al. 1991). Thus, direct observations of known-age crabs in earthen ponds may be the most accurate method (to date) for obtaining length-at-age data and quantifying growth (DeVries & Frie 1996; but see Chapter 3).

Von Bertalanffy growth function

The model most commonly used in fisheries to describe growth in length is the three-parameter VBGF (Haddon 2001). Although the model was originally intended for estimating the growth of finfish, it has consistently been used in studies on crustacean growth (Rothschild et al. 1992, Rugolo et al. 1998, Oh et al. 1999, Ju et al. 2001, Loher et al. 2001, Lee and Hsu 2003). Extensive use of the model stems from its ability to provide a good description of growth for diverse taxa, the extensive compilation of model parameters useful for comparative analyses, and the utility of parameters in stock assessments and life history studies (Jennings et al. 2001). Yet, the model has been widely criticized (Roff 1980, Haddon 2001, Hernandez-Llamas and Ratkowsky 2004), particularly when applied to model crustacean growth (Smith

1997, Brylawski 2002). The criticisms are largely related to the underlying assumptions and statistical properties of the model. Accurate length-at-age data is difficult to obtain for crustacean species, thus, age must often be assumed (e.g., Rugolo et al. 1998, Ju et al. 2001) or estimated indirectly from length frequency analysis (France et al. 1991, Oh et al. 1999). By assuming continuous growth, the VBGF fails to depict the incremental nature of crustacean growth (Smith 1997, Brylawski 2002). Further, growth patterns influenced by spawning date and seasonal temperature fluctuations, typical in mid-Atlantic estuaries, reduce the effectiveness of VBGF for estimating growth rates. Lastly, extrapolation is often required to obtain estimates for parameters CW_∞ and t_0, which represent the extremes of the growth curve where data tends to be least adequate (Hilborn and Walter 1992, Haddon 2001).

In this study, I used known-age pond-reared cohorts and a modification of the VBGF (Pitcher and MacDonald 1973). By doing so, I minimized the length-at-age quandary and incorporated seasonal growth dynamics that previous stock assessments of Chesapeake Bay blue crabs (Rothschild et al. 1992, Rugolo et al. 1998) failed to capture. For instance, summer settlers (e.g. pond cohort 3), although the minority, are initially subjected to favorable temperatures for growth over several months and may grow at rates greater than 1 mm d^{-1}, potentially reaching c. 70 mm prior to overwintering. In contrast, fall settling cohorts (e.g. pond cohorts 1 and 2) are exposed to a short initial growth season and grow at rates less than 0.2 mm d^{-1}. Once released from temperature constraints the following season, fall settling cohorts may grow at rates two-fold higher than summer settling cohorts (c. 1 mm d^{-1} vs. 0.5 mm d^{-1}). After completing one growth season (i.e., 1+ years of age), sub-annual cohorts

overlap in size and converge with older year classes, indicative of size-dependent growth rates or a compensatory mechanism among inter-cohort growth dynamics.

The estimates of CW_{∞} for pond cohorts 1 and 2 (171 mm and 216 mm, respectively) were similar to those reported in previous studies (Table 2.5) and to the largest blue crabs caught in trawl (197 mm) and WDS (230 mm) collections. Yet, the estimates of CW_{∞} obtained in this study may underestimate the true asymptotic CW of blue crabs in Chesapeake Bay (i.e., $\geq$ 260 mm CW, Rugolo et al. 1998). Underestimation of CW_{∞} results in an overestimation of K because the two parameters negatively covary. Despite extensive criticisms, Bunnell and Miller (2005) noted that the VBGF resulted in reference points for Chesapeake Bay blue crabs that were similar to those derived from discontinuous growth models.

Comparisons of growth curves among sexes indicated that male and female blue crabs grew at similar rates and to similar sizes over the duration of this study (c. 1.5 years of age), which included the transition to maturity for females. Likewise, de Lestang et al. (2003) indicated that seasonalized VBGF were similar among male and female *Portunus pelagicus* up to an estimated age of 20 months. Sexual dimorphism would likely be more pronounced over an extended time period as females essentially cease to grow (i.e., terminal molt) and males continue growing. Pond cohorts 1 and 2 obtained a similar size (c. 140 mm) after one full growing season, essentially growing c. 70 % of their estimated lifetime asymptotic size. Although length-at-age appears to converge near 1.5 years of age, estimates of CW_{∞} derived from seasonalized VBGF were moderately higher for cohort 2. It should be noted that cohort 2 was reared five months longer than cohort 1 and that growth rate (K) discrepancies may be due to the

unrealistically young age of cohort 1. Still, differences in model parameters suggest that cohort-specific growth variability may be an important component of accurately modeling the population dynamics of blue crabs in Chesapeake Bay.

Length frequency analysis

For relatively short-lived species that lack accurate age estimates, length-frequency analysis has provided useful growth data that could not be obtained by other means (Jennings et al. 2001). Yet, the underlying assumptions of pulsed production, unimodal distributions, no sampling bias, and no net immigration/emigration have lead to skepticism in the validity of length-based methods (Hilborn and Walters 1992). The length frequency analysis presented here subjectively designated modal progression of cohorts and assumed that early production was pulsed and modes were unimodally and normally distributed. Although spawning is generally considered to be protracted, there is evidence that peaks in spawning occur during July and August (Prager 1996), with corresponding settlement peaks in September and October (van Montfrans et al. 1990). Pulsed production should, theoretically, result in distinct size modes among sub-annual cohorts. Still, saltatory growth can amplify small discrepancies in individual growth rates, which can affect modal width (e.g., asynchronously molting crabs within a cohort) and shape (e.g., skewed distributions of number of molts at date for a given cohort). In this study, modal means of field cohorts differed by more than one molt increment until September and October when modes became less discrete.

The largest potential for bias in size distributions of field-collected crabs was likely associated with habitat association, which is partitioned by size, sex, and molt

stage (Hines et al. 1987). Small juveniles preferentially utilize near-shore shallow habitats where they obtain refuge from predation (Hines and Ruiz 1995). Therefore, consistently obtaining representative samples of the smallest cohorts (< 20 mm CW) by trawling is difficult. Hines et al. (1987) also documented the use (typically by males) of tidal creeks as molting habitats, presumably for osmotic advantages or to minimize mortality by predation. Hence, by concentrating our sampling efforts in rivers, the snap shot of monthly size distributions may be biased towards the largest (shortest IMP) individuals within a cohort; although in an asynchronously molting population one can assume that at any time, the individuals in a population are uniformly distributed among IMP (Hoenig and Restrepo 1989). In addition, a fishing effect (principally trot liners) may reduce the bias towards capture of 'fast growers' in riverine systems by selectively harvesting the largest and presumably fastest growing crabs from these habitats (i.e., size truncation, Jennings et al. 2001).

Ideally, the same fishing gear with the same selectivity should be used to collect samples for length frequency analysis. In 2003, samples from the Choptank River were collected using the same gear, with the notable exception of dip net collections in July. Crabs collected with a dip net in July were used for lipofuscin analysis of wild juveniles (Chapter 3), but were not included in the length frequency modal analysis. In 2004, four different gear types were utilized, all with differential effort and presumably differential selectivity. Biases introduced by the different mesh sizes among summer and fall trawl deployments were probably minimal because by that time (July-October) cohort modal means had progressed to sizes larger than the minimum size captured. Seining efforts in June were aimed at

collecting crabs less than 120 mm (i.e. summer and fall cohorts), which is likely the upper limit of sizes vulnerable to the gear due to avoidance behaviors or habitat preferences.

Although multiple sampling gears were employed, seasonal growth differences between sub-estuaries were typically absent. Across system/year, intra-cohort modal means were within 2 standard deviations, except in October 2004. Higher growth rates estimated for Patuxent River cohort 1 when compared to its counterpart from the Choptank River were likely attributable to the detection of only a single large mode in October 2004 (Figure 2.14), but may also be due to higher condition of Patuxent River cohorts, sampling regime, or environmental covariates (e.g., temperature, salinity). Still, the similarity of intra-cohort monthly modal means and ensuing partial recruitment over consecutive years in two sub-estuaries suggests that 1) diverse sampling may still result in representative samples for cohort designations and 2) cohort dynamics are similar in the mid-bay portion of Chesapeake Bay.

Seasonal (June-October) growth rates estimated from length frequency modal analysis were similar to cohort dynamics observed in pond-rearing experiments and to growth rates estimated by modal progression in Mississippi Sound, Gulf estuaries of Texas, and Louisiana (Table 2.13, Guillory et al. 2001). Yet, uncertainties remain in applying these results to the entire Chesapeake Bay. Alternatives interpretations of modal structure and progression could profoundly affect estimates of growth and

Table 2.13. Study site, time period, and size range of blue crabs included in growth rate estimates reported in this study and in Gulf States. Growth rates were determined from length frequency modal analysis.

Study site	Time period	Size range (mm)	Growth (mm d^{-1})	Source
Chesapeake Bay Choptank R.	June-Oct.	10-195	0.6	This study
Chesapeake Bay Patuxent R.	June-Oct.	10-180	0.6—1.1	This study
LA estuaries		< 85 > 85	0.5 0.5-0.7	Adkins 1972*
MS Sound	July-Jan.	-	0.8	Perry 1975*
TX estuaries	Feb.-Aug.	-	0.7-0.8	Hammerschmidt 1982*

*Cited in Guillory et al. 2001

recruitment rates. Furthermore, samples for modal analysis were collected near mid-Bay where ambient conditions are similar. Although the environmental factors that affect growth, such as temperature, salinity, and forage vary spatially throughout the Bay, De Lestang et al. (2003) documented similar modal progression and subsequent growth of juvenile *Portunus pelagicus* in disparate systems (i.e., marine embayment and estuary). Therefore, I suggest that modal analysis may not be sensitive to small differences in growth and that average growth rates determined from modal analysis may be applicable to large systems with spatial variability in abiotic/biotic characteristics.

Temperature-dependent growth model

An increase in temperature typically increases the growth rate of crustaceans via increases in GPM (unlikely), reductions in IMP, or a combination of the two (Hartnoll 2001). Leffler (1972) reported that growth rates of blue crabs increased from 13 to 27 °C, were maximal from 20 to 27 °C, and decreased below 15 °C and above 30 °C. Temperature records from earthen ponds, field-sampling sites, HPL and VIMS did not exceed 30 °C implying that Chesapeake Bay spring, summer, and fall temperatures may provide near optimal growth conditions for blue crabs.

Molt-process growth models directly simulate the molting cycle, and as such are a promising method for overcoming inadequacies in the length-at-age relationship for wild populations of crustacean (Smith 1997). For this reason, molt-process growth models have been developed for several crustacean spp. including Alaska king crab (McCaughran and Powell 1977), stone crab (Restrepo 1989), Norway lobster (Castro 1992), Dungeness crab (Wainwright and Armstrong 1993), and blue

crab (Smith 1997, Brylawski 2002, Bunnell and Miller 2005). The models recently constructed for blue crabs have incorporated a TD day-IMP function, which provides a valuable means of capturing the physiological effects of temperature while simultaneously accounting for chronological time.

In this study, I used a molt-frequency function based upon a TD day framework (sensu Ju 2000), assumed a constant GPM (20% or 50%, depending on crab instar), and incorporated natural and fishing mortality in an attempt to back-calculate settlement date, predict seasonal size distributions, and estimate partial recruitments. I assumed that molting ceased below 10 °C, yet growth stasis has been reported to occur at 8.9 °C (Smith 1997), 11 °C (Brylawski 2002), and 15 °C (Tagatz 1968). The molt-process growth model slightly underestimated observed pond growth from c. 70 to 100 mm CW, potentially biasing predictions of recruitment to the peeler/soft crab fishery. Underestimations were linked to the molt-frequency function, which underestimated the number of molts during this time period. However, it should be noted that underestimations over this limited size range occurred primarily for a single cohort (cohort 3). Model predictions were accurate over the remainder of pond observations and given the duration of time over which I am predicting size distributions, the overall bias is likely small.

In model simulations, predicted settlement dates compared favorably with observed peaks in settlement, which typically occur from late September to mid-October (van Montfrans et al. 1990). Predicted recruitment and seasonal size distributions corresponded well with expected patterns in fishery yields (excluding spring landings) and the subsequent larger adult size distribution within the WDS.

Peeler/soft crab landings in May and hard crab landings in July (2002) or August (2003) may be partially dependent on the small percentage of new recruits entering these fisheries (Table 2.11). Further, the secondary peak in peeler/soft crab landings in July (2002) and August (2003) coincided with near complete recruitment of the winter-time juvenile cohort to the fishery. Declines in peeler/soft crab landings from September to December (Figure 2.1) may be explained by the reduction in predicted molting events, hence sustained vulnerability to the fishery, during those months. However, it should be noted that effort in the peeler/soft crab fishery is diminished during the fall (Miller 2001a). Predicted recruitment to the hard crab fishery peaked in October, coincident with the largest peak in landings; afterwards, partial recruitment declined (by c. 10%) as recruits were removed by the fishery without replacement due to seasonal growth cessation. Although partial recruitment to both fisheries was predicted to have occurred earlier during simulations in 2002, differences in monthly commercial landings between years were not apparent.

Chi-square analyses indicated that mortality scenarios provided a better fit to observed WDS data than non-mortality scenarios, although the two scenarios resulted in only subtle differences in size distributions. Surprisingly, model simulations (both years, both mortality scenarios) resulted in the majority of crabs obtaining the same instar by the end of the growth season. Further, the smallest crabs at the beginning of model simulations did not necessarily comprise the smallest individuals at the end of simulations providing additional evidence for the effects of body size on growth or compensatory growth.

An underlying assumption, with the potential to bias much of the model, is constant GPM. Although, GPM in blue crabs (> 20 mm) has been reported to be highly variable, 7.8 to 50 % (aggregated by size, Tagatz 1968), several studies have indicated that mean GPM is a relatively constant proportional increase of premolt CW: 22% (Leffler 1972), 20.9% (Fitz and Weigert 1991), 19.4% (Brylawski 2002), or 25.3 (Tagatz 1968). Theoretically, GPM must be geometrically constrained; Cheng and Chang (1994) suggested that GPM was constrained by the amount of the new cuticle that could be folded under the old exoskeleton. The variability in GPM may be due to a suite of biotic (e.g., autonomy and body size) and abiotic (e.g., temperature, salinity, and rearing condition) factors (Tagatz 1968, Leffler 1972, Cadman and Weinstein 1988, Smith 1990, Cheng and Chang 1993, Cheng and Chang 1994). The effects of temperature on GPM are contradictory; in a meta-analysis of several crustacean spp., Hartnoll (2001) generally reported a decrease in GPM with an increase in temperature. However, Brylawski (2002) reported that temperatures ranging from 16 to 28 °C had a minimal affect on GPM. Thus, the 20% GPM used in this study appears to be representative of the mean GPM for blue crabs (> 20 mm) during much of the growing season in Chesapeake Bay. While the 50% GPM applied to individuals less than 20 mm likely represents the upper boundary of growth, such growth increments have been reported for crabs larger than 20 mm (Tagatz 1968). Therefore, it is not unreasonable to assume that individuals less than 20 mm are capable of increasing in size by 50% following each molt. Nevertheless, incorporating estimates of GPM that are maximal would delay estimated settlement date and partial recruitments in model simulations.

While IMP (i.e., molt-frequency in this study) is affected by several factors, the likely dominant effect of temperature is a consistent and substantial shortening of IMP with increasing temperatures (Hartnoll 2001), or an increase in molt-frequency as observed in this study. During favorable temperatures for growth, reductions in IMP (increases in molt-frequency) are complicated by a significant size and temperature interaction (Tagatz 1968), such that IMP increases (molt-frequency decreases) as size increases regardless of temperature. Variations in molt-frequency as it pertains to size and individual initial sizes were accounted for in the model, but individual variations in GPM and the molt-frequency function were not incorporated. While individual variability is common, Bunnell and Miller (2005) indicated that including individual variation yielded the same reference points as a model that included blue crabs of the same average size and growth rate.

Additional biases in model inputs may be attributed to observations of pond-reared blue crabs and temperature histories used in simulations. While growth rates of pond-reared blue crabs were similar to those estimated for field-collected crabs using length frequency modal analysis, several rearing artifacts could bias model inputs. Pond-reared crabs were not subjected to intense predation or competition from larger conspecifics. Furthermore, movement and proximity to resources in a pond setting is restricted by the pond dimensions, which may act to reduce energy expenditures. Therefore, the sublethal affects often associated with competition and increased migratory costs, such as reduced growth, were not evident in pond-reared individuals, and thus, were not incorporated into model inputs.

Discrepancies between actual temperatures encountered by individuals and the environmental records used in the model could have large effects on predicted settlement dates and partial recruitments. The difficulties of obtaining a representative temperature record are related to the complex life history of blue crabs. Due to proximity to the Bay mouth where most settlement occurs, temperature records from near the mouth of the York River (Virginia Institute of Marine Science) were used to back-calculate date of settlement. Following settlement, juveniles (c. 20 mm) begin to disperse throughout the estuary (Hines 2003). Hence, the application of a single temperature record is not likely to be representative of temperatures experienced by blue crabs (> 20 mm) collected throughout Chesapeake Bay. To predict seasonal size distributions, I attempted to account for dispersive behavior by utilizing water temperatures from the Choptank River (S. Tobash-Alexander unpubl. data), located near mid-Bay. Yet, water temperatures can be quite variable at large spatial scales. For instance, daily temperature records during 2002 were, on average, 1.3°C warmer at mid-Bay locations (HPL data) than at lower-Bay locations (VIMS data). Seasonal and daily temperature contrasts between the upper- and lower-Bay are likely to be even more striking. The incorporation of a single temperature record during model simulations could explain, in part, the similarity in final predicted crab instar and the reduced variance of the final predicted size distribution when compared to the corresponding WDS mode (Table 2.12).

I initiated simulations by assuming that only crabs less than 70 mm CW were juveniles (i.e., young of the year). In part, this assumption was justified by a persistent modal gap at this size within WDS samples. Brylawski (2002) used a

similar cutoff (65 mm) based on the same criteria. Growth of pond-reared juveniles certainly indicated that summer settling cohorts were capable of obtaining sizes larger than 70 mm CW prior to overwintering. If this were the case, I failed to incorporate all juveniles, effectively reducing the size range of individuals at the beginning of simulations and potentially the variance at the end of simulations. An additional bias is the omission of a large mode of crabs < 15 mm CW, which are not efficiently captured in the WDS. Model simulations beginning with an initial WDS mode (< 70 mm) skewed towards smaller individuals would delay estimates of peak settlement and partial recruitment and reduce monthly modal means of predicted size distributions. Delays in settlement would suggest that the pond cohort released in June (cohort 1) was highly atypical of wild cohorts, particularly with respect to age. The under sampling of small crabs (< 15 mm) and subsequent inclusion in the WDS at sizes larger than minimal commercial size limits potentially strengthens the notion of rapid growth leading to recruitment within one full growing season. More important to the discrepancy between observed and predicted final size distributions, is the composition of the larger WDS mode (> 70 mm CW), which may include early-summer settling cohorts, previous year summer and fall settling cohorts (1+), and any additional older age classes (Sharov et al. 2003). The inclusion of YOY (summer settling) cohorts overwintering at sizes > 70 mm may effectively reduce the modal mean of the larger adult size WDS mode, potentially explaining the overestimation of predicted size distributions in model simulations. In addition, the range of year classes potentially incorporated into the larger WDS mode would most likely lead to an increase in variance of the mode.

Lastly, differences between observed and predicted size distributions may be due to several mortality and fishery simplifications inherent in model simulations. Natural mortality was modeled as a constant and independent of molt status and size, despite evidence that vulnerability to predation increases during molting (Hines et al. 1987) and crabs smaller than 50 mm CW suffer significantly higher natural mortality than their larger conspecifics (Hines and Ruiz 1995). In this study, I applied the fixed value of M reported by Miller et al. (2005) in a recent stock assessment. A natural mortality of 0.9 yr^{-1} was decided as the most likely value of M, because this estimate was consistent with several indirect and empirical estimates (Miller et al. 2005). While the value of natural mortality applied in model simulations may have been incorrect, it is unlikely that inaccuracies in M would affect predicted modal size distributions because M was applied uniformly among all individuals.

Given the uncertainty in estimating M, the poor knowledge of recreational harvests, and suspected under-reporting, estimates of F may be biased as well (T. Miller, Chesapeake Biological Laboratory, pers. comm.). In model simulations, I attempted to incorporate the complexity of the Chesapeake Bay blue crab fishery by partitioning F among sizes and shell status. I accounted for the change in MD commercial fishing regulations and only subjected peeler/soft shell recruits to F for seven days per predicted molt, during which time they were not vulnerable to the hard crab fishery. The model did not account for gender/maturity, size selectivity within each fishery, or temporal/spatial variations in fishing mortality. Although not accounted for in the model, there appears to be strong temporal and spatial variability in patterns of exploitation (Miller 2003). Peaks in landings appear to be concentrated

at discrete times within the year and temporal commercial gear restrictions are also employed (e.g., winter dredge fishery). Spatially, commercial gear types are restricted to specific areas (e.g., pot fishery is restricted to the main stem of the Bay) (Miller 2003).

Despite the aforementioned concerns, the temperature-dependent molt-process growth model appears generally applicable in predicting seasonal recruitment and potential fishery yields. Because it is impossible to know the temperature that a population will experience, model scenarios incorporating cold, average, and warm years will be needed to provide a range of likely recruitment rates. While the molt-process model was sensitive to annual fluctuations in temperature, partial recruitments were relatively similar across years, despite a c. 10% difference in temperature histories between 2002 and 2003. Further, across years, seasonal yields suggest a pattern consistent with recruitment of juveniles to the mid-summer peeler/soft crab fishery and the large recruitment of juveniles to the fall hard crab fishery. Moreover, declines in peeler/soft crab landings in the fall coincided with drastic reductions in predicted molt frequencies and, thus, vulnerability to the fishery). In combination with more traditional stock assessment techniques, molt-process growth models can be used to provide information to managers with regards to natural variation in growth and subsequent recruitment to the commercial fishery.

Appendices

Appendix 2a. MATLAB code used for calculating number of molts from settlement for individuals (< 70 mm) originating from the Winter Dredge Survey (WDS). Note, the code shown below is for simulations involving the 2001/2002 WDS, but the code for simulations during 2002/2003 is nearly identical.

```
function [WDSCW, WDSmolts, WDSinstar] = WDSlengthmolts (n);

% function [WDSCW, WDSmolts, WDSinstar] = WDSlengthinstar (n)
%
% This function imports an excel file ('01-02wdslength') that contains the initial
% length of each individual, and calculates the initial number of molts from
% settlement and corresponding instar for each individual based on this initial
% length.
%
% Arguments
% n--number of individuals at the beginning of simulations--1041
%
% Returns
% WDSCW--initial CW of each individual as measured by the WDS
% WDSmolts--initial number of molts for each individual
% WDSinstar--initial instar for each individual at capture in WDS
%
% Brandon Puckett -- 22 Dec, 2005

num = xlsread('01-02wdslength'); %importing excel file
WDSCW=num;
WDSmolts=zeros(n,1); %preallocating matrix
WDSinstar=zeros(n,1);
for i=1:n;
   if WDSCW(i,1) < 24;
      WDSmolts(i,1)=(log(WDSCW(i,1)/2.5))/(log(1.5)); %growth per molt 50%
         % when initial size < 24mm
   elseif WDSCW(i,1) >= 24;
      WDSmolts(i,1)=(log(WDSCW(i,1)/18.9844))/(log(1.2))+5; %growth per molt
         % 20  when initial size >= 24mm
   end;
   WDSmolts=round(WDSmolts); %rounds estimated WDSinstar to nearest whole
         % number
   WDSinstar(i,:)=WDSmolts(i,:)+1; %calculating corresponding crab instar
end;
WDSCW;
WDSmolts;
WDSinstar;
```

Appendix 2b. MATLAB code used for calculating accumulated temperature-degree days from settlement until capture in the Winter Dredge Survey (WDS), which was assumed to have occurred on January 1. Note, the code shown below is for simulations involving the 2001/2002 WDS, but the code for simulations during 2002/2003 is nearly identical.

```
function predtddsettle = tddsettle (n);

% function predtddsettle = tddsettle (n)
%
% This function calls WDSlengthmolts, which returns individual WDS lengths,
% number of molts from settlement, and corresponding instar.  Using number of
% molts from settlement, and the join point molt-frequency function, the function
% calculates the predicted temperature degree days accumulated by each individual
% from settlement until Jan 1, 2002.
%
% Arguments
% n--number of individuals at the beginning of simulations--1041
%
% Returns
% predtddsettle--Temperature degree day accumulated since settlement
%
% Brandon Puckett -- 22 Dec, 2005

predtddsettle=zeros(n,1); %preallocating matrix
[WDSCW, WDSmolts, WDSinstar] = WDSlengthmoltsinstar (n); %calling function
for i=1:n;
   predtddsettle(i,:)=(WDSmolts(i,:)-(-1.230497))/0.013855; %using inverse
         % prediction to estimate TD days from settlement
   if predtddsettle(i,:)>1093 %join point threshold at which linear regressions used to
         % describe relationship between TD days and number of molts changes.
      predtddsettle(i,:)=(WDSmolts(i,:)-12.91033)/0.000915; %using inverse
         % prediction to estimate TD days from settlement
   end;
end;
predtddsettle;
```

Appendix 2c. MATLAB code used for back calculating date of settlement for individuals originating from the Winter Dredge Survey. Note, the code shown below is for simulations involving the 2001/2002 WDS, but the code for simulations during 2002/2003 is nearly identical.

```
function settle = settle (n)

% function settledate = settle (n)
%
% This function imports an excel file ('01-02backcalctddanddate') that contains back-
% calculated (1 Jan 2002 to 1 Jan 2001) cumulative temperature degree days (TD
% days) and corresponding date. When back-calculated TD days equal or exceed TD
% days from settlement. TD days from settlement is obtained by calling function
% predtddsettle tddsettle (n).
%
% Arguments
% n--number of individuals at the beginning of simulations--1041
%
% Returns
% settle--date of settlement for each individual
%
% Brandon Puckett -- 22 Dec, 2005

settle = zeros(n,1); %preallocating matrix
predtddsettle = tddsettle (n); %calling function
num = xlsread('01-02backcalctddanddate'); %importing excel file
TDbackcalc=num;
for t = 2:139; %time steps day 1 is 1 Jan 2002, day 2 is 31 Dec 2001, day 139 is 15
        % Aug 2001
   for i = 1:n; %individuals
      if settle(i,:)<1;
         if TDbackcalc(t,2)>=predtddsettle(i,:);
            settle(i,:)=TDbackcalc(t,1);
         end;
      else settle(i,:)=settle(i,:);
      end;
   end;
end;
settle;
```

Appendix 2d. MATLAB code used for increasing temperature degree days from settlement on a daily time step. Note, the code shown below is for simulations involving the 2001/2002 WDS, but the code for simulations during 2002/2003 is nearly identical.

```
function dailytdd = tddupdate (n);

% function dailytdd = tddupdate (n)
%
% This function imports an excel file ('01-02cumdailytdd') that contains daily
% cumulative temperature degree days, calls the function tddsettle, and updates
% temperature degree days from settlement on a daily time step from Jan 2, 2002 to
% Dec. 15, 2002.
%
% Arguments
% n--number of individuals at the beginning of simulations--1041
%
% Returns
% dailytdd--cumulative TDD accumulated on each day of simulations for each
% individual
%
% Brandon Puckett -- 22 Dec, 2005

num = xlsread('01-02cumdailytdd'); %importing excel file
tdddailyincr=num;
predtddsettle = tddsettle (n); %calling function tddsettle
dailytdd=zeros(n,349); %preallocating matrix
dailytdd(:,1)=predtddsettle; %setting the first value for each individual equal to
        % predicted tdd since settlement
for t=2:349;
dailytdd(:,t) = predtddsettle+tdddailyincr(t,1); %updating cumulative temp degree day
        % accumulated for each individual
end;
dailytdd;
```

Appendix 2e. MATLAB code used for predicting daily carapace width (CW) and number of molts for individuals originating from the winter dredge survey in the absence of mortality. Note, the code shown below is for simulations involving the 2001/2002 WDS, but the code for simulations during 2002/2003 is nearly identical.

```
function [CW,numbermolts, instar] = dailypredcwmolt(n);

% function [CW, numbermolts] = dailypredcwmolt (n)
%
% This function calls WDSlengthmolts and tddupdate and calculates number of molts
% and CW for each individual on each day of model simulation. Simulation run from
% Jan 2,2002 to Dec. 15, 2002. Number of molts estimated using joint point
% regression, hence the different equations used for calculating number of molts at
% the join point threshold (1093 TD days).
%
% Arguments
% n--number of individuals at the beginning of simulations--1041
%
% Returns
% CW--daily CW of each individual
% numbermolts--daily number of molts for each individual
%
% Brandon Puckett -- 22 Dec, 2005

[WDSCW, WDSmolts, WDSinstar] = WDSlengthmoltsinstar (n); %calling function
CW=zeros(n,349); %preallocating matrix
CW(:,1)=WDSCW; %setting initial CW equal to CW measured from WDS
totalmolts=zeros(n,349); %preallocating matrix
totalmolts(:,1)=WDSmolts; %setting initial total number of molts from settlement to
        % number of molts calculated from WDS lengths.
instar=zeros(n,349); %preallocating matrix
instar(:,1)=WDSinstar; %setting initial instar equal to instar calculated from WDS
        % lengths

dailytdd = tddupdate (n); %calling function
dailytdd=dailytdd;
numbermolts=zeros(n,349); %preallocating matrix
for t=2:349; %time steps
   for i=1:n; %individuals
      if dailytdd(i,t)<1093; %join point threshold
         numbermolts(i,t)=round(((-1.2305+(0.01386*dailytdd(i,t))))-totalmolts(i,t-1));
      else numbermolts(i,t)=round(((12.91033+(0.000915*dailytdd(i,t))))-
totalmolts(i,t-
      1));
      end;
      totalmolts(i,t)=numbermolts(i,t)+totalmolts(i,t-1);
```

```
        instar(i,t)=numbermolts(i,t)+instar(i,t-1);
        if instar(i,t)<=5;
           CW(i,t)=CW(i,t-1)*(1.5^numbermolts(i,t));
        else CW(i,t)=CW(i,t-1)*(1.2^numbermolts(i,t));
        end;
    end;
end;
CW;
CWmonth=zeros(1041,10); %preallocating matrix
CWmonth(:,1)=CW(:,1); %CW on Jan 1 (same as WDSlengths)
CWmonth(:,2)=CW(:,91); %CW on April 1
CWmonth(:,3)=CW(:,121); %CW on May 1
CWmonth(:,4)=CW(:,152); %CW on June 1
CWmonth(:,5)=CW(:,182); %CW on July 1
CWmonth(:,6)=CW(:,213); %CW on Aug. 1
CWmonth(:,7)=CW(:,244); %CW on Sept. 1
CWmonth(:,8)=CW(:,274); %CW on Oct. 1
CWmonth(:,9)=CW(:,305); %CW on Nov. 1
CWmonth(:,10)=CW(:,349); %CW on Dec. 15
CWmonth;
numbermolts;
instar;
xlswrite('01-02CWmonthnomort',CWmonth); %exporting excel file
```

Appendix 2f. MATLAB code used for predicting daily carapace width (CW) and number of molts for individuals originating from the winter dredge survey with both natural and fishing mortality incorporated. Note, the code shown below is for simulations involving the 2001/2002 WDS, but the code for simulations during 2002/2003 is nearly identical.

```
% Script file dailycwmoltwithmort(n)
% This script outputs CW and number of molts at specified time periods while
% incorporating fishing and natural mortality
% Brandon Puckett--December 22, 2005

n=1041; %number of individuals at the beginning of simulations
M = 0.0024657; %Daily instantaneous natural mortality reported by Miller et al.
        % 2005(0.9 yr-1/365)
% Instantaneous fishing mortality reported in Miller et al. 2005 (1.3146 yr-1) is
% partitioned among peeler and hard crab recruits
% Peeler landings (# of individuals) were 10.1% of total landings
Fpeeler = 0.000512643; %Daily instantaneous fishing mortality for peeler recruits
        % ((1.3146 yr-1*0.101)/259)
Fhard =0.004563032 ; %Daily instantaneous fishing mortality for hard crab recruits
        % ((1.3146 yr-1*0.899)/259)
Zpeeler = Fpeeler+M; %Daily instantaneous total mortality (F+M) for peeler
        % recruits
Zhard = Fhard+M; %Daily instantaneous total mortality (F+M) for hard crab
        % recruits
[CW, numbermolts] = dailypredcwmolt(n); %calling function dailypredmolt, which
        % returns matrices CW and number of molts for each individual each day

for t=2:90; %time steps iterated daily from Jan. 2, 2002 (2) to March 31, 2002 (90).
        % During this time period the fishing season is closed.
    Ninitial=size(CW); %size returns the dimensions of the matrix CW
    Ninitial=Ninitial(1,1); %first row and column of Ninitial corresponds to the
        % number of individuals
    Nt=Ninitial*exp(-M); %Using the exponential decay equation to determine the
        % number of individuals remaining during the next time step
    surviveprob=Nt/Ninitial; %determining the survival probability
    randomnumber=rand(1,Ninitial); %generating uniform random number between
        % 0 and 1 for each individual
    NewPop=0; %setting initial value for row place holder to be used when creating
        % NewCW and Newnumbermolts (see below)
    NewCW = zeros(Ninitial,349); %preallocating matrix NewCW
    Newnumbermolts = zeros(Ninitial,349); %preallocating matrix
        % Newnumbermolts
    for i=1:Ninitial;
        if randomnumber(:,i)<=surviveprob; %when random number for each
```

```
            % individual is less than the probability of surviving the individual
            % survives and is incorporated into NewCW and Newnumbermolts
          NewPop = NewPop + 1; %updating row NewPop for each individual that
            % survives
          NewCW(NewPop,:) = CW(i,:); %creating new matrix of CW and
            % numbermolts  for surviving individuals
          Newnumbermolts(NewPop,:) = numbermolts(i,:);
        end;
      end;
   CW = NewCW(1:NewPop,:); %setting matrix of survivors equal to CW and
        % numbermolts for next time step iteration
   numbermolts = Newnumbermolts(1:NewPop,:);
end;
CW90=CW(:,90);
CW;
numbermolts;

for t=91:121; %time steps iterated daily from April 1, 2002 (91) to May 1, 2002 (121)
   rownon=0; rowpeeler=0; rowhard=0; %initializing row values
   NewPopnon=0; NewPoppeeler=0;  NewPophard=0;
   Newnonrecruits=zeros(1,349); Newnumbermoltsnon=zeros(1,349); %creating
        % 'dummy'  variables as placeholders
   Newpeeler=zeros(1,349); Newnumbermoltspeeler=zeros(1,349);
   Newhard=zeros(1,349); Newnumbermoltshard=zeros(1,349);
   nonrecruits=zeros(1,349); numbermoltsnon=zeros(1,349);
   peeler=zeros(1,349); numbermoltspeeler=zeros(1,349);
   hard=zeros(1,349); numbermoltshard=zeros(1,349);
   lengthCW=length(CW); %determining the largest dimension (# rows) in matrix
        % CW
   for i=1:lengthCW;
      if ((CW(i,t) < 82.5) | ((numbermolts(i,t) < 1) & (CW(i,t) < 127))); %criteria used
        % for determining nonrecruits, peeler recruits and hard crab recruits
        rownon=rownon+1; %updating row
        nonrecruits(rownon,:)=CW(i,:); %placing individuals from CW and
            % numbermolts  that meet nonrecruit criteria in new matrices
            % nonrecruits and numbermoltsnon
        numbermoltsnon(rownon,:)=numbermolts(i,:);
      end;
      if ((numbermolts(i,t) > 0) & (CW(i,t) >= 82.5));
        rowpeeler=rowpeeler+1;
        peeler(rowpeeler,:)=CW(i,:);
        numbermoltspeeler(rowpeeler,:)=numbermolts(i,:);
      end;
      if ((CW(i,t) >= 127) & (numbermolts(i,t) < 1));
        rowhard=rowhard+1;
        hard(rowhard,:)=CW(i,:);
```

```
        numbermoltshard(rowhard,:)=numbermolts(i,:);
    end;
end;
sizenon=size(nonrecruits); %determining size (number of individuals) that are
        % nonrecruits
sizenon=sizenon(1,1);
if nonrecruits(1,:) > 0;
    Newnonrecruits=zeros(sizenon,349); %preallocating matrices
    Newnumbermoltsnon=zeros(sizenon,349);
    for i=1:sizenon;
        randomnumber(:,i)=rand(1,1); %generating uniform random number between
                % 0 and 1 for each nonrecruit
        Ninitnon=sizenon; %determining number of nonrecruits at time t
        Ntnon=Ninitnon*exp(-M); %using exponential decay equation to determine
                % number of non recruits at time t+1
        surviveprobnon=Ntnon/Ninitnon; %determining the survival probability
        if randomnumber(:,i)<=surviveprobnon; %when random number for each
                %individual is less than the probability of surviving the individual
                % survives and is incorporated into Newnonrecruits and
                % Newnumbermoltsnon
            NewPopnon = NewPopnon+1; %updating row number
            Newnonrecruits(NewPopnon,:)=nonrecruits(i,:);
            Newnumbermoltsnon(NewPopnon,:)=numbermoltsnon(i,:);
        end;
    end;
end;
sizepeeler=size(peeler); %determining size (number of individuals) that are peeler
        % recruits
sizepeeler=sizepeeler(1,1);
if peeler(1,:)> 0;
    Newpeeler=zeros(sizepeeler,349); %preallocating matrices
    Newnumbermoltspeeler=zeros(sizepeeler,349);
    for i=1:sizepeeler;
        randomnumber(:,i)=rand(1,1); %generating uniform random number between
                % 0 and 1 for each peeler recruit
        Ninitpeeler=sizepeeler; %determining number of peeler recruits at time t
        Ntpeeler=Ninitpeeler*exp(-Zpeeler*7); %using exponential decay equation to
                % determine number of peeler recruits at time t+1, multiplying Z by 7
                % because peelers are susceptible for 7 days per molt
        surviveprobpeeler=Ntpeeler/Ninitpeeler; %determining the survival
                % probability
        if randomnumber(:,i)<=surviveprobpeeler; %when random number for each
                % individual is less than the probability of surviving the individual
                % survives and is incorporated into Newpeeler and
                % Newnumbermoltspeeler
            NewPoppeeler=NewPoppeeler+1; %updating row number
```

```
            Newpeeler(NewPoppeeler,:)=peeler(i,:);
            Newnumbermoltspeeler(NewPoppeeler,:)=numbermoltspeeler(i,:);
         end;
      end;
   end;
   sizehard=size(hard); %determining size (number of individuals) that are hard crab
         % recruits
   sizehard=sizehard(1,1);
   if hard(1,:)> 0;
      Newhard=zeros(sizehard,349); %preallocating matrices
      Newnumbermoltshard=zeros(sizehard,349);
      for i=1:sizehard;
         randomonumber=rand(1,1); %generating uniform random number between 0
               % and1 for each hard crab recruit
         Ninithard=sizehard; %determining number of hard crab recruits at time t
         Nthard=Ninithard*exp(-Zhard); %using exponential decay equation to
               % determine number of hard crab recruits at time t+1
         surviveprobhard=Nthard/Ninithard; %determining the survival probability
         if randomnumber(:,i)<=surviveprobhard; %when random number for each
               % individual is less than the probability of surviving
               % the individual survives and is incorporated into Newhard and
               % Newnumbermoltshard
            NewPophard=NewPophard+1; %updating row number
            Newhard(NewPophard,:)=hard(i,:);
            Newnumbermoltshard(NewPophard,:)=numbermoltshard(i,:);
         end;
      end;
   end;
   CW = cat(1,Newnonrecruits(1:NewPopnon,:),Newpeeler(1:NewPoppeeler,:),
Newhard(1:NewPophard,:)); %concatenating matrices of nonrecruits, peeler, and
         % hard crab recruits to create CW and numbermolts
      numbermolts=cat(1,Newnumbermoltsnon(1:NewPopnon,:),
Newnumbermoltspeeler(1:NewPoppeeler,:),
Newnumbermoltshard(1:NewPophard,:));
   for i=1:length(CW);
      if CW(i,:)<1;
         CW(i,:)=[ ];%deleting 'dummy' variable if present
         numbermolts(i,:)=[ ];
      end;
   end;
end;
CW121=CW(:,121);
CW;
numbermolts;
.
.
```

```
.
.

for t=196:213; %time steps iterated daily from July 15, 2002 (196) to August 1, 2002
        % (213).  Note, commercial size limits change on July 15
   rownon=0; rowpeeler=0; rowhard=0; %initializing row values
   NewPopnon=0; NewPoppeeler=0;  NewPophard=0;
   Newnonrecruits=zeros(1,349); Newnumbermoltsnon=zeros(1,349); %creating
        % 'dummy'  variables as placeholders
   Newpeeler=zeros(1,349); Newnumbermoltspeeler=zeros(1,349);
   Newhard=zeros(1,349); Newnumbermoltshard=zeros(1,349);
   nonrecruits=zeros(1,349); numbermoltsnon=zeros(1,349);
   peeler=zeros(1,349); numbermoltspeeler=zeros(1,349);
   hard=zeros(1,349); numbermoltshard=zeros(1,349);
   lengthCW=length(CW); %determining the largest dimension (# rows) in matrix
        % CW
   for i=1:lengthCW;
      if ((CW(i,t) < 88.9) | ((numbermolts(i,t) < 1) & (CW(i,t) < 133.3))); %criteria
         % used  for determining nonrecruits, peeler recruits and hard crab recruits
         rownon=rownon+1; %updating row
         nonrecruits(rownon,:)=CW(i,:); %placing individuals from CW and
                % numbermolts that meet nonrecruit criteria in new matrices
                % nonrecruits and numbermoltsnon
         numbermoltsnon(rownon,:)=numbermolts(i,:);
      end;
      if ((numbermolts(i,t) > 0) & (CW(i,t) >= 88.9));
         rowpeeler=rowpeeler+1;
         peeler(rowpeeler,:)=CW(i,:);
         numbermoltspeeler(rowpeeler,:)=numbermolts(i,:);
      end;
      if ((CW(i,t) >= 133.3) & (numbermolts(i,t) < 1));
         rowhard=rowhard+1;
         hard(rowhard,:)=CW(i,:);
         numbermoltshard(rowhard,:)=numbermolts(i,:);
      end;
   end;
   sizenon=size(nonrecruits); %determining size (number of individuals) that are
        % nonrecruits
   sizenon=sizenon(1,1);
   if nonrecruits(1,:) > 0;
      Newnonrecruits=zeros(sizenon,349); %preallocating matrices
      Newnumbermoltsnon=zeros(sizenon,349);
      for i=1:sizenon;
         randomnumber(:,i)=rand(1,1); %generating uniform random number between
                % 0 and 1 for each nonrecruit
         Ninitnon=sizenon; %determining number of nonrecruits at time t
```

```
      Ntnon=Ninitnon*exp(-M); %using exponential decay equation to determine
            % number of non recruits at time t+1
      surviveprobnon=Ntnon/Ninitnon; %determining the survival probability
      if randomnumber(:,i)<=surviveprobnon; %when random number for each
            % individual is less than the probability of surviving the individual
            % survives and is incorporated into Newnonrecruits and
            % Newnumbermoltsnon
        NewPopnon = NewPopnon+1; %updating row number
        Newnonrecruits(NewPopnon,:)=nonrecruits(i,:);
        Newnumbermoltsnon(NewPopnon,:)=numbermoltsnon(i,:);
      end;
   end;
end;
sizepeeler=size(peeler); %determining size (number of individuals) that are peeler
      % recruits
sizepeeler=sizepeeler(1,1);
if peeler(1,:)> 0;
   Newpeeler=zeros(sizepeeler,349); %preallocating matrices
   Newnumbermoltspeeler=zeros(sizepeeler,349);
   for i=1:sizepeeler;
      randomnumber(:,i)=rand(1,1); %generating uniform random number between
            % 0 and 1 for each peeler recruit
      Ninitpeeler=sizepeeler; %determining number of peeler recruits at time t
      Ntpeeler=Ninitpeeler*exp(-Zpeeler*7); %using exponential decay equation to
            % determine number of peeler recruits at time t+1, multiplying Z by 7
            % because peelers are susceptible for 7 days per molt
      surviveprobpeeler=Ntpeeler/Ninitpeeler; %determining the survival
            % probability
      if randomnumber(:,i)<=surviveprobpeeler; %when random number for each
            % individual is less than the probability of surviving the individual
            % survives and is incorporated into Newpeeler and
            % Newnumbermoltspeeler
        NewPoppeeler=NewPoppeeler+1; %updating row number
        Newpeeler(NewPoppeeler,:)=peeler(i,:);
        Newnumbermoltspeeler(NewPoppeeler,:)=numbermoltspeeler(i,:);
      end;
   end;
end;
sizehard=size(hard); %determining size (number of individuals) that are hard crab
      % recruits
sizehard=sizehard(1,1);
if hard(1,:)> 0;
   Newhard=zeros(sizehard,349); %preallocating matrices
   Newnumbermoltshard=zeros(sizehard,349);
   for i=1:sizehard;
      randomonumber=rand(1,1); %generating uniform random number between 0
```

```
            % and 1 for each hard crab recruit
        Ninithard=sizehard; %determining number of hard crab recruits at time t
        Nthard=Ninithard*exp(-Zhard); %using exponential decay equation to
            % determine number of hard crab recruits at time t+1
        surviveprobhard=Nthard/Ninithard; %determining the survival probability
        if randomnumber(:,i)<=surviveprobhard; %when random number for each
            % individual is less than the probability of surviving the individual
            % survives and is incorporated into Newhard and
            % Newnumbermoltshard
          NewPophard=NewPophard+1; %updating row number
          Newhard(NewPophard,:)=hard(i,:);
          Newnumbermoltshard(NewPophard,:)=numbermoltshard(i,:);
        end;
      end;
   end;
   CW = cat(1,Newnonrecruits(1:NewPopnon,:),Newpeeler(1:NewPoppeeler,:),
Newhard(1:NewPophard,:)); %concatenating matrices of nonrecruits, peeler, and
        % hard crab recruits to create CW and numbermolts
      numbermolts=cat(1,Newnumbermoltsnon(1:NewPopnon,:),
Newnumbermoltspeeler(1:NewPoppeeler,:),
Newnumbermoltshard(1:NewPophard,:));
   for i=1:length(CW);
      if CW(i,:)<1;
        CW(i,:)=[ ]; %deleting 'dummy' variable if present
        numbermolts(i,:)=[ ];
      end;
   end;
end;
CW213=CW(:,213);
CW;
numbermolts;
.
.
.
.
for t=306:349; %time steps iterated daily from November 2, 2002 (306) to December
        % 15,2002 (349). Note, fishery closed after Dec. 15.
   rownon=0; rowpeeler=0; rowhard=0; %initializing row values
   NewPopnon=0; NewPoppeeler=0;  NewPophard=0;
   Newnonrecruits=zeros(1,349); Newnumbermoltsnon=zeros(1,349); %creating
        % 'dummy' variables as placeholders
   Newpeeler=zeros(1,349); Newnumbermoltspeeler=zeros(1,349);
   Newhard=zeros(1,349); Newnumbermoltshard=zeros(1,349);
   nonrecruits=zeros(1,349); numbermoltsnon=zeros(1,349);
   peeler=zeros(1,349); numbermoltspeeler=zeros(1,349);
   hard=zeros(1,349); numbermoltshard=zeros(1,349);
```

```
sizeCW=size(CW); %determining the largest dimension (# rows) in matrix CW
sizeCW=sizeCW(1,1);
for i=1:sizeCW;
   if ((CW(i,t) < 88.9) | ((numbermolts(i,t) < 1) & (CW(i,t) < 133.3))); %criteria
      % used for determining nonrecruits, peeler recruits and hard crab recruits
      rownon=rownon+1; %updating row
      nonrecruits(rownon,:)=CW(i,:); %placing individuals from CW and
            % numbermolts that meet nonrecruit criteria in new matrices
            % nonrecruits and numbermoltsnon
      numbermoltsnon(rownon,:)=numbermolts(i,:);
   end;
   if ((numbermolts(i,t) > 0) & (CW(i,t) >= 88.9));
      rowpeeler=rowpeeler+1;
      peeler(rowpeeler,:)=CW(i,:);
      numbermoltspeeler(rowpeeler,:)=numbermolts(i,:);
   end;
   if ((CW(i,t) >= 133.3) & (numbermolts(i,t) < 1));
      rowhard=rowhard+1;
      hard(rowhard,:)=CW(i,:);
      numbermoltshard(rowhard,:)=numbermolts(i,:);
   end;
end;
sizenon=size(nonrecruits); %determining size (number of individuals) that are
      % nonrecruits
sizenon=sizenon(1,1);
if nonrecruits(1,:) > 0;
   Newnonrecruits=zeros(sizenon,349); %preallocating matrices
   Newnumbermoltsnon=zeros(sizenon,349);
   for i=1:sizenon;
      randomnumber(:,i)=rand(1,1); %generating uniform random number between
            % 0 and 1 for each nonrecruit
      Ninitnon=sizenon; %determining number of nonrecruits at time t
      Ntnon=Ninitnon*exp(-M); %using exponential decay equation to determine
            % number of non recruits at time t+1
      surviveprobnon=Ntnon/Ninitnon; %determining the survival probability
      if randomnumber(:,i)<=surviveprobnon; %when random number for each
            % individual is less than the probability of surviving the individual
            % survives and is incorporated into Newnonrecruits and
            % Newnumbermoltsnon
         NewPopnon = NewPopnon+1; %updating row number
         Newnonrecruits(NewPopnon,:)=nonrecruits(i,:);
         Newnumbermoltsnon(NewPopnon,:)=numbermoltsnon(i,:);
      end;
   end;
end;
sizepeeler=size(peeler); %determining size (number of individuals) that are peeler
```

```
        % recruits
sizepeeler=sizepeeler(1,1);
if peeler(1,:)> 0;
   Newpeeler=zeros(sizepeeler,349); %preallocating matrices
   Newnumbermoltspeeler=zeros(sizepeeler,349);
   for i=1:sizepeeler;
      randomnumber(:,i)=rand(1,1); %generating uniform random number between
            % 0 and 1 for each peeler recruit
      Ninitpeeler=sizepeeler; %determining number of peeler recruits at time t
      Ntpeeler=Ninitpeeler*exp(-Zpeeler*7); %using exponential decay equation to
            % determine number of peeler recruits at time t+1, multiplying Z by 7
            % because peelers are susceptible for 7 days per molt
      surviveprobpeeler=Ntpeeler/Ninitpeeler; %determining the survival
            % probability
      if randomnumber(:,i)<=surviveprobpeeler; %when random number for each
            % individual is less than the probability of surviving the individual
            % survives and is incorporated into Newpeeler and
            % Newnumbermoltspeeler
         NewPoppeeler=NewPoppeeler+1; %updating row number
         Newpeeler(NewPoppeeler,:)=peeler(i,:);
         Newnumbermoltspeeler(NewPoppeeler,:)=numbermoltspeeler(i,:);
      end;
   end;
end;
sizehard=size(hard); %determining size (number of individuals) that are hard crab
      % recruits
sizehard=sizehard(1,1);
if hard(1,:)> 0;
   Newhard=zeros(sizehard,349); %preallocating matrices
   Newnumbermoltshard=zeros(sizehard,349);
   for i=1:sizehard;
      randomonumber=rand(1,1); %generating uniform random number between 0
            % and 1 for each hard crab recruit
      Ninithard=sizehard; %determining number of hard crab recruits at time t
      Nthard=Ninithard*exp(-Zhard); %using exponential decay equation to
            % determine number of hard crab recruits at time t+1
      surviveprobhard=Nthard/Ninithard; %determining the survival probability
      if randomnumber(:,i)<=surviveprobhard; %when random number for each
            %individual is less than the probability of surviving the individual
            % survives and is incorporated into Newhard and
            % Newnumbermoltshard
         NewPophard=NewPophard+1; %updating row number
         Newhard(NewPophard,:)=hard(i,:);
         Newnumbermoltshard(NewPophard,:)=numbermoltshard(i,:);
      end;
   end;
```

```
   end;
   CW = cat(1,Newnonrecruits(1:NewPopnon,:),Newpeeler(1:NewPoppeeler,:),
Newhard(1:NewPophard,:)); %concatenating matrices of nonrecruits, peeler, and
         % hard crab recruits to create CW and numbermolts
   numbermolts=cat(1,Newnumbermoltsnon(1:NewPopnon,:),
Newnumbermoltspeeler(1:NewPoppeeler,:),
Newnumbermoltshard(1:NewPophard,:));
   sizeCW=size(CW);
   sizeCW=sizeCW(1,1);
      for i=1:sizeCW;
      if CW(i,:)<1;
         CW(i,:)=[ ]; %deleting 'dummy' variable if present
         numbermolts(i,:)=[ ];
      end;
   end;
end;
CW349=CW(:,349);
CW;
numbermolts;
```

Chapter 3: Quantification of extractable lipofuscins in known-age pond-reared juvenile blue crabs and application for age determination of field-collected blue crabs in Chesapeake Bay.

Abstract

Given the commercial value and current depleted status of Chesapeake Bay blue crabs, demographic analyses independent of traditional size-based approaches are necessary for establishing defensible fisheries management targets and thresholds. Lipofuscin (LF), a metabolic byproduct of oxidation accumulating in post-mitotic cells, has been proposed as an alternative approach to traditional ageing methodologies for crustacean. Here, I calibrated LF accumulation rates with known-age pond-reared blue crabs and used LF-based ageing to evaluate age-specific partial recruitment to the blue crab commercial fisheries. Three cohorts of known-age juvenile blue crabs (c. 70 days old), produced at a research hatchery, were released (June 2003, October 2003, and September 2004) into separate earthen brackish-water ponds, reared up to 1.8 years of age and sampled bimonthly. To collect wild juveniles, monthly (June-October) sampling was conducted in two Chesapeake Bay sub-estuaries. Lipofuscin was extracted from eyestalks, and fluorimetrically assayed. Lipofuscin accumulated exponentially with chronological age. Significant accumulation occurred within at least a 4-month period, except during winter months when blue crabs were in a state of torpor. Lipofuscin accumulated at a similar rate (1.74 $\log_e$ LF index yr^{-1}) among pond cohorts and between genders. Mean age prediction errors for LF and carapace width were c. 0.2 years over the duration of pond-rearing. Lipofuscin-based age designations for field-collected blue crabs support my hypothesis that peeler fisheries in the summer and hard crab fisheries in

the fall/winter are predominately dependent on recruits younger than 18 months of age.

Introduction

The blue crab (*Callinectes sapidus* Rathbun) is a commercially and ecologically important species throughout much of its range, which extends from Brazil to New England (Norse 1977). In North America, the largest fishery occurs in Chesapeake Bay (Sharov et al. 2003), commercially comprised of two primary sectors: the peeler/soft crab and hard crab fisheries (for more details see Chapter 2). Given the commercial value and current depleted status (Lipcius and Stockhausen 2002) of Chesapeake Bay blue crabs, demographic analyses independent of traditional size-based approaches are paramount in establishing defensible fisheries management targets and thresholds aimed at facilitating sustainable exploitation of the species.

Age composition is often used to determine vital rates (e.g., growth, maturation, and mortality), assess recruitment, compare relative abundance of year classes, and estimate longevity (DeVries & Frie 1996). Examination of hard parts (i.e. otoliths, scales, fin spines) is the most frequently used method for ageing fish (Weatherly and Gill 1987). Such age determinations are not possible for crustacean, which periodically molt their exoskeleton, thereby removing any evidence of age (Sheehy 1990, Ju et al. 1999, Hartnoll 2001). Consequently, length frequency modal analysis has been used as an alternative to direct/indirect ageing (see Chapter 2, France et al. 1991, Sheehy et al. 1997, Ju et al. 2003). Yet, discontinuous and seasonal growth patterns (see Chapter 2), combined with protracted spawning reduce the effectiveness of morphometric approaches for age determination of blue crab.

Recently, the use of lipofuscin (LF) has been proposed as an alternative approach to traditional ageing methodologies for crustacean. For well over a century the scientific community has recognized that normal senescence in eukaryotic organisms was accompanied by the progressive cellular accumulation of fluorescent pigments, classically referred to as LF (Hammer and Braum 1988, Katz and Robison 2002, Chowdhury et al. 2004). Generation of the fluorescent granular pigments in post-mitotic cells is believed to be the product of free radical-mediated lipid peroxidation and the accumulation of non-degradable oxidized macromolecules at the primary site of cellular waste disposal—the lysosome (Hill and Womersley 1993, Brunk and Terman 2002, Chowdhury et al. 2004). Because the amount of LF increases positively with chronological age and the rate of accumulation is negatively correlated with longevity, LF is often referred to as an age pigment (Brunk & Terman 2002). Quantification of LF, by several approaches, has been applied to estimate age, growth, and longevity for a diverse range of taxa including: lobsters (Wahle et al. 1996, Sheehy et al. 1997), shrimps (Vila et al. 2000, Bluhm & Brey 2001), crayfish (Belchier et al. 1998), crabs (Ju et al. 2001, 2003), amphipods (Bluhm et al. 2001), fishes (Vernet et al. 1988), and bivalves (Lomovasky et al. 2002).

The generally accepted mechanisms for LF formation suggest that alterations in metabolic rate must be taken into consideration if lipofuscin is to be applied as an indicator of age (O'Donovan and Tully 1996, Ju and Harvey 2002). In poikilotherms, metabolic processes and presumably LF accumulation are, at least partially, dependent on environmental temperature (Tully et al. 2000). The affects of temperature on metabolic rate may be particularly pronounced in temperate estuarine

habitats (e.g. Chesapeake Bay) where large temperature fluctuations occur seasonally (Leffler 1972). Although several studies have investigated the affects of temperature on LF accumulation, such studies have typically been confined to laboratory settings (Hill and Womersley 1993, O'Donovan and Tully 1996, Tully et al. 2000, Ju and Harvey 2002), which may introduce additional interactions (e.g., physiological stress, unnatural forage, and container effects). Thus, examining LF accumulation rates in more natural settings (i.e., earthen ponds) may be more appropriate when extrapolating effects to wild populations. Furthermore, given the uncertainty in structure and formation of LF, studies investigating the pattern of accumulation with respect to age should be calibrated by direct observation of known-age individuals.

Here, I reared three hatchery-produced known-age cohorts of juvenile blue crabs in separate earthen ponds to (1) calibrate LF accumulation rates, (2) investigate the effects of temperature on LF accumulation, and (3) assess the applicability of LF as an indicator of age in wild populations of Chesapeake Bay blue crabs. The juvenile (0+) stage of the blue crab life cycle was emphasized for two primary reasons. Lipofuscin accumulation has not been previously measured for juvenile blue crabs. Additionally, recent research has documented high growth rates and rapid (fishery) recruitment (see Chapter 2, Ju et al. 2003). Two sub-estuaries of Chesapeake Bay were sampled to assess the LF-based age composition of field-collected blue crabs and estimate age-specific partial recruitment to peeler/soft and hard crab fisheries. I hypothesize that LF provides a more robust measure of age than length-based measures and that age composition of peeler/soft crab and hard crab

recruits in the summer and fall, respectively, are skewed towards recruits younger than 18 months of age.

Methods

Pond Rearing

Known-age juvenile blue crabs were provided by the Center of Marine Biotechnology (University of Maryland Biotechnology Institute) through their blue crab hatchery program (Zmora et al. 2005). Three cohorts were released into separate earthen brackish water ponds at Horn Point Laboratory (Cambridge, MD) in June 2003, October 2003, and September 2004 to simulate summer and fall settling cohorts (for more details see Chapter 2). Cohorts were released at 63, 66, and 83 days of age (see Table 2.1) and reared for 14 months (cohort 1), 20 months (cohort 2), and 9 months (cohort 3). Ponds received constant flow of ambient water from the Choptank River, and temperature was monitored at1hr intervals from a depth of 1 m with a HOBO ® data logger. Temperature degree-day (TD day) was calculated for each pond cohort from assumed date of settlement (33 days post hatch, Zmora et al. 2005):

$$TDdays = \sum_{i} (t_a - t_b) \qquad (2.1)$$

where i is the number of days, t_a is the mean daily temperature (°C) and t_b is the base temperature (10 °C, Ju 2000) below which growth (molting) was assumed to cease.

Natural refuge was provided by submerged aquatic vegetation (principally *Ruppia maritima*) and the forage base consisted of abundant small fishes (*Fundulus heteroclitus* and *F. majalis*, *Cyprinodon variegates, Gobiasoma bosc*), the brackish water clam (*Rangia cuneata*), polychaetes, and amphipods. From March to October, cohorts were sampled at bimonthly intervals with a seine (1 by 3.1 m with 6 mm

mesh) and/or baited traps (wrapped with 6 mm wire mesh). Individuals were transported back to the laboratory and sacrificed for LF analysis (see below). Carapace width (CW—measured from tips of lateral spines) and sex were recorded for each individual.

Field collection

We conducted monthly (June to October) trawls in two Chesapeake Bay sub-estuaries (see Figure 2.3) to test spatial and temporal variation of age composition of collected crabs. In 2003, we conducted bottom trawls in the lower Choptank River and adjoining Broad Creek, sampling from six fixed stations (see Figure 2.3a). Crabs were collected with a 4.9 m semi-balloon otter trawl with a tickler chain and 12 mm cod-end mesh. On 12 July, c. 25% of samples were collected in floating vegetation with a dip net, comprising the majority of individuals ranging from 11-30 mm CW. Choptank River sampling sites were those designated as part of a MD Department of Natural Resource (DNR) sampling program; MD DNR collected (with the same trawl) blue crabs as part of their program and provided supplemental samples during June, July, and August.

In 2004, blue crabs were collected from the Choptank River in June (same trawl as in 2003) and from the Patuxent River (June-October) with four different gear types (see Figure 2.3). Crabs were collected with a 1.5 by 30.5 m beach seine with 3.2 mm bag mesh in June and with a 4.9 m bottom trawl (as in 2003) in July. From August through October, blue crabs were collected monthly on two consecutive days with one of two gear types, an obliquely towed 18 m^2 midwater trawl with 6 mm cod-end mesh and a 9 m otter trawl with 6 mm cod-end mesh. Monthly sampling sites (16

for otter trawl, 10 for midwater trawl) were randomly selected and distributed throughout c. 45 river km. All collected crabs were wrapped within wet burlap and transported live back to the laboratory for LF analysis; CW (mm) and sex were recorded for each individual (for more sampling details see Chapter 2).

Analytical methods

I used a modified biochemical approach, as reported in Ju et al. (1999) for LF analysis. Crabs were transported to the lab live. Morphological measurements were taken and individuals were anesthetized on ice. External eyestalk(s) were carefully excised. Only the left eyestalk was removed from crabs > 40 mm CW, both eyestalks were removed from crabs < 40 mm. Retinal tissues were separated from external eyestalks (with calcareous exoskeleton) and discarded due to high levels of pigment that may interfere with fluorescence emission of LF (Hill and Womersley 1991). Each excised tissue sample was transferred to a 4 mL amber vial and stored in 2 mL mixture of dichloromethane (CH_2Cl_2) and methanol (MeOH) (2:1, v/v) at -70 °C prior to analysis. Eyestalks were crushed and sonicated for 10 minutes at 25 °C to initiate solvent extraction of lipofuscin with a bath sonicator (Branson 5510). A fraction (½ mL) of the extract was transferred to 1.8 mL vials, dried under N_2, and redissolved in (½ mL) methanol. Afterwards, fluorescent intensity was measured with a fluorescence detector using an Agilent 1100 Series HPLC. Volumes of 10uL from each extract were injected by an auto-sampler (Agilent 1100 Series) with methanol as the carrier solvent (0.8 mL min^{-1}). Fluorescence intensities of extractable LF were measured at a maximum emission wavelength of 405 nm using a maximum excitation

at 340 nm. After LF measurement, the remaining extract in the 1.8 mL vials was retransferred to the original 4 mL amber vials for protein measurement.

To provide a quantitative measure of LF, fluorescence intensities of extracted material were calibrated using quinine sulfate (range 0-0.5 μg/mL) dissolved in 0.1 N sulfuric acid (H_2SO_4) with water as the carrier solvent (Figure 3.1a). Fluorescence intensities were measured at a maximum emission wavelength of 450 nm using a maximum excitation at 340 nm. Fluorescence intensities were converted to concentrations (μg/mL) and normalized to protein content of extracted tissues measured by the modified bicinchoninic acid (BCA) assay described by Nguyen and Harvey (1994). To measure protein content, vials containing eyestalks were dried under N_2 and redissolved with 2 mL of 0.16% deoxycholic acid (DOC) to assist protein dissolution. Samples were sonicated for 10 minutes at 25 °C to extract protein. A 20 μL sample was taken from each vial containing eyestalks and diluted with 80 μL of 0.16% DOC in 5 mL test tubes. For protein standards, 0-40 μL of 1mg/mL Bovine Serum Albumin (Figure 3.1b) and the appropriate amount of 0.16% DOC were added to make a final volume of 100 μL in test tubes. BCA reagents (2 mL) were added to each test tube and then samples were incubated at 60 °C for 1 hour. The absorbance of protein standards and extracts were measured at 562 nm with a Genesys 10 UV spectrophotometer. The protein-normalized LF content

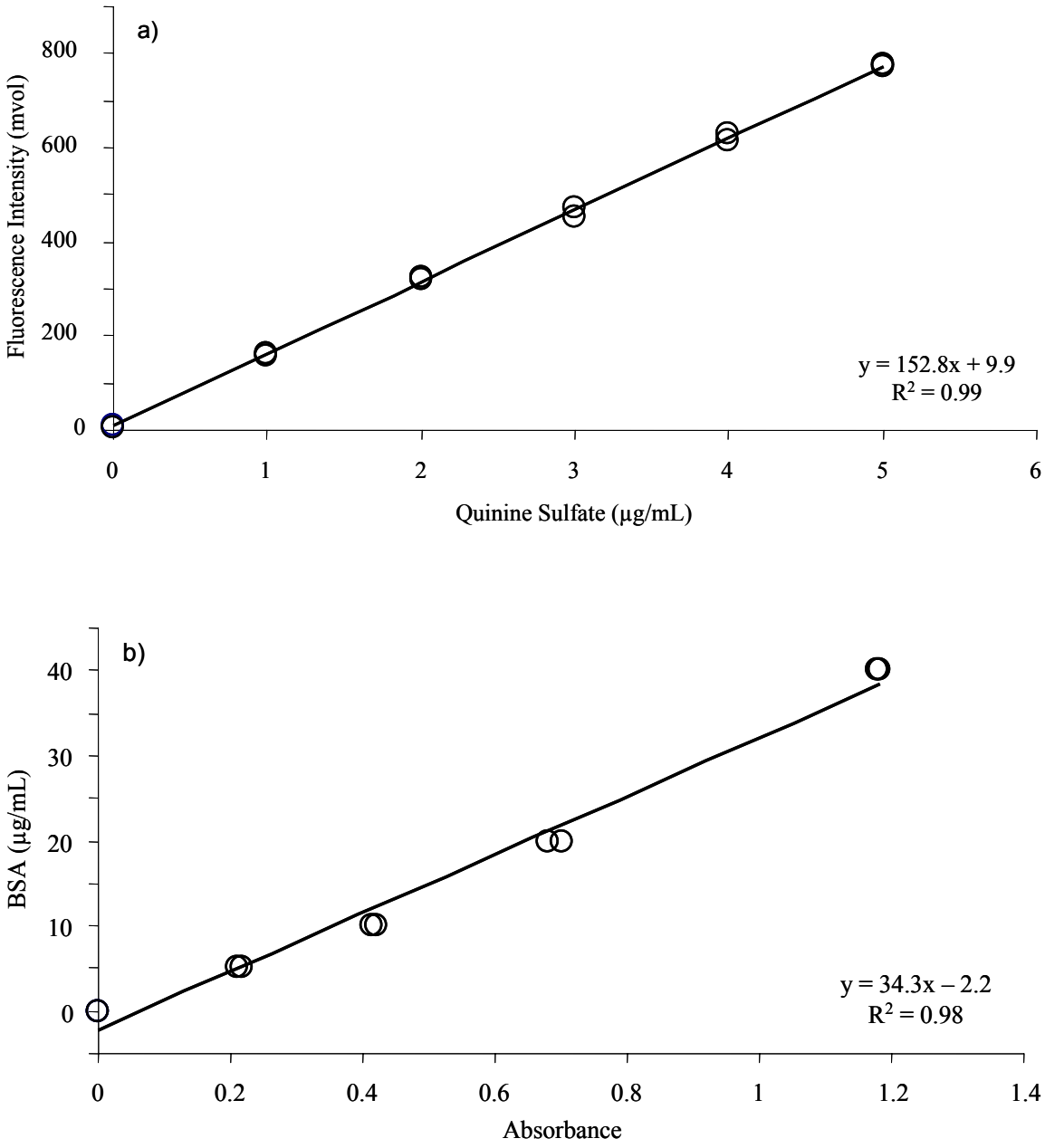

Figure 3.1. (a) An example of the quinine sulfate standard curve used to quantify fluorescence intensity of extracted lipofuscin. Fluorescence intensity measured in millivolts. (b) An example of the Bovine Serum Albumin (BSA) standard curve used to quantify extracted protein. Absorbance measured at 562 nm in absorbance units.

was used to calculate LF index:

$$LFindex(\frac{\mu g\ lipofuscin}{mg\ protein}) = \frac{Lipofuscin\ concentration\ (\frac{\mu g}{mL})}{Total\ protein\ content\ (\frac{mg}{mL})} \quad (2.2)$$

Statistical analyses

For parametric analyses, LF index was $\log_e$ transformed to satisfy assumptions of homogeneity of variance and normality of residuals. Linear regression was used to obtain parameter estimates for LF index as a function of age, CW, and TD day. Analysis of covariance (ANCOVA), with age, CW, and TD day as covariates, was used to determine significant differences in LF accumulation rates between pond-reared cohorts, between river system, and between gender (among pond cohorts and among field collections). Differences of least squares means (Tukey-Kramer adjusted) were calculated to determine time intervals at which significant lipofuscin accumulation occurred and to compare intercepts obtained from linear regression. Multiple linear regression was used to determine explanatory variables needed to construct a predictive model of $\log_e$ LF index. To determine the most appropriate predictive model, several model selection methods were utilized (forward selection, adjusted R^2, and Mallows' C(p) criterion).

For known-age pond-reared cohorts (combined), the relationship between age and LF index was well described by a linear model. The relationship between age and CW was defined with a seasonalized von Bertalanffy growth model (Pitcher and MacDonald 1973):

$$CW(t) = CW_{\infty}[1 - e^{-[C\sin(2\pi(t-t_s)+K(t-t_0)]}] \quad (2.3)$$

where *CW(t)* is carapace width (mm) at age *t*, CW_∞ is the asymptotic maximum carapace width (mm), *K* is the annual growth coefficient (yr^{-1}), t_0 is the theoretical age (yr) when length is zero, *C* is related to the magnitude of the seasonal oscillation, and t_s is the starting age (yr) for the sine curve. Nonlinear regression was used to estimate von Bertalanffy model parameters. The models used to describe the relationship between the independent variable, age, and the dependent variables (either LF index or CW), were appropriately reorganized for back-calculation of age (Table 3.1). Age prediction errors, the absolute difference between predicted and true age, were calculated to assess and compare the accuracy of the age estimates between dependent variables. Individual prediction errors were used to calculate mean age prediction errors (MAPE) for each age group and each dependent variable. Differences of least squares means were used to compare $\log_e$ transformed MAPE between dependent variables. Confidence limits (95%) for LF index-based age estimates were calculated for inverse predictions as described by Sokal and Rohlf (1995).

Results

Known-age validation

A total of 132 known-age blue crabs were collected and analyzed for lipofuscin (Table 3.2). Lipofuscin index accumulated exponentially with respect to age in all pond-reared cohorts (Figure 3.2). Significant $\log_e$ LF index accumulation occurred within at least a 4-month period (0.33 yr), except during winter months when blue crabs were in a state of torpor (Table 3.3, Figure 3.2). Log_e LF index

Table 3.1. Regression models and parameter estimates describing the relationship between age and $\log_e$ LF index (μg mg^{-1}protein) and carapace width (mm) for three cohorts (combined) of known-age pond-reared blue crabs. Table adapted from Belchier et al. 1998.

Predictor variable	Descriptive model	Age prediction models	Parameter estimates
Log_e LF index (μg mg^{-1}protein)	$Log_e\ LF = a(age) + b$	$Age = \frac{\log_e\ LF - b}{a}$	a = 1.74 b = 3.82
CW (mm)	$C_t = CW_\infty(1 - e^{-(C\sin(2\pi(age - t_s)) + K(age - t_0))})$	$Age = \frac{\log_e(-(\frac{CW}{CW_\infty} - 1)) + C\sin 2\pi\ t_s + Kt_0}{-C\sin 2\pi + K}$	CW_∞ = 187 K = 1.50 t_0 = 0.20 C = 0.31 t_s = 1.12

Table 3.2. Sampling dates, age, range of carapace widths (CW) and number of blue crabs analyzed for lipofuscin among three known-age pond-reared cohorts released in June 2003 (cohort 1), October 2003 (cohort 2), and September 2004 (cohort 3).

Cohort 1			
Date	Age in days (years)	CW (mm) range	n (Σn = 48)
6-2-2003	66 (0.18)	12-29	14
7-22-2003	116 (0.32)	73-76	4
9-26-2003	182 (0.50)	112-155	10
3-10-2004	348 (0.95)	112-157	5
5-25-2004	424 (1.16)	138-140	3
7-7-2004	467 (1.28)	142-161	5
8-23-2004	514 (1.41)	153-180	2
8-31-2004	522 (1.43)	153-177	5

Cohort 2			
Date	Age in days (years)	CW (mm) range	n (Σn = 54)
10-8-2003	83 (0.23)	15-33	25
3-10-2004	237 (0.65)	27-33	5
6-1-2004	320 (0.88)	86-111	5
7-7-2004	356 (0.98)	92-137	6
9-18-2004	429 (1.18)	122-135	2
10-19-2004	460 (1.26)	133-183	5
3-29-2004	621 (1.70)	157-156	3
5-19-2005	672 (1.84)	166-167	3

Cohort 3			
Date	Age in days (years)	CW (mm) range	n (Σn = 30)
9-7-2004	63 (0.17)	15-33	17
10-19-04	105 (0.29)	27-33	5
3-29-05	266 (0.73)	86-111	5
5-19-05	317 (0.87)	92-137	3

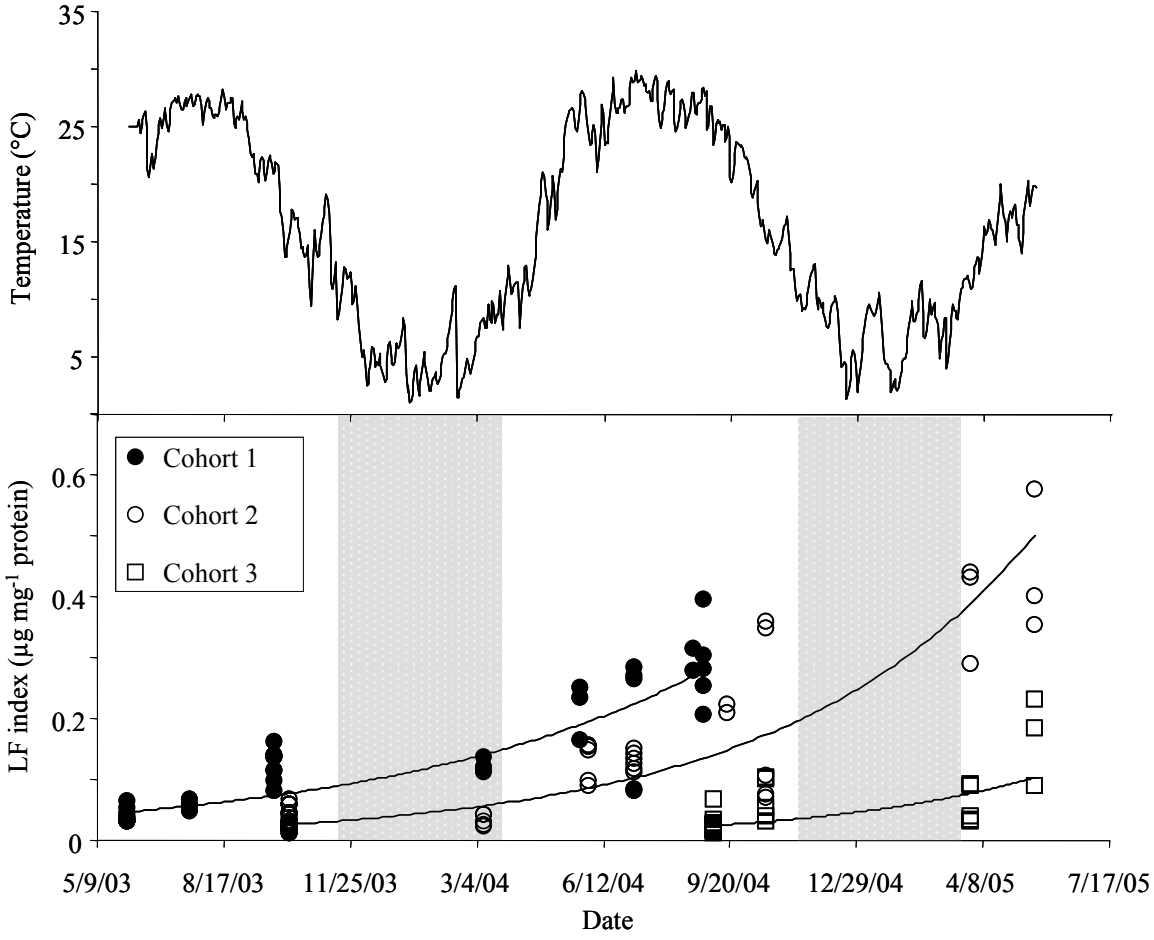

Figure 3.2. Temperature history (upper panel) and lipofuscin index (LF index, µg mg^{-1} protein) accumulation for three pond-reared blue crab cohorts released in June 2003 (cohort 1, n = 48), October 2003 (cohort 2, n = 54), and September 2004 (cohort 3, n = 30). Lipofuscin indices for each cohort were fitted with exponential functions (solid line); linearized parameter estimates for each cohort are provided in Table 3.4. Shaded regions represent dates during which water temperatures were below 10 °C.

Table 3.3. Mean $\log_e$ lipofuscin index (LF index) levels and standard error of the means in relation to age for three known-age pond-reared cohorts of blue crabs released in June 2003 (cohort 1), October 2003 (cohort 2), and September 2004 (cohort 3).

Cohort 1		Cohort 2		Cohort 3	
Age days (years)	Mean LN LF index (s.e.)	Age days (years)	Mean LN LF index (s.e.)	Age days (years)	Mean LN LF index (s.e.)
66 (0.18)	-3.30 (0.07)[a]	83 (0.23)*	-3.62 (0.10)[a]	63 (0.17)	-3.85 (0.11)[a]
116 (0.32)	-2.89 (0.14)[a]	237 (0.65)*	-3.46 (0.21)[a]	105 (0.29)*	-2.86 (0.20)[b]
182 (0.50)*	-2.10 (0.09)[b]	320 (0.88)	-2.08 (0.21)[b]	266 (0.73)*	-3.01 (0.20)[b]
348 (0.95)*	-2.12 (0.12)[b]	356 (0.98)	-2.05 (0.20)[b]	317 (0.87)	-1.86 (0.26)[c]
424 (1.16)	-1.55 (0.16)[bc]	429 (1.18)	-1.54 (0.34)[bc]	-	-
467 (1.28)	-1.78 (0.12)[bc]	460 (1.26)*	-1.92 (0.21)[bc]	-	-
514 (1.41)	-1.22 (0.20)[c]	621 (1.70)*	-0.97 (0.28)[c]	-	-
522 (1.43)	-1.27 (0.12)[c]	672 (1.84)	-0.84 (0.28)[c]	-	-

*Indicates collection dates in October and March (of the following year), which I assumed to represent pre- and post-winter torpor for blue crabs in earthen ponds.

[abc] Letters denote significantly different mean $\log_e$ LF indices between ages

accumulated positively and linearly with respect to age, CW, and TD day in pond-reared cohorts (Figure 3.3). Yet, there was no correlation (Pearson correlation coefficient, $P > 0.1$) between the incremental increase in mean $\log_e$ LF index and the days or accumulated TD days between sampling dates.

Analysis of covariance, with age as the covariate, for cohorts 1 and 2 indicated that cohort had a marginal affect on $\log_e$ LF index ($F = 3.86$, $P = 0.05$, Table 3.4). However, when cohort 3 was included and range of covariate reduced, $\log_e$ LF index accumulation with respect to age was not significantly different between pond cohorts ($F = 0.22$, $P = 0.8$). Cohort effects on $\log_e$ LF index were more pronounced with CW (cohort 1 and 2: $F = 10.2$, $P = 0.002$; all cohorts: $F = 5.2$, $p = 0.007$, Table 3.4) and TD day (cohort 1 and 2: $F = 4.2$, $P = 0.04$; all cohorts: $F = 7.2$, $P = 0.001$, Table 3.4) as covariates. Although not always significantly different, intercept estimates between cohorts followed a general pattern (cohort 1 > cohort 2 > cohort 3, Table 3.4).

Despite apparent cohort differences in $\log_e$ LF index accumulation, cohorts were combined to investigate the affects of gender on $\log_e$ LF index (Table 3.5, Figure 3.4). Gender-specific differences in accumulation of $\log_e$ LF index with respect to age and CW were not apparent from ANCOVA analysis ($F = 2.0$, $P = 0.2$; $F = 2.6$, $P = 0.1$, respectively), whereas $\log_e$ LF index accumulation was moderately different between genders with TD day as the covariate ($F = 3.8$, $P = 0.05$; Table 3.5). Slope and intercept estimates were typically lower for females than males. Because there were no apparent trends in slopes among cohorts, and $\log_e$ LF index (adjusted for age and CW) was not significantly influenced by gender, data for all three cohorts

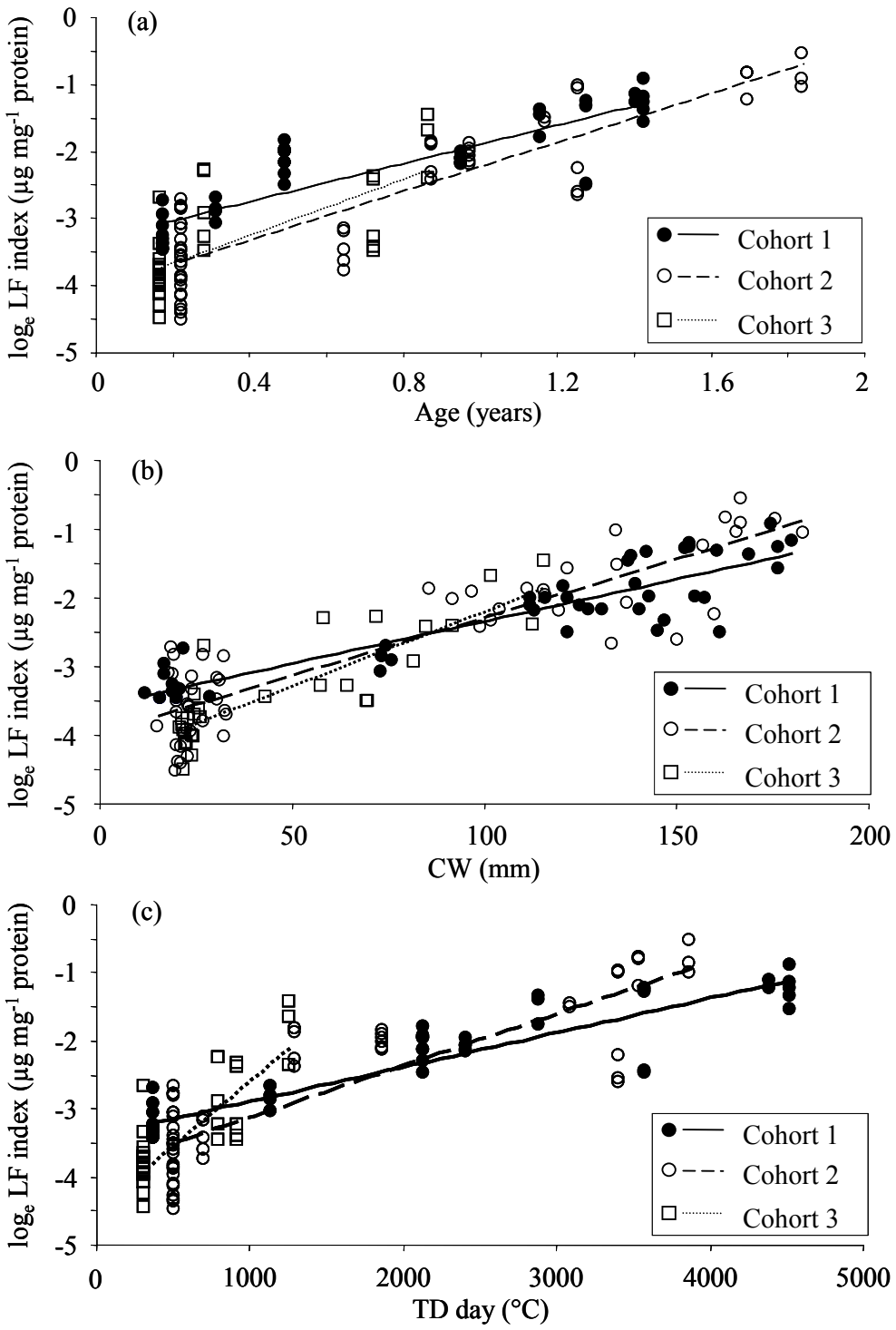

Figure 3.3. Log$_e$ lipofuscin (LF index, µg mg^{-1} protein) accumulation as a function of a) age (years), b) carapace width (mm), and c) temperature degree-days (TD day, °C) for three known-age pond-reared cohorts of blue crabs released in June 2003 (cohort 1, n = 48), October 2003 (cohort 2, n = 54), and September 2004 (cohort 3, n = 30). Parameter estimates and slope and intercept comparisons for each relationship are provided in Table 3.4.

Table 3.4. Parameter estimates ± 95 % confidence intervals for slopes and intercepts obtained from linear regression of $\log_e$ lipofuscin index (LF index, μg mg^{-1}protein) at age (years), carapace width (CW, mm), and temperature degree-days (TD day, °C) for three pond-reared blue crab cohorts (released in June 2003-cohort 1, October 2003-cohort 2, and September 2004-cohort 3). Parameter estimates, coefficients of determination, and P values from linear regression were estimated over the entire rearing duration for each individual cohort (cohort 1: n = 48, cohort 2: n = 54, cohort 3: n = 30). Slopes (ANCOVA) and intercepts (difference of LS means) were compared over common covariate ranges.

	slope	intercept	r^2	P
		LF index vs. Age		
Cohort 1	1.43 ± 0.24	-3.32[a] ± 0.20	0.76	<0.0001
Cohort 2	1.83 ± 0.27	-4.05[b] ± 0.24	0.79	<0.0001
Cohort 3	2.08 ± 0.81	-4.08[b] ± 0.36	0.50	<0.0001
ANCOVA				0.05*
		LF index vs. CW		
Cohort 1	0.01 ± 0.002	-3.57[a] ± 0.20	0.83	<0.0001
Cohort 2	0.02 ± 0.002	-3.98[ab] ± 0.22	0.80	<0.0001
Cohort 3	0.02 ± 0.005	-4.38[b] ± 0.28	0.73	<0.0001
ANCOVA				0.002[+]
		LF index vs. TD day		
Cohort 1	0.0005 ± 0.00006	-3.40[a] ± 0.16	0.85	<0.0001
Cohort 2	0.0008 ± 0.0001	-3.88[a] ± 0.25	0.73	<0.0001
Cohort 3	0.0022 ± 0.0005	-4.49[a] ± 0.36	0.67	<0.0001
ANCOVA				0.04[#]

*Reported P value denotes slope comparisons between cohorts 1 (n = 41) and 2 (n = 48) up to 1.3 years of age. Slope comparisons between all cohorts (up to c. 0.9 years of age, Σn = 98) were non-significant (P = 0.8).

[+]Reported P value denotes slope comparisons between cohorts 1 and 2 over entire CW range. Slope comparisons between all cohorts (up to c. 115 mm CW, Σn = 91) were also significant (P = 0.007).

[#]Reported P value denotes slope comparisons between cohorts 1 (n = 41) and 2 (n = 51) up to c. 3600 TD days. Slope comparisons between all cohorts (up to c. 1300 TD days, Σn = 83) were also significant (P = 0.001).

[ab] Letters denote significantly different intercepts

Table 3.5. Parameter estimates ± 95 % confidence intervals for slopes and intercepts obtained from linear regression of $\log_e$ lipofuscin index (LF index, μg mg^{-1}protein) at age (years), carapace width (CW, mm), and temperature degree-days (TD day, °C) for male (n = 79) and female (n = 53) blue crabs (cohorts combined). Slopes and intercepts were compared by ANCOVA and difference of LS means, respectively.

Regression	slope	intercept	r^2	P
LF index vs. Age				
Male	1.82 ± 0.22	-3.78[a] ± 0.18	0.77	<0.0001
Female	1.54 ± 0.34	-3.82[b] ± 0.26	0.62	<0.0001
ANCOVA				0.2
LF index vs. CW				
Male	0.02 ± 0.002	-3.92[a] ± 0.16	0.84	<0.0001
Female	0.01 ± 0.002	-3.93[b] ± 0.22	0.73	<0.0001
ANCOVA				0.1
LF index vs. TD day				
Male	0.0007 ± 0.00008	-3.72[a] ± 0.17	0.79	<0.0001
Female	0.0006 ± 0.0001	-3.68[a] ± 0.24	0.60	<0.0001
ANCOVA				0.05

[ab] Letters denote significantly different intercepts

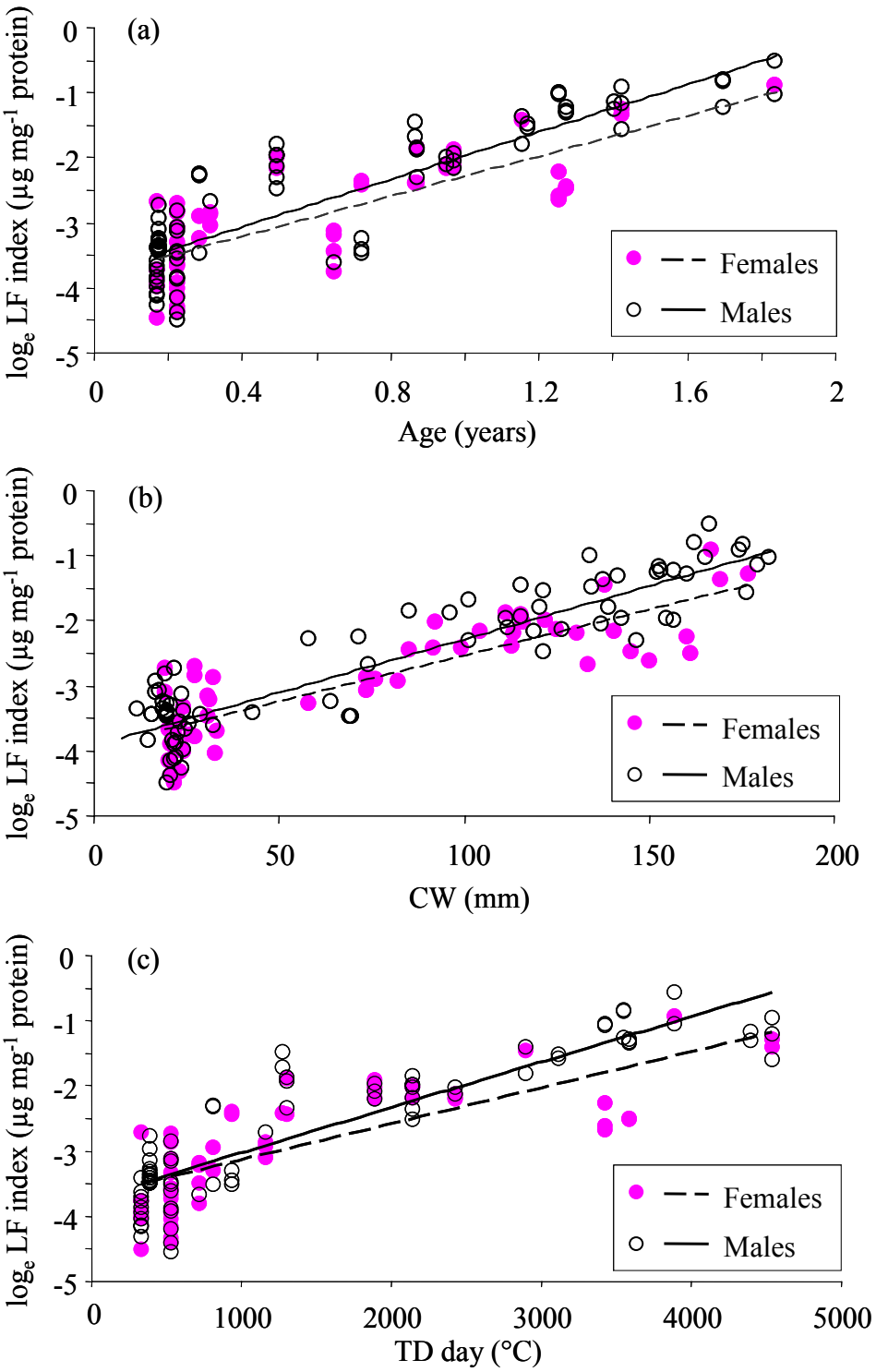

Figure 3.4. Log$_e$ lipofuscin index (LF index, µg mg^{-1} protein) accumulation as a function of a) age (years), b) carapace width (mm), and c) temperature degree-days (TD day, °C) for female (n = 53) and male (n = 79) blue crabs from three known-age pond-reared cohorts (combined) of blue crabs. Parameter estimates and slope and intercept comparisons for each relationship are provided in Table 3.5.

and both sexes were combined (Figure 3.5) to construct a predictive model for $\log_e$ LF index. The predictive model was used to evaluate the appropriateness of $\log_e$ LF index and CW as age predictors, and to predict age of field-collected cohorts.

Predictive model

Multiple regression analysis of the initial general linear model (Log_e LF index = β_1*age + β_2*CW + β_3*TD day) resulted in an adjusted R^2 of 0.81 and parameter estimates in which only the slope of TD day was not significantly greater than 0. Analysis of the squared partial correlation coefficients using type I sum of squares indicated that age alone explained c. 72% of variability in $\log_e$ LF index, and CW explained an additional 33% of the variation in $\log_e$ LF index given that age was already included in the model. All model selection techniques and factor orders indicated that including age and CW provided the most robust description of $\log_e$ LF index.

Age prediction error

Log_e LF index-based age was predicted with a linear function and CW-based age was predicted with a seasonalized von Bertalanffy growth function over the duration of pond rearing (i.e., 1.8 years of age, Table 3.1 and Figure 3.6). The linear relationship between $\log_e$ LF index and age derived here was nearly identical to regression equation estimated in Ju et al. 2001 (Figure 3.6a). Mean age prediction errors ranged from 0.003 to 0.64 years using $\log_e$ LF index and from 0.02 to 1.5 years using CW. Over the duration of pond rearing, MAPE were c. 0.2 yrs (2.5 months) for both $\log_e$ LF index- and CW-based age estimates and were not significantly

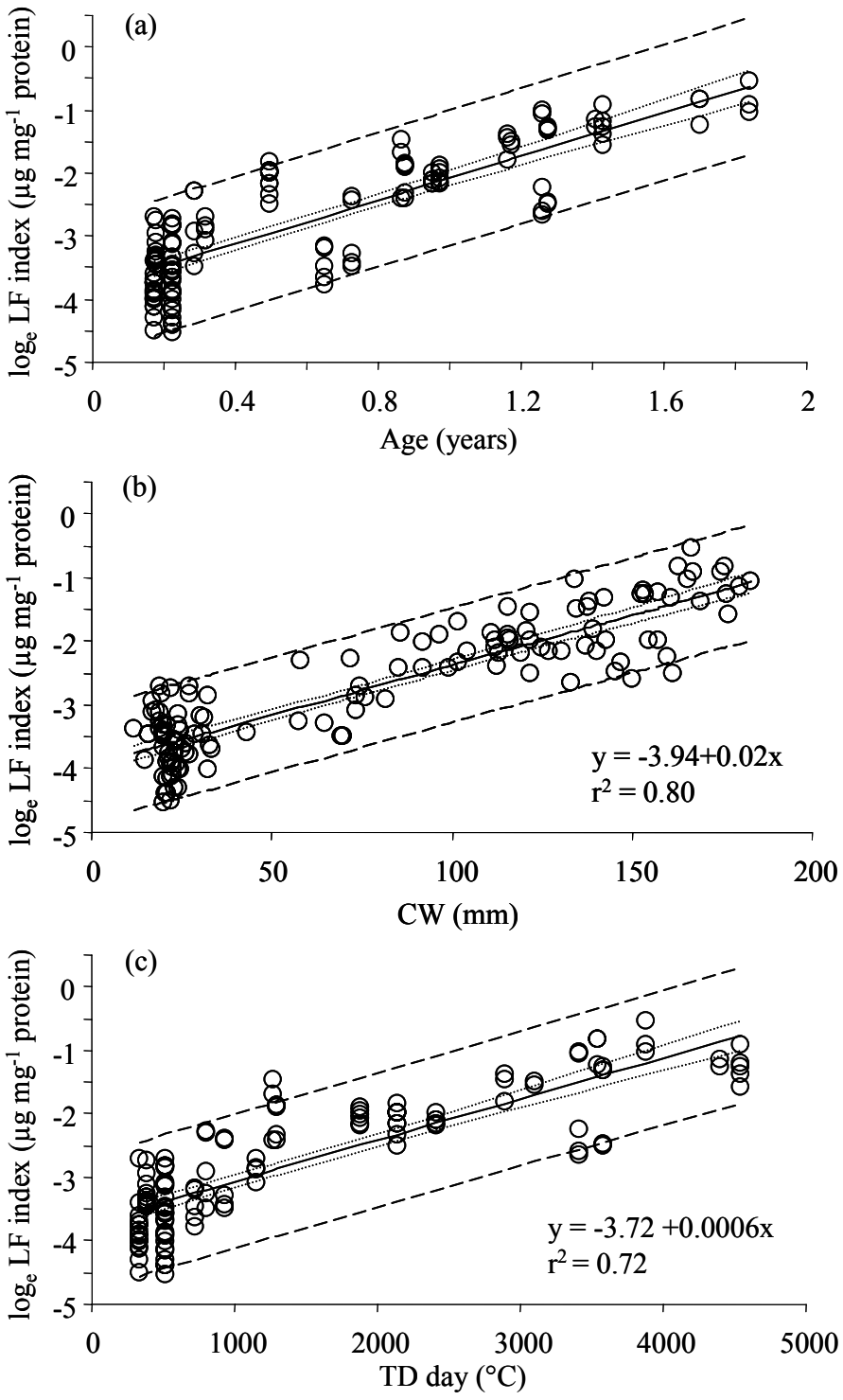

Figure 3.5. Log$_e$ lipofuscin (LF index, µg mg^{-1} protein) accumulation as a function of a) age (years), b) carapace width (mm), and c) temperature degree-day (TD day, °C) for three known-age pond-reared blue crab cohorts (combined, n = 132). Solid, dotted, and dashed lines represent best fitting linear regressions and their 95% confidence limits and prediction limits, respectively.

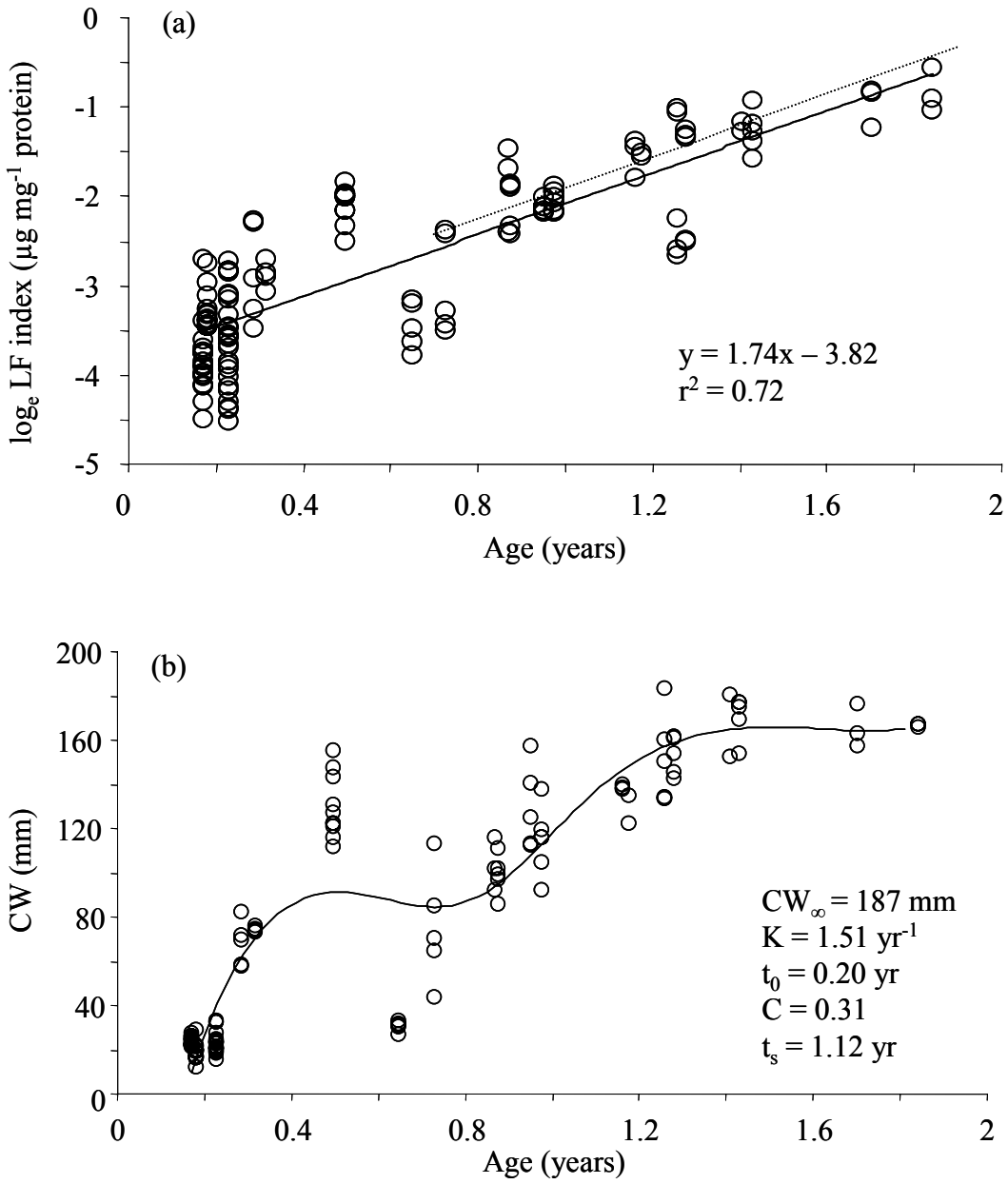

Figure 3.6. Relationship between a) $\log_e$ lipofuscin index (LF index, µg mg^{-1} protein) and age (years) or b) carapace width (mm) and age (years) for three known-age pond-reared blue crab cohorts (combined). Solid lines represent best fitting linear and nonlinear regressions. For reference, the regression line derived for blue crabs (0.7-1.9 years of age) from Ju et al. 2001 is shown (dotted line: $y = 1.75x – 3.65$, $r^2 = 0.7$) in (a).

different from one another ($t = -0.95$, $P = 0.3$). Mean age prediction error for the youngest year class (≤ 0.5 years of age) was significantly lower using CW than that for $\log_e$ LF index ($t = -3.7$, $P = 0.0003$, Figure 3.7). Although MAPE between CW and $\log_e$ LF index did not differ significantly in older year classes ($P > 0.07$), errors for age estimates based on CW correlated positively with age (Pearson correlation; $r = 0.4$, $P < 0.0001$), while error in age estimates using $\log_e$ LF index were negatively correlated with age ($r = -0.2$, $P = 0.02$).

Lipofuscin accumulation in field-collections

A total of 552 crabs from the Choptank River and 366 crabs from the Patuxent River were analyzed for LF (Table 3.6). Interestingly, individuals with the highest LF index were not necessarily the largest individuals (Figure 3.8). Still, $\log_e$ LF index and CW were positively related in field-collected crabs (Choptank River: $r = 0.3$, $P < 0.0001$; Patuxent River: $r = 0.1$, $P = 0.06$), although there was high variability in $\log_e$ LF index at a given size (Table 3.7, Figure 3.8). Although the regression of $\log_e$ LF index on CW was significant for all subcategories (river and gender) except for males collected from the Patuxent River ($t = 0.04$, $P = 0.97$), coefficients of determination were quite low ($r^2 < 0.2$, Table 3.7, Figure 3.8, Figure 3.9). Analysis of covariance indicated that $\log_e$ LF index (adjusted for CW) accumulated at significantly different rates between Choptank and Patuxent River collections ($F = 9.22$, $P = 0.003$), males and females in the Choptank River ($F = 9.13$, $P = 0.003$), and pond cohorts (combined) and field-collections (Choptank R.: $F = 81.5$, $p < 0.0001$; Patuxent R.: $F = 129.7$, $p < 0.0001$).

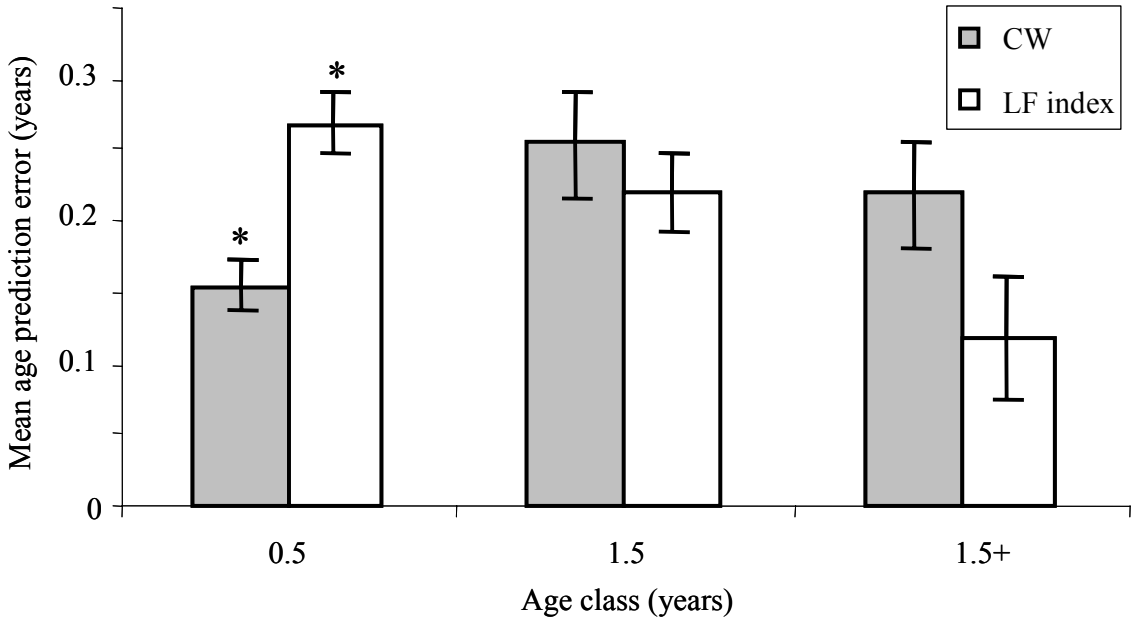

Figure 3.7. Mean age prediction errors for different year classes of blue crabs based on the relationships between age and lipofuscin index (LF index, μg mg^{-1} protein) or carapace width (CW, mm) as presented in Figure 3.6 for three known-age pond-reared blue crab cohorts. Year class designations are as follows: 0—0.5, n = 75; 0.5—1.5, n = 51; 1.5+, n = 6. Note, the 1.5+ age class is represented by a single cohort. Significant differences between mean age prediction errors within age class are indicated by *.

Table 3.6. Sampling dates, range of carapace widths (CW), number of males and females collected, and total monthly sample size of blue crabs collected from the Choptank and Patuxent Rivers and analyzed for lipofuscin. For map of sampling stations see Figure 2.3 and for gear types employed at each date see Table 2.2.

Choptank River 2003 and 2004				
Date	CW (mm) range	# males	# females	n (Σn = 552)
6-16-2003	41-160	78	27	105
7-15-2003	11-160	94	70	164
8-25-2003	60-177	37	79	116
9-26-2003	54-175	19	55	74
10-24-2003	86-178	26	9	35
6-1-2004	97-169	51	7	58

Patuxent River 2004				
Date	CW (mm) range	# males	# females	n (Σn = 366)
6-17-2004	23-121	29	13	42
7-12-2004	23-171	44	34	78
8-4-2004	53-174	54	42	96
9-10-2004	20-178	36	25	61
10-6-2004	19-194	39	50	89

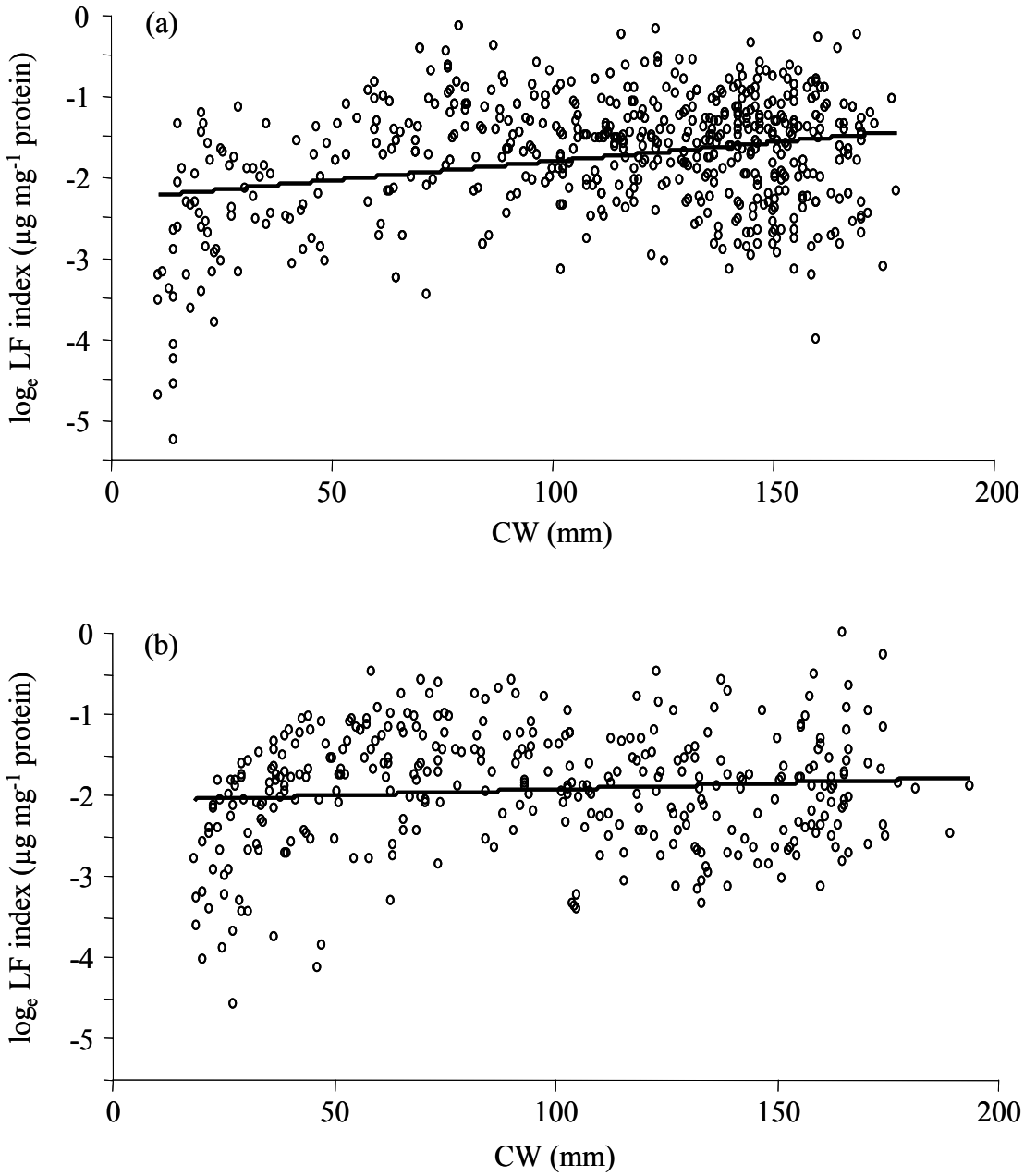

Figure 3.8. Log$_e$ lipofuscin index (LF index, µg mg^{-1} protein) accumulation as a function of carapace width (mm) for blue crabs collected in the a) Choptank River (June-October 2003 and June 2004, n = 552) and b) Patuxent River (June-October 2004, n = 366). Solid line represents best fitting regression line. Parameter estimates and slope and intercept comparisons are provided in Table 3.7.

Table 3.7. Parameter estimates ± 95 % confidence intervals for slopes and intercepts obtained from linear regression of $\log_e$ lipofuscin index (LF index, μg mg^{-1}protein) at carapace width (mm) for blue crabs collected in the Choptank and Patuxent Rivers. Slopes and intercepts were compared by ANCOVA and difference of LS means, respectively.

LF index vs. CW	slope	intercept	r^2	P
Gender combined				
Choptank R.	0.005 ± 0.001	-2.27[a] ± 0.24	0.08	<0.0001
Patuxent R.	0.002 ± 0.002	-2.07[a] ± 0.17	0.01	0.06
ANCOVA				0.003
Choptank R.				
Male	0.007 ± 0.002	-2.40[a] ± 0.20	0.17	<0.0001
Female	0.003 ± 0.002	-2.22[b] ± 0.25	0.03	<0.0001
ANCOVA				0.003
Patuxent R.				
Male	0.00005 ± 0.002	-1.91[a] ± 0.23	0.00	0.97
Female	0.003 ± 0.002	-2.24[a] ± 0.23	0.04	0.008
ANCOVA				0.06

[ab] Letters denote significantly different intercepts

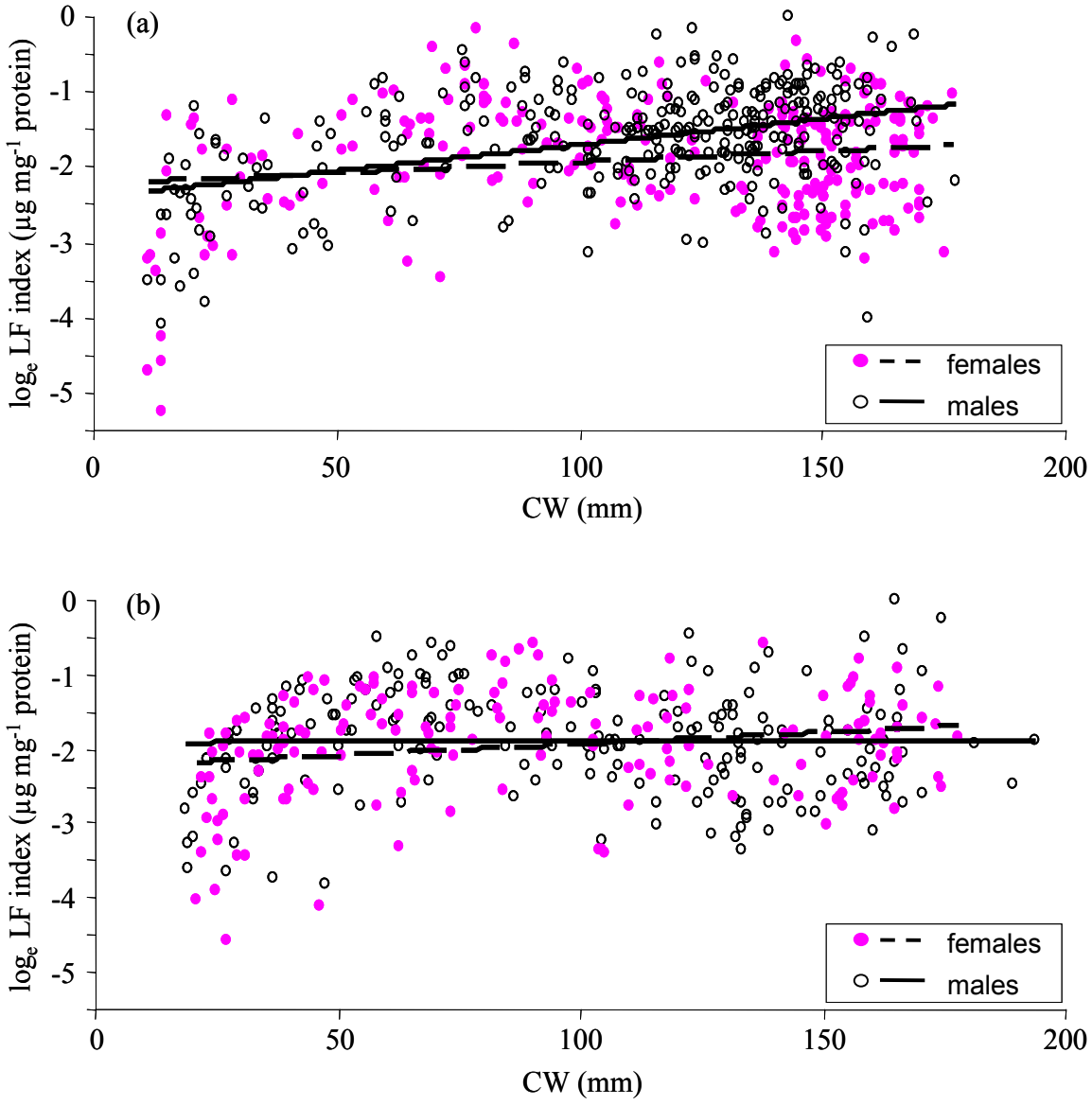

Figure 3.9. Log$_e$ lipofuscin index (µg mg^{-1} protein) accumulation as a function of carapace width (mm) for male and female blue crabs collected in the a) Choptank River (June-October 2003 and June 2004, n = 305 males and 247 females) and b) Patuxent River (June-October 2004, n = 211 males and 167 females). Solid and dashed lines represent best fitting regression line for males and females, respectively. Parameter estimates and slope and intercept comparisons are provided in Table 3.7.

Age composition of field-collections

Based on linear regression analysis of $\log_e$ LF index at age for known-age pond-reared cohorts (Table 3.1), I estimated age for field-collected crabs. Because pond-reared crabs were reared to a maximum age of 1.8 years, I grouped all individuals estimated to be older than 1.5 years of age into a single age class (1.5+). Confidence limits of age estimations indicated that LF-based age estimates provided c. yearly resolution (Figure 3.10). Therefore, age estimates for field-collected individuals were grouped into three age classes (≤ 0.5, $0.5 < \text{age} \leq 1.5$, and > 1.5). Lipofuscin index-based age class designations for field-collected crabs indicated that c. 75% of peeler/soft crab recruits and c. 60% of hard crab recruits were less than 1.5 years of age in summer months (Table 3.8, Figure 3.11 and 3.12). By the fall, both fisheries were almost completely (> 90%) comprised of recruits younger than 1.5 years of age. Further, LF-based age designations suggested that size modes, which are often used in modal analysis, were comprised of multiple age classes.

Discussion

Lipofuscin is a measure of physiological or metabolic age; therefore, the main prerequisite for relating LF to chronological age is a calibration of the accumulation rate (Belchier et al. 1998). In this study, I used known-age pond-reared blue crabs to verify LF accumulation as a function of chronological age. Lipofuscin was quantifiable in juvenile blue crabs c. 0.2 years of age and accumulated exponentially with respect to age in three known-age cohorts of blue crabs raised to ≤ 1.8 years of age. The mean LF accumulation rate and variability reported here (1.74 $\log_e$ LF index year^{-1}, $r^2 = 0.72$) were virtually identical to a previous study conducted by

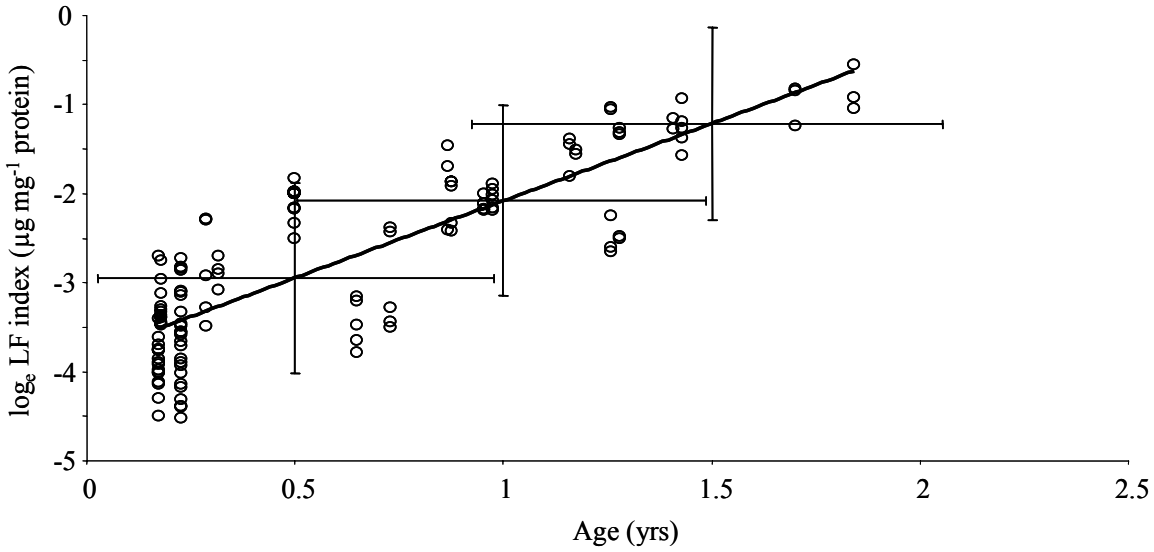

Figure 3.10. Log$_e$ lipofuscin index (LF index, µg mg^{-1} protein) accumulation rate as a function of age as in Figures 2.4a and 2.5a for three known-age pond-reared cohorts of blue crabs. Also shown are 95% confidence limits at 0.5, 1, and 1.5 years of age for lipofuscin estimates (vertical error bars) and inverse prediction of age from lipofuscin (horizontal error bars).

Table 3.8. Age class (years), number, and cumulative relative frequency (in parentheses) of non-recruits, peeler/soft crab recruits, and hard crab recruits within each age class estimated by lipofuscin index (LF index, μg mg^{-1}protein) during summer (June-August) and fall (September-October). Crabs were collected from the Choptank (June-October, 2003 and June, 2004) and Patuxent (June-October 2004) Rivers. Commercial size limits (peeler/soft crabs: > 88.9 mm CW; hard crabs: 133.3 mm CW) assigned to peeler and hard crab recruits are enforced from 15 July to 15 December in Maryland waters.

Choptank River: summer			
Age class (yrs)	Non-recruits (< 88.9mm*)	Peeler/soft crab recruits (>88.9*)	Hard crab recruits (> 133.3 mm*)
≤ 0.5	21 (0.15)	1 (0.01)	0
0.5 < age ≤ 1.5	89 (0.80)	99 (0.75)	106 (0.62)
> 1.5	28 (1)	34 (1)	65 (1)
Σn = 443	138	134	171
Choptank River: fall			
≤ 0.5	0	2 (0.08)	7 (0.08)
0.5 < age ≤ 1.5	1 (0.50)	21 (0.96)	74 (0.98)
> 1.5	1 (1)	1 (1)	2 (1)
Σn = 109	2	24	83

*Commercial size limits in June for peeler/soft crabs and hard crabs are > 82.5 mm and > 127 mm, respectively

Patuxent River: summer			
Age class (yrs)	Non-recruits (< 88.9mm*)	Peeler/soft crab recruits (88.9 < CW < 133.3 mm*)	Hard crab recruits (> 133.3 mm*)
≤ 0.5	1 (0.01)	4 (0.05)	5 (0.1)
0.5 < age ≤ 1.5	65 (0.73)	61 (0.84)	31 (0.73)
> 1.5	24 (1)	12 (1)	13 (1)
Σn = 216	90	77	49
Patuxent River: fall			
≤ 0.5	16 (0.22)	1 (0.06)	2 (0.03)
0.5 < age ≤ 1.5	54 (0.96)	17 (1)	53 (0.93)
> 1.5	3 (1)	0 (1)	4 (1)
Σn = 150	73	18	59

* Commercial size limits in June for peeler/soft crabs and hard crabs are > 82.5 mm and > 127 mm, respectively

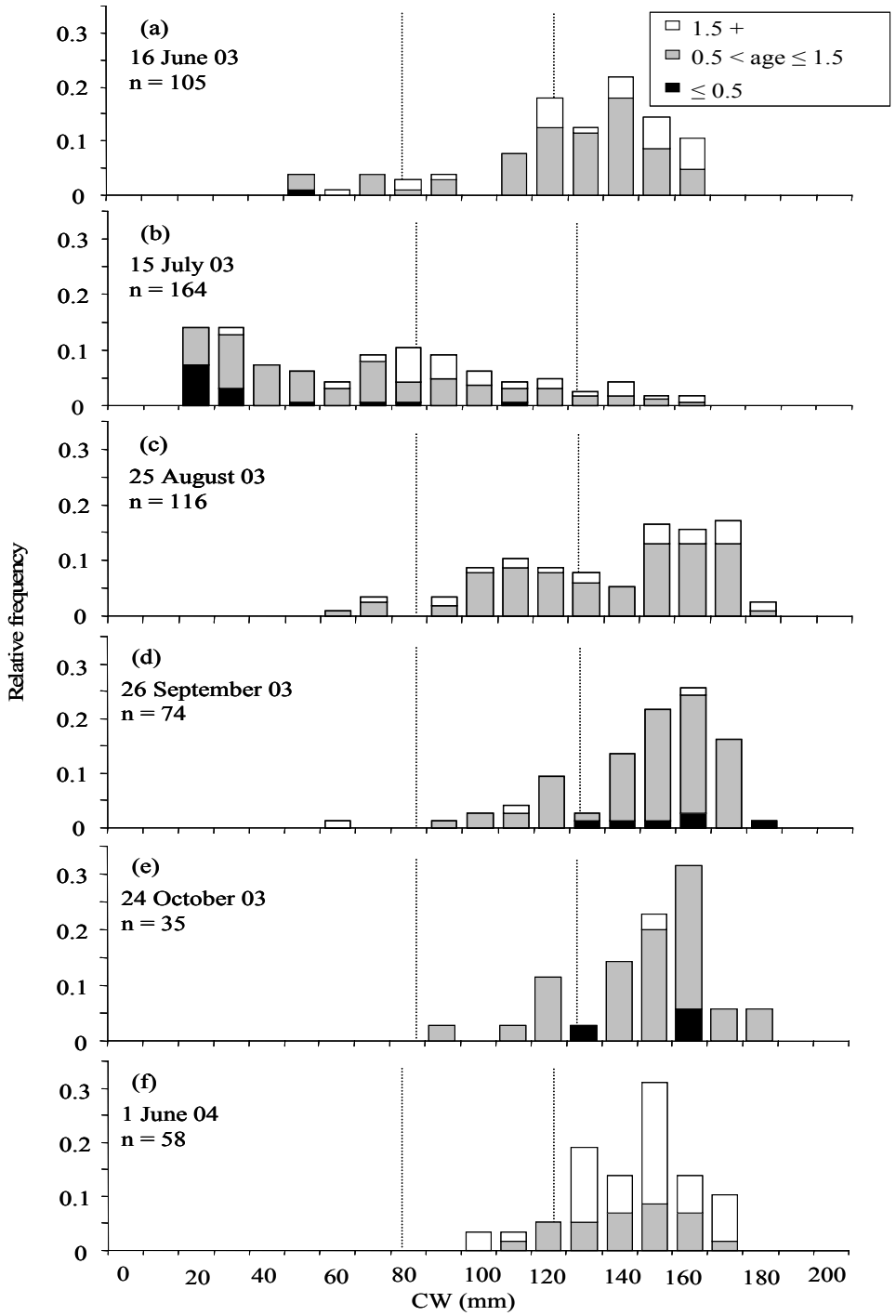

Figure 3.11. Length frequency distributions of blue crabs collected from the Choptank River in a) June 2003, b) July 2003, c) August 2003, d) September 2003, e) October 2003, and f) June 2004 partitioned into lipofuscin-estimated age classes (≤ 0.5, 0.5 < age ≤ 1.5, and 1.5+ years of age). Vertical dotted bars represent minimum commercial size limits for peeler/soft crab and hard crab fisheries. Size limits (peeler/soft crab: 82.5 mm-1 April to 14 July, 88.9 mm-15 July to 15 December; hard crab: 127 mm-1 April to 14 July, 133.3 mm- 15 July to 15 December) vary by season.

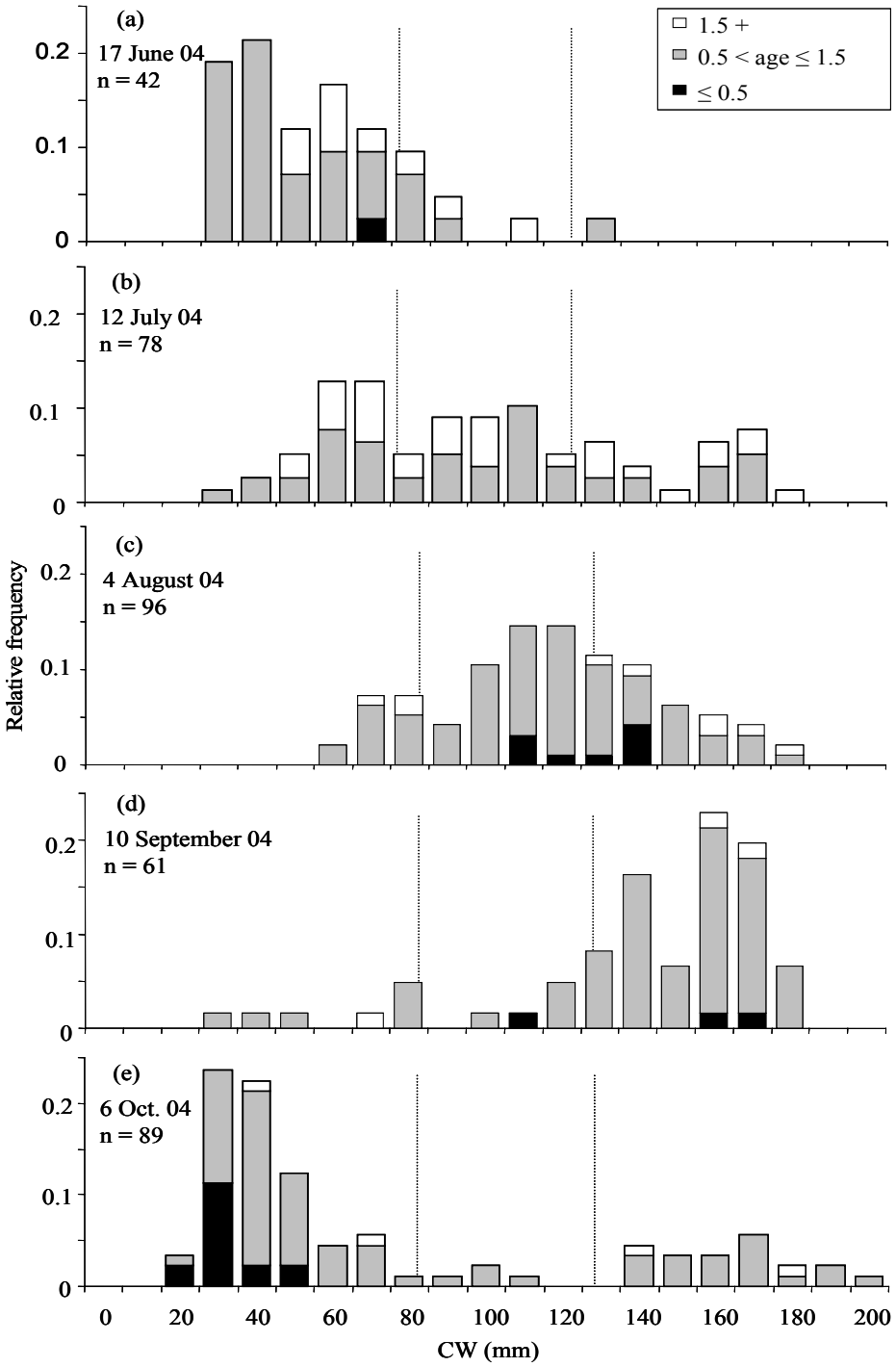

Figure 3.12. Length frequency distributions of blue crabs collected from the Patuxent River in a) June 2004, b) July 2004, c) August 2004, d) September 2004, and e) October 2004 partitioned into lipofuscin-estimated age classes (≤ 0.5, 0.5 < age ≤ 1.5, and 1.5+ years of age). Vertical dotted bars represent minimum commercial size limits for peeler/soft crab and hard crab fisheries. Size limits (peeler/soft crab: 82.5 mm-1 April to 14 July, 88.9 mm-15 July to 15 December; hard crab: 127 mm-1 April to 14 July, 133.3 mm- 15 July to 15 December) vary by season.

Ju et al. (2001, 1.75 $\log_e$ LF index $year^{-1}$, $r^2 = 0.70$). Significant accumulation of LF occurred within a 4 month interval during spring, summer, and fall months. Because LF accumulation is coupled with metabolic rate, temperature-induced torpor (< 10 °C) during winter months significantly reduced the rate of accumulation. Still, similarities in LF accumulation rate (with respect to age) between gender and pond cohorts experiencing vastly different temperature fluctuations at a common age, suggests that lipofuscin is a robust alternative to traditional length-based methods used for crustacean demographic analyses. Application of LF-based age estimates to field-collected blue crabs supported my hypothesis that peeler/soft crab and hard crab fishery recruits were primarily comprised of recruits younger than 1.5 years of age. Yet, uncertainties remain in applying these results to the entirety of Chesapeake Bay due to potentially different spatiotemporal LF accumulation rates and the limited age range over which LF accumulation was calibrated.

Accumulation of lipofuscin

The relationship between $\log_e$ LF index and age, CW, and TD day in known-age pond-reared blue crabs was linear, although seasonal variations in accumulation rates were detected. As a result, the residuals of the exponential function used to depict the accumulation of LF index over time were typically positive during mid-summer months and negative during winter months, likely due to the combination of reduced LF accumulation rates in winter months and the use of least squares regression for fitting purposes. Despite the positive relationship between LF index and age or TD day, there was not a discernible trend in the incremental increase in LF as a function of elapsed time or accumulated TD days between sampling events. The

lack of a positive relationship between elapsed time and LF accumulation is likely attributable to the duration of overwintering (c. 180 days) during which LF accumulation was reduced. Moreover, the elapsed time (< 80 days, with the exception of winter) and the accumulated TD days (< 1200, equivalent to 60 chronological days at 20 °C) between sampling events may have been insufficient for significant accumulation of LF to occur.

Although TD day expresses the physiological effects of temperature while simultaneously accounting for chronological time, the variation in LF index explained by TD day ($r^2 = 0.72$) for three pond-reared cohorts was no better than chronological age alone. Ju et al. (2001) suggested that differences in rearing temperatures (in this study differences in spring, summer, and fall temperatures) may not be sufficient to alter those metabolic rates that affect LF accumulation. Indeed, Sheehy (2002) reported reduced sensitivity of LF accumulation to temperatures in the thermal mid-range, a range that may have been experienced in experimental ponds where temperatures rarely attained super-optimal levels for growth (see Chapter 2, Leffler 1972). Multiple linear regression of LF index with age, CW, and TD day as explanatory variables indicated that LF accumulation was primarily a function of age and CW in pond-reared blue crabs.

Although CW explained the most variability (i.e., $r^2 = 0.80$) in LF index for pond-reared blue crabs, several studies have indicated that LF accumulation is not influenced by size (O'Donovan and Tully 1996, Wahle et al. 1996, Belchier et al. 1998, Ju et al. 2001). Due to the homogeneity within the pond system, crabs were not spatially subjected to various forage bases, temperature regimes, and other

abiotic/biotic environmental conditions that appear to influence CW more than LF index. Ju et al. (2001) reported similar LF indices at highly variable CW between pond- and laboratory-reared blue crabs exposed to different temperature regimes and forage. In support of this notion, the relationship between LF index and CW was highly variable in field-collected crabs, indicating large variability in individual growth rates. Further, differences in the accumulation of LF as a function of CW between pond-reared cohorts, sub-estuaries, and pond and field-collections suggests that the metabolic processes responsible for lipofuscin formation may not be directly linked to the allocation of energy for growth (O'Donovan and Tully 1996, Wahle et al. 1996). As such, lipofuscin can provide an alternative method for age determination that is independent of traditional size-based approaches.

My results confirm that LF index is highly correlated with chronological age and compare favorably with previous LF investigations on blue crabs (Ju et al. 1999, 2001). The apparent absence of a gender-specific lipofuscin accumulation rate across a range of ages is consistent with previous studies conducted on other crustacean species (Sheehy et al. 1994, Vila et al. 2000). More importantly for the utility of LF as an age determinant in the field are inter-cohort similarities in age-related LF accumulation. In Chesapeake Bay, protracted spawning and the onset of winter torpor at different sizes and ages requires separate sub-annual cohort growth models to describe the CW at age relationships (see Chapter 2). The accumulation of LF index with respect to age was statistically similar between three pond-reared cohorts up to c. one year of age. However, after one year of age (and up to 1.8 years of age) LF accumulation rates as a function of age were marginally different among pond

cohorts. If differences in accumulation rates become more apparent at older ages, the effectiveness of LF for age determination when applied to field crabs would be reduced, and potentially no better than morphological approaches.

While a linearized relationship between age and LF is convenient for parametric analyses and prediction of age, such a relationship implies that the accumulation rate of LF is constant throughout the life span of the species (Vila et al. 2000). Yet, LF accumulation rates in tissues have been shown to decelerate with age, prompting the use of curvilinear models to describe the relationship (Sheehy 1992, Sheehy et al. 1994). Here, the approximately linear age-lipofuscin relationship may be attributed to the limited age range examined (≤ 1.8 years), relative to longevity of the species (3-8 years; Van Engel 1958, Rugolo et al. 1998) (O'Donovan and Tully 1996) or a growth dilution effect, when the rapid synthesis of neural tissues during summer months disproportionately exceeds the seasonal accumulation of LF (Ju et al. 1999). Given the initial exponential increase in LF as a function of age, a logistic model (Figure 3.13) may be appropriate and provide insights into longevity, although calibration with older individuals would be required before extrapolating the rate of LF accumulation over the life span of blue crabs.

Age prediction and composition

Both LF and CW were relatively accurate age predictors for pond-reared blue crabs. In fact, mean age prediction errors using either LF or CW were c. 0.2 years over the duration of pond-rearing (1.8 years). However, pond-rearing studies indicated that CW was less variable than LF for early juveniles (< 0.5 years of age)

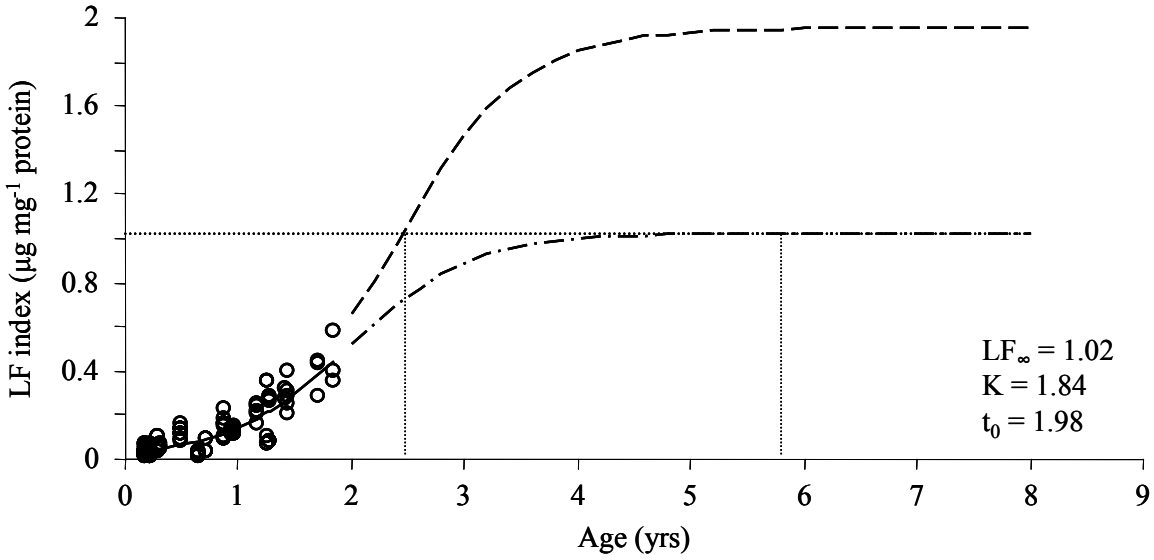

Figure 3.13. Logistic model fit to LF index at age for three known-age pond-reared cohorts (combined). Solid line represents the portion of the curve where data are available; the dotted-dashed line represents extrapolation of the logistic function to ages outside of those observed in this study; the dashed line represents the upper 95% confidence interval of the function; the horizontal and vertical dotted lines represent the asymptotic LF index and the corresponding ages (2.4 and 5.8 years) at which the asymptote is intersected, respectively. Parameter estimates are defined as follows: LF_∞ is the asymptotic lipofuscin index, K is the rate at which the asymptote is approached (yr^{-1}), and t_0 is the theoretical age at a LF index of 0.

and hence a more accurate age predictor. The increased variability in LF at youngest ages (i.e., at release) may be related to the frequency of molts at small size, which can occur within days (see Chapter 2, Tagatz 1968). Molting involves a cascade of physiological and biochemical events (Smith 1997), part of which (molting inhibition) is controlled by the X-organ gland in the eyestalk (Freeman et al. 1987). To the author's knowledge, no studies have explicitly investigated the affects of the molt cycle (i.e., molt stage) on LF accumulation for post-larval individuals. However, Hirche and Anger (1987) documented a loss in LF between zoeal molts (in *Hyas araneus*) and concluded that the molting cycle affects LF accumulation in larvae. Despite the initial variability and imprecise age determination of LF, the range of errors over the duration of pond-rearing were reduced when LF was used as an age predictor. Moreover, LF-based age estimates appeared to increase in accuracy with increasing age, whereas accuracy of age predictions using CW decreased with age. Thus, it appears that asymptotic CW may be reached at an earlier age than asymptotic LF, reducing the effectiveness of morphometric measures for age determinations.

Confidence limits for age predictions at 0.5, 1.0, and 1.5 years of age were ± c. 0.5 years. The confidence limits about the mean obtained from this study at 1.5 years of age (-0.56 and +0.58 years) were similar to those reported for *Cherax quadricarinatus* (longevity 3-5 years) at 2 years of age (-0.33 and +0.76 years) and *Pacifastacus leniusculus* (longevity 12+) at 2 years of age (-0.75 and +0.89 years) (Belchier et al. 1998). However, due to the degree of initial variability in the LF-age relationship, resolution at 0.5 years of age (-0.48 and +0.47 years) was far worse than

the ± c. 0.1 years reported for *C. quadricarinatus* at 0.4 years of age (Sheehy 1992). Given the confidence limits for LF-based age predictions obtained in this study, age class designations (≤ 0.5, ≤ 1.5, and 1.5+) can confidently be grouped as 0+, 1+, and 2+ (i.e., ≤ 1, ≤ 2, and 2+).

Although the resolution of LF-based age predictions in comparison to other crustacean was not exceptional, the technique did highlight the predominance (c. 70%) of blue crabs ≤ 1.5 years of age that were recruited to the peeler/soft and hard crab fisheries. In support of this view, growth rates observed under favorable conditions suggest that cohorts overwintering at small sizes (c. 20 mm) are capable of obtaining legal commercial size within one full growth season (see Chapter 2, Ju et al. 2001). Further, Ju et al. (2003) concluded from LF-based modal analysis that crabs > 2 years of age were a minor contributor (< 10%) to the harvestable stock in Chesapeake Bay. Despite the capability to grow rapidly during spring, summer, and fall months, LF-based age estimates suggest that individual growth rates may be highly variable in the field, hence, similar sized blue crabs can be found in age classes ranging from ≤ 1 to 2+. Such a notion has ramifications for the use of length-frequency modal analysis, which assumes that cohorts are represented by distinct size modes (see Chapter 2, France et al. 1991).

Still, uncertainties remain in applying these results to the entire wild population of blue crabs in Chesapeake Bay. The variance in the LF-age relationship is sufficient to cause significant inaccuracies in individual age determinations (Ju et al. 2003). Moreover, samples used in this study did not cover the entire Chesapeake Bay system. Lipofuscin accumulation rates may fluctuate spatiotemporally as a result

of diet (Castro et al. 2002) and pollution/heavy metal concentrations (Totaro et al. 1985), and individually as a result of genotype (and the ensuing affects on metabolic rate) (O'Donovan and Tully 1996), activity level (e.g., female spawning migration) (Sheehy 1990) or degree of parasitic infestation (Sukhotin et al. 2002). Here, the relationship between LF and age was calibrated with individuals < 2 years of age; additional studies incorporating a more comprehensive range of ages (particularly those > 2 years) are needed to investigate the accumulation of LF throughout the life span of blue crabs.

Chapter 4: Summary, Conclusions, and Future Work

The lack of reliable demographic information has complicated the estimation of vital rates (e.g., growth, mortality, etc.) and the application of stock assessments to commercially important crustaceans (Smith and Addison 2003). Estimating growth rates of crustaceans in temperate regions is further complicated by the discontinuous and highly seasonal growth pattern. The goal of my thesis was to estimate seasonal growth and recruitment rates of juvenile blue crabs in Chesapeake Bay by incorporating three principal independent approaches: direct observation, length-based analysis, and indirect analysis-lipofuscin. Three broad objectives were identified and met:

1) Construct a continuous growth model of known-age pond-reared juvenile blue crabs, and conduct a length frequency modal analysis to estimate seasonal growth rates of field-collected blue crabs.
2) Construct a temperature-dependent molt-process growth model based on empirical observations of known-age pond-reared blue crabs to predict settlement dates and partial recruitments of individuals originating from the Chesapeake Bay Winter Dredge Survey.
3) Calibrate lipofuscin (LF) accumulation rates with respect to chronological age in known-age crabs to estimate age structure of field-collected crabs.

Objective 1: Continuous growth model and length frequency modal analysis

In pond-rearing studies, growth rates peaked (c. 1.5 mm d^{-1}) at small juvenile sizes coincident with favorable temperatures and declined to c. 0.5 mm d^{-1} at sizes

larger than 115 mm. Seasonalized von Bertalanffy growth models indicated that juveniles were capable of reaching c. 140 mm after one full growing season. Parameter estimates were similar to those reported in previous studies although K (the growth coefficient) was c. 2 fold-higher than previously reported from laboratory studies or length-frequency analysis of wild blue crabs. Growth rates obtained from length frequency modal analysis of wild blue crabs in this study were similar to those observed in pond systems and from length frequency modal analysis in Gulf of Mexico states.

Despite the unknown effects of pond-rearing and the subjective interpretation of the length frequency modal analysis, the similarity in estimated growth rates and cohort dynamics obtained from the two methods was striking, and suggests that the estimates derived from these methods represent robust patterns of seasonal and annual growth rates of juvenile blue crab in Chesapeake Bay. Continuous models, such as the von Bertalanffy growth model, have been extensively applied to model blue crab growth, primarily due to the utility of parameter estimates in stock assessments (Rugolo et al. 1998, Helser and Kahn 1999, Miller et al. 2005). While the von Bertalanffy growth model incorporated in the most recent stock assessment of Chesapeake Bay blue crabs (Miller et al. 2005) represents a significant improvement from previous assessments, the growth coefficient (K, 0.82 yr^{-1}) may still underestimate blue crab growth according to pond-rearing experiments (1.0-1.9 yr^{-1}). Errors or biases in the growth model integrated into assessments can cause inaccuracies in recommended levels of exploitation (Miller and Smith 2003). Helser and Kahn (1999) reported an increase in F_{max} (F where yield per recruit is highest,

Jennings et al. 2001) from 1.02 to 1.26 as the value of the von Bertalanffy growth coefficient decreased from 0.75 to 0.62 yr^{-1}. Moreover, the growth models applied for assessment purposes typically fail to incorporate the seasonal and cohort-specific growth patterns that were evident in both pond-rearing experiments and length frequency modal analysis. Such simplifications may reduce the accuracy of population dynamic models.

Objective 2: Molt-process growth model

Predicted settlement dates from the temperature-dependent molt-process growth model compared favorably with reported peaks in settlement (late-September to mid-October). Predicted peaks in recruitment of juveniles (< 70 mm) emerging from winter torpor occurred in July/August and October, concurrent with peaks in summer peeler/soft crab landings and fall hard crab landings. Differences in annual temperature (i.e., 10%) moderately affected predicted entry and mean recruitment to the primary commercial fisheries.

Molt-process growth models based on a temperature degree-day formula (sensu Smith 1997) provide the capacity to simulate the physiological components of growth, individual and inter-annual growth variability, and complex mortality scenarios. As such, molt-process models provide a method for more accurately depicting growth and potentially improving reference points (sensu Bunnell and Miller 2005) and, ultimately, management. While molt process models do not account for all of the factors influencing blue crab population dynamics (e.g., density-dependence), I suggest that the underlying growth component of the modeling framework provides a robust depiction of growth for crustaceans, and therefore, will

be increasingly incorporated into the assessment of such species. To this end, Miller and Smith (2003) concluded that a diversity of models, including molt process models, would provide the most insight into blue crab population dynamics, and in turn, lead to more realistic models of greater utility.

Objective 3: Lipofuscin based-ageing

Lipofuscin accumulated exponentially as a function of chronological age in pond-reared blue crabs, with significant accumulation occurring within a 4 month period (excluding winter months). Accumulation rates were similar among pond cohorts and gender. Mean age prediction errors for LF were c. 0.2 years over the duration of pond-rearing. Lipofuscin-based ageing indicated highly variable growth rates, but still suggested that > 70% of peeler/soft crab and hard crab recruits were ≤ 1.5 years of age.

Results presented in this study, in combination with previous studies (Sheehy 1992, O'Donovan and Tully 1996, Wahle 1996, Belchier et al. 1998, Vila et al. 2000) verify the potential of LF-based methods for directly determining age composition of crustaceans. Because LF accumulation does not appear to be solely dependent on size, LF-based approaches provide a method for demographic analyses independent of traditional morphometric approaches. The value of any ageing technique is ultimately linked to accuracy and precision. Lipofuscin-based age estimates improved in accuracy with increasing age, whereas age estimates obtained from morphometric approaches decreased in accuracy. Additionally, my results support the generality with which LF-based ageing can be applied to crustacean taxa (Sheehy 1990). Given the apparent ubiquity in cellular LF deposition, LF appears to be a

valuable ageing technique with potentially widespread use (Belchier et al. 1998). Indeed, LF has been shown to accumulate with age in species with longevities ranging from days (e.g., *Musca domestica*, Donato and Sohal 1978, cited in Hammer and Braum 1988) to decades (e.g., *Homarus gammarus*, Sheehy et al. 1999).

Conclusions

The high juvenile growth rates, rapid recruitment to the fisheries, and lipofuscin-based age designations all support my hypothesis that peeler fisheries in the summer and hard crab fisheries in the fall/winter are predominately dependent on new recruits younger than 18 months of age. Van Engel (1958) initially suggested that rapid growth during the juvenile stage led to recruitment within 1 to 1.5 years of age. More recent research has supported this notion. Based on estimates of absolute abundance of crabs designated as age 1+ and total commercial landings, Sharov et al. (2003) concluded that fishery removals equaled or exceeded abundance of age 1+ crabs, and therefore, crabs designated as age-0 in winter (< 60 mm) recruit to and subsidize the hard crab fishery during the ensuing summer. Furthermore, Ju et al. (2003) conducted a lipofuscin modal analysis and concluded that crabs < 2 years of age were a significant fraction of the harvestable stock in Chesapeake Bay.

Species such as the blue crab, which are relatively small, fast growing, rapidly maturing, and short-lived are characterized as having high intrinsic rates of population growth with irregular population dynamics (King and McFarlane 2003). Life history characteristics contributing to a shorter generation time typically result in increased fishery production, resiliency to harvest pressures, and rapid recovery from overfishing (Adams 1980). Yet, Miller (2001b) suggested that increased proportions

of juveniles recruiting to the fishery significantly decreased population growth rate, presumably due to reductions in reproductive potential. If post-mating behavior precludes the majority of females from producing even a single brood before winter as suggested by Turner et al. (2003) and there is a temporal overlap between the onset of maturity and recruitment, I speculate that a large fraction of mature females are harvested prior to reproducing. Subsequently, growth overfishing (Abbe 2002) may transition to recruitment overfishing. Thus, the consequences of a shorter generation time are that recruitment and landings will be increasingly correlated with environmental factors affecting growth and annual variations in egg production, settlement, and post-settlement survival (Ju et al. 2003). Accordingly, the recent declines in spawning stock biomass, larval abundance, and post-settlement recruitment (Lipcius and Stockhausen 2002) are alarming for a fishery that essentially depends on an annual crop of new recruits.

Future Work

In addition to the applications of this study, which provided empirical estimates of juvenile blue crab seasonal growth, earthen ponds may also provide a setting to investigate other factors affecting growth and blue crab population dynamics. Although temperature is likely to be a controlling factor of growth, additional environmental parameters could be continuously monitored in earthen pond systems. Dissolved oxygen may profoundly influence growth of benthic organisms such as the blue crab, either directly through physiological responses or indirectly by altering predator prey dynamics (Bell et al. 2003). Differences in salinity and osmoregulatory costs have also been linked to growth discrepancies

(Leffler 1972). Continuously monitoring salinity and determining its effects on growth may prove useful, particularly with regards to improving knowledge of spatial variation in growth throughout Chesapeake Bay.

Stocking earthen ponds with different densities of juvenile blue crabs may provide insight into the effects of intrinsic factors (i.e., density dependent) on growth. Releasing two similar size/age cohorts simultaneously, but with different SAV coverage may further reveal the effects of density on growth, and provide empirical evidence for the benefits of SAV in terms of increasing growth and decreasing natural mortality. Releasing multiple cohorts of different age/size may provide a means of investigating size-dependent patterns of natural mortality. Moreover, long term monitoring of pond cohorts could be conducted to establish blue crab longevity in the absence of fishing pressure.

The tagging of individual crabs, particularly small crabs, released into ponds would likely provide more accurate estimates of growth per molt and potentially intermolt period, while providing insight into the degree of individual variability. Replicating pond-rearing experiments at different latitudes (e.g., the Gulf of Mexico, North Carolina, and New England) where temperatures and seasonal signals are markedly different will be necessary if the seasonalized von Bertalanffy growth function and molt-process growth model are to be applied in different environments. Model simulations involving the molt-process growth model could be further applied for management purposes by incorporating a range of natural and fishing mortalities, seasonal temperature records, and commercial size limits.

Further studies investigating the accumulation of LF for older blue crabs (> 2 years of age) are necessary, particularly given the uncertainty surrounding longevity and its link to vital rates such as natural mortality. Studies investigating LF dynamics as a function of molt cycle are needed to more completely demonstrate the robustness of LF in age determination, although quantifying LF in peeler and soft crabs would require some methodological adaptations to remove eyestalks and separate retinal tissue. Experimental ponds may also provide the opportunity to investigate density dependent LF accumulation (i.e., changes due to physiological stress associated with increased densities). Finally, the release of marked known-age juvenile blue crabs for stock enhancement purposes may provide an opportunity to determine LF accumulation rates in situ.

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