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[Article]

Validation and Application of Lipofuscin-Based Age Determination for Chesapeake Bay Blue Crabs *Callinectes sapidus*

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Abstract.—Quantifying lipofuscin (LF), a metabolic byproduct that accumulates in postmitotic cells, serves as one of the principal approaches for aging crustaceans, but the accuracy of this method remains an important issue. Here, we quantified LF accumulation as a function of chronological age and temperature (degree-days) in an economically important crustacean, the blue crab *Callinectes sapidus*, to test the accuracy of LF-based age estimates and determine the age-specific partial recruitment of juveniles to summer and fall commercial fisheries. Three known-age juvenile cohorts (63–83 d) were reared in ponds up to 1.8 years of age. Field collections were conducted in two subestuaries of Chesapeake Bay from June to October during 2003 and 2004. Lipofuscin accumulation oscillated seasonally in known-age cohorts. Significant ($\log_e$ transformed) LF accumulation occurred at average intervals of 2.5 months, with the exception of winter months (mean temperature = 8°C). Seasonalized von Bertalanffy functions accurately depicted age-specific LF accumulation but were cohort specific. The relationship between LF and temperature degree-day (TD day) was similar among cohorts and genders, and a single LF–TD day model was applied to field-collected crabs. The mean age prediction error of this model was 2.0 months. Lipofuscin-based age composition of field collections indicated that the peeler–soft crab and hard crab fisheries were predominately composed of recruits less than age 1.5 from August to October. The consequences of this short (approximately annual) generation time is that recruitment and landings will be responsive to environmental factors affecting growth and the annual variations in egg production, settlement, and postsettlement survival.

Age-dependent assessments are a cornerstone in fisheries management, and careful attention is warranted in developing age determination procedures (Beamish and McFarlane 1983; Campana 2001). Crustaceans present unique challenges for management owing to their lack of traditional aging structures (i.e., otoliths, scales, and fin spines) and the periodic loss of their only hard parts through molting (Hartnoll 2001; Smith and Addison 2003). Typically, length frequency modal analysis has been used as an alternative to direct aging (Jennings et al. 2001), but the effectiveness of resolving length modes is often compromised by gear selectivity, protracted spawning periods, and seasonal growth dynamics (Hilborn and Walters 1992).

Beginning with Ettershank's (1983) work on Antarctic krill *Euphausia superba*, quantifying lipofuscin (LF) has become an alternative approach for age determination in crustaceans. Lipofuscin occurs as fluorescent granular pigments in postmitotic tissue (i.e., neural tissue), and is believed to be the product of free radical-mediated lipid peroxidation and the accumulation of nondegradable oxidized macromolecules in lysosomes (Hill and Womersley 1993; Brunk and Terman 2002; Chowdhury et al. 2004). The amount of LF increases with age and senescence, often showing a positive relationship with chronological age (Brunk and Terman 2002). Accordingly, several studies have quantified LF to estimate age, growth, and longevity for diverse taxa, including Antarctic krill (Ettershank 1983), lobster (Wahle et al. 1996; Sheehy et al. 1998, 1999), shrimp (Vila et al. 2000; Bluhm and Brey 2001; Kodama et al. 2005), crayfish (Sheehy 1992; Belchier et al. 1998), swimming crab *Callinectes sapidus* (Ju et al. 2001, 2003), amphipod *Waldeckia obesa* (Bluhm et al. 2001), fish (Vernet et al. 1988), and clam *Eurhomalea exalbida* (Lomovasky et al. 2002).

Several methods of quantifying LF have been

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developed, but fluorescent microscopy– and extraction spectrofluorimetric–based methods are most often used (Sheehy 1996; Ju et al. 1999; Sheehy 2002a). The extraction spectrofluorimetry approach used here, where LF is defined as chemically extractable aging-related fluorescent compounds, has advantages in permitting analysis of larger sample sizes and increased objectivity in measures. Still, this approach has seen only recent application and remains controversial because the mixture of extractable fluorescent compounds is not fully defined (Sheehy 2008; Harvey et al. 2008). Here, we follow rigorous steps in verifying the accuracy and precision of this age determination approach for blue crabs *Callinectes sapidus* by using known-age individuals (Campana 2001).

Previous research, which successfully developed and applied the LF-based extraction approach for age determination of Chesapeake Bay blue crabs, indicated that LF accumulation was positively and linearly related to chronological age (Ju et al. 1999, 2001). In addition, Ju et al. (1999, 2001, 2003) demonstrated that LF-based measures provided greater resolution of demographic structure than size-based measures, and that individuals less than 2 years of age comprised a significant fraction of the harvestable blue crab stock in Chesapeake Bay. Missing from the initial applications were (1) a complete validation of LF accumulation (because individuals of an assumed age and limited size range [>60 mm] were reared for 1 year [Ju et al. 1999]) and (2) the incorporation of temperature when applying LF accumulation rates for blue crab age determination (Ju et al. 2001, 2003). In poikilotherms, metabolic processes, and presumably LF accumulation, are at least partially dependent on environmental temperature (Tully et al. 2000; Sheehy 2002b). The effects of temperature on metabolic rate may be particularly pronounced in temperate estuarine habitats like Chesapeake Bay, where large temperature fluctuations occur seasonally (Leffler 1972).

The seasonal effects of temperature are further compounded by the complex life history of blue crabs, which produces subannual cohorts that overwinter at different ages. In Chesapeake Bay, the spawning season of blue crabs is protracted from May to September (Prager 1996). Larvae develop on the continental shelf for 30–45 d (Epifanio 1995; Epifanio and Garvine 2001) and settle near the mouth of Chesapeake Bay from June to November, although the fall cohorts tend to be dominant (van Montfrans et al. 1990). Thereafter, these subannual juvenile cohorts disperse throughout the estuary (Hines 2003). Movement, growth, and possibly LF accumulation cease during winter months at temperatures near 10°C when blue crabs enter a state of torpor. During the ensuing summer, juvenile blue crabs may begin recruiting to the commercial fishery (Ju et al. 2001; Sharov et al. 2003).

The Chesapeake Bay blue crab commercial fishery is composed of two primary sectors: the peeler–soft crab and hard crab fisheries. Minimum size limits for peeler–soft crabs (82.5–88.9 mm) and hard crabs (127–133.4 mm) vary by season. Landings of peeler (just prior to molt) and soft-shell (after molt) crabs typically peak in May, a smaller secondary peak occurring in July–August (NMFS 2006). Hard crab landings typically peak in October and account for approximately 90% of the annual Chesapeake Bay commercial harvest (NMFS 2006). Documented declines in commercial landings, total abundance, and other stock metrics (such as the percentage of legal-sized crabs [Miller et al. 2005]) have stimulated concerns about the status of the stock and sustainability of the fishery.

Here, we (1) reared three hatchery-produced, known-age cohorts of juvenile blue crabs in separate earthen ponds to validate LF accumulation rates as a function of age and temperature (degree-days); (2) developed a general predictive LF-based model applicable to subannual juvenile cohorts; and (3) applied the predictive model to estimate the age-specific partial recruitment of field-collected blue crabs to the commercial fishery. Based on reported growth rates (Van Engel 1958; Ju et al. 2001), we hypothesized that blue crabs were capable of recruiting to the primary commercial fisheries by age 1 and that due to high annual exploitation (~50%; Miller et al. 2005), fishery recruits predominately comprised individuals age 1.5 or less.

Methods

Pond rearing.—Known-age juvenile blue crabs (age 63–83 d) 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 ponds at Horn Point Laboratory (University of Maryland, Cambridge) in June 2003, October 2003, and September 2004 to simulate summer and fall settling cohorts (Table 1). It should be noted that cohort 1 (spawned on March 28, 2003) is atypical. Ponds were approximately 360 m^2 and 1.2 m deep. Initial stocking densities approached 0.84 crabs/m^2 (within the range) but were, on average, an order of magnitude lower than the mean densities reported for similar-sized juveniles in seagrass beds of Chesapeake Bay (Orth and van Montfrans 1987). Cohorts 1, 2, and 3 were reared to 1.4, 1.8, and 1.2 years of age, respectively.

Ponds received constant flow of ambient water from

TABLE 1.—Initial stocking information for three known-age blue crab cohorts released into separate earthen ponds on three dates.

Cohort	Birth date	Age (d) at release	Date of release	Carapace width (mm; mean ± SD) at release	Number released
1	28 Mar 2003	68	4 Jun 2003	16.8 ± 3.8	272
2	17 Jul 2003	83	8 Oct 2003	21.3 ± 5.9	302
3	6 Jul 2004	63	7 Sep 2004	20.5 ± 4.2	293

the Choptank River (pond salinity = 7–11), a subestuary of the Chesapeake Bay (Figure 1), and contained natural refuge (primarily widgeon grass *Ruppia maritima*) and forage consisting of small fishes (mummichog *Fundulus heteroclitus*, striped killifish *F. majalis*, sheepshead minnow *Cyprinodon variegatus*, and naked goby *Gobiosoma bosc*), polychaetes, and amphipods. Pond water temperatures were monitored at 1-h intervals from a depth of 1 m with a HOBO data logger. Temperature and salinities were similar to locations in the Choptank River and other locations in Chesapeake Bay where juvenile blue crabs are abundant. Temperature degree-days (TD day) were calculated for each pond cohort from assumed date of settlement (33 d post hatch; Zmora et al. 2005) as

$$\text{TD day} = \sum_i (t_a - t_b), \qquad (1)$$

where i is the number of days, t_a is the mean daily temperature (°C), and t_b is the basal temperature (10°C; Smith 1997; Ju 2000) below which growth (molting) and LF accumulation were assumed to cease. The assumed age at settlement was toward the lower end of the 30–45-d range reported by Epifanio and Garvine (2001 and references therein). From March to October, cohorts were sampled at bimonthly intervals with a seine, baited traps, or both. Individuals from each cohort (n = 48, 54, and 40, respectively) were transported back to the laboratory and sacrificed for LF analysis. Carapace width, measured in millimeters from tips of lateral spines, and sex were recorded for each individual.

Field collection.—Partial recruitments were estimated for blue crabs collected during the commercial fishing season at monthly intervals from June to October in the Choptank and Patuxent rivers, two large subestuaries in the mid to upper portion of Chesapeake Bay (Figure 1). Important trotline (hard crab) fisheries occur in the regions sampled. In 2003, we conducted bottom trawls in the lower Choptank River and adjoining Broad Creek, sampling from six fixed stations with a 4.9-m semiballoon otter trawl with 12-mm cod end mesh (Figure 1A). The Maryland Department of Natural Resources provided supplemental samples from these sites using the same gear during June, July, and August.

During June–October 2004, samples were collected from the Patuxent River by means of four different gear types (Figure 1B). Crabs were collected using a 1.5-m × 30.5-m beach seine with 3.2-mm bag mesh in June and 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 a 6-mm cod end mesh or a 9-m otter trawl with a 6-mm cod end mesh. All collected crabs (Choptank River: n = 494; Patuxent River: n = 366) were transported live back to the laboratory for LF analysis; CW (mm) and sex were recorded for each individual.

Daily temperatures recorded at the Chesapeake Biological Laboratory (CBL) research pier (University of Maryland, Solomons) from January 1, 2000, to October 6, 2004, were used to back-calculate the age of field-collected blue crabs in the Choptank and Patuxent rivers using the validated LF–TD day model obtained from pond-reared cohorts (see the section on statistical analyses below). Interpolation was used to fill gaps in the temperature record (typically less than three consecutive days). A temperature record from this midbay location was assumed to be representative of the average temperatures experienced by crabs distributed baywide.

Analytical methods.—We used a modification of the biochemical approach for LF analysis reported by Ju et al. (1999). Individuals were anesthetized, and external eyestalk(s) were carefully excised. The left eyestalk was removed from crabs larger than 40-mm CW; both eyestalks were removed from crabs smaller than 40-mm CW. Retinal tissues were removed due to high levels of pigment that may interfere with fluorescence emission of LF (Hill and Womersley 1991). Each excised tissue sample was stored in a 2-mL mixture of dichloromethane and methanol (CH_2Cl_2:MeOH; 2:1 by volume). Samples were sonicated for 10 min at 25°C to initiate solvent extraction of LF. The extract was stored at −70°C prior to analysis. The total extract was dried under N_2 and redissolved in MeOH (0.5 mL). Flow injection, high-performance liquid chromatography was used to quantify extractable LF. Volumes of 10

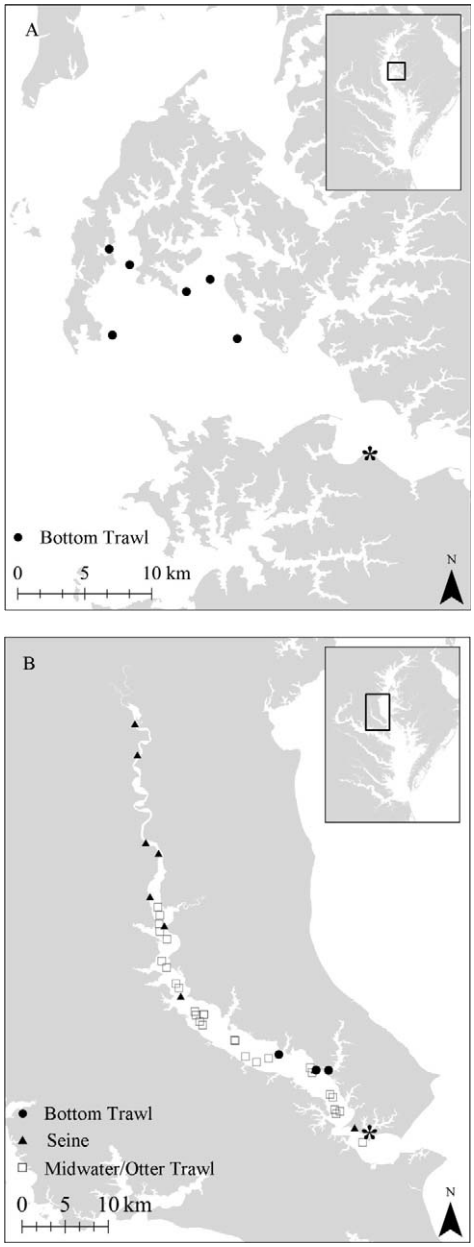

FIGURE 1.—Panel **(A)** shows the bottom-trawl sampling sites for blue crabs in the Choptank River from June to October 2003. The asterisk indicates the location of the earthen ponds at Horn Point Laboratory. Panel **(B)** shows the seine (June–July 2004), bottom-trawl (July 2004), and midwater otter-trawl (August–October 2004) sampling sites in the Patuxent River. The midwater otter-trawl sites were randomly selected each month; those shown here were sampled in August. The asterisk indicates the location of the research pier at Chesapeake Biological Laboratory.

μL from each extract were injected by an autosampler, methanol serving as the carrier solvent (0.8 mL/min). Fluorescence intensities of extractable LF were measured with a fluorescence detector at a maximum emission wavelength of 405 nm using a maximum excitation at 340 nm. Fluorescence intensities of extracted material were calibrated using quinine sulfate (range = 0–0.5 μg/mL) dissolved in 0.1 normal sulfuric acid, water serving as the carrier solvent. Fluorescence intensities were converted to concentrations (μg/mL) and normalized to protein content of extracted tissues measured by the modified bicinchoninic acid assay described by Nguyen and Harvey (1994). The absorbance of protein standards and extracts were measured at 562 nm using a spectrophotometer. The protein-normalized LF content (μg LF/mg protein), or "LF index," was used to represent the absolute LF content per unit of neural tissue.

Statistical analyses.—The LF index was $\log_e$ transformed to satisfy the assumptions of homogeneity of variance and normality of residuals. Linear regression and analysis of covariance were used to compare age-related $\log_e$ LF accumulation rates in known-age, pond-reared cohorts (pooled) with the accumulation rate reported in Ju et al. (2001). A Durbin–Watson test was conducted to test for autocorrelation among residuals from linear regression analysis. Differences in least-squares means (Tukey–Kramer adjusted) were calculated to determine the time intervals at which significant $\log_e$ LF accumulation occurred in each pond-reared cohort. Least-squares regression was used to fit seasonalized von Bertalanffy functions (VBF) to model mean LF index as a function of age according to

$$\mathrm{LFindex}(t) = \mathrm{LFindex}_\infty \left\{ 1 - e^{\left[-K(t-t_0) - \left(C\frac{K}{2\pi}\right)\sin(2\pi)(t-t_w+0.5)\right]} \right\}, \quad (2)$$

where LFindex(t) is the LF index (μg LF/mg protein) at age t, $\mathrm{LFindex}_\infty$ is the maximum asymptotic LF index, K is a curvature parameter that determines how fast $\mathrm{LFindex}_\infty$ is approached, t_0 is the theoretical age (year) when LFindex is zero, C is related to the magnitude of the seasonal oscillation, and t_w is the time at which LF accumulation is lowest. Parameter estimates of $\mathrm{LFindex}_\infty$ and C were constrained by upper bounds of 1.5 μg LF/mg protein and 1.0, respectively. Least-squares regression was also used to fit linear, power, and logarithmic models to the TD day–mean $\log_e$ LF index relationship for each pond-reared cohort. Adjusted coefficients of determination, Akaike's information criteria (AIC) adjusted for sample size bias, and tests of coincident models among cohorts were used to

determine the most appropriate model for predictive purposes. Likelihood ratio tests were conducted to test for coincident models among cohorts and between genders.

For age estimation, the LF–TD day model was appropriately rearranged for inverse prediction of TD days accumulated since settlement. From date of capture, estimated TD days accumulated from settlement was incrementally reduced by TD days accumulated during the previous day. This iterative back-calculation procedure was continued until TD days accumulated from settlement was less than or equal to zero, at which point settlement was assumed to have occurred and the date was noted. The number of days from capture until settlement was calculated and increased by 33 d (settlement was assumed to occur 33 d posthatch) to estimate age. Pond and CBL pier temperature records were used for back-calculation of age for pond-reared cohorts. Because pond temperature collection was initiated when cohorts were released in ponds, there was no record available for back-calculating age of individuals that were predicted to have accumulated more TD days from settlement than accumulated over the duration of pond temperature collection (i.e., negative residuals). Accordingly, CBL pier temperature data were concatenated to the beginning of the pond temperature data for such back-calculations. Only CBL pier temperature data were used for back-calculation of age for field-collected crabs.

Predicted ages were grouped into four age-classes: 0.5 ($\leq$0.5 years), 1 ($>$0.5 to $\leq$1 years), 1.5 ($>$1 to $\leq$1.5 years), and 1.5+ ($>$1.5 years). Individual age prediction error was calculated as the absolute difference between known and predicted age. Mean age prediction error (MAPE) was compared among known-age cohorts, genders, and age-classes using difference of least-squares mean (Tukey–Kramer adjusted). Back-calculated settlement dates of field-collected individuals were converted to relative frequencies and grouped into monthly bins. Partial recruitments were calculated for both commercial fishery sectors as the proportion of individuals in each age-class that obtained the minimum size limit during a sampling period. A two-sample Kolmogorov–Smirnov test was used to compare settlement and age probability distributions between Choptank and Patuxent River samples.

Results

Pond and Field Temperature

Pond and CBL pier temperature records were highly correlated ($r = 0.95$; $P < 0.0001$). In both systems, recorded temperatures ranged from 1–30°C and were below 10°C (i.e., no TD days accumulated) from early–mid-December to late March. During 2004, the only year with complete pond and river temperature records, cumulative annual TD day accumulation differed by only 3%. Pond cohorts 1, 2, and 3 accumulated 4.5×10^3, 3.9×10^3, and 2.9×10^3 TD days over 1.4, 1.8, and 1.2 years, respectively. Cumulative annual TD day accumulation in the Choptank River ranged from 2.5×10^3 to 2.7×10^3 during 2000 to 2004, a difference of less than 10%.

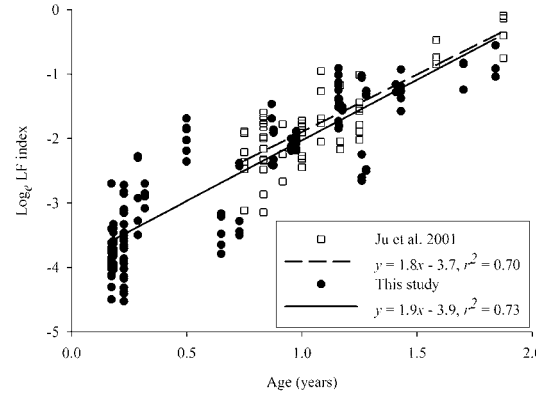

FIGURE 2.—Value of the $\log_e$ transformed lipofuscin (LF) index (μg LF/mg protein) as a function of age in three blue crab cohorts of known age (combined; $n = 142$) in this study and in the study by Ju et al. (2001).

Lipofuscin Accumulation: Known-Age Cohorts

The linear relationship between $\log_e$ LF index and age for three known-age, pond-reared cohorts (combined; $n = 142$) was nearly identical to the regression equation reported in Ju et al. 2001 for crabs of assumed age ($F_{1,194} = 2.12$, $P = 0.15$; Figure 2). Residuals of $\log_e$ LF index regressed on age from this study were autocorrelated (d = 1.12). Within each cohort significant $\log_e$ LF index accumulation occurred at an average interval of 2.5 months (0.21 years) with the exception of winter months (when the mean temperature was 8°C; Table 2). Seasonal oscillations in mean LF index were accurately modeled with seasonalized VBFs (Figure 3). The seasonalized VBFs were not coincident among cohorts ($\chi^2 = 10.6$; df = 3; $P = 0.02$) and therefore not useful for a general predictive LF–age model.

Mean $\log_e$ LF index accumulated nonlinearly with TD day as the explanatory variable. Linear models fit to each cohort explained the least variability, ranked lowest in AIC, and were cohort specific (Table 3). Of the additional models tested (e.g., power and logarithmic), only the power model was coincident among cohorts ($\chi^2 = 6.5$, df = 3, $P = 0.09$; Table 3; Figure 4A)

TABLE 2.—Mean (SE) $\log_e$ transformed lipofuscin (LF) index (values μg LF/mg protein) in relation to age for three known-age blue crab cohorts (see Table 1). Values in bold italics are from collections in October and March (of the following year), which represent pre- and postwinter torpor for blue crabs in earthen ponds. Within cohorts, values with the same letter are not statistically different ($P > 0.05$).

Cohort 1 ($n = 48$)		Cohort 2 ($n = 54$)		Cohort 3 ($n = 40$)	
Age (years)	LF index	Age (years)	LF index	Age (years)	LF index
0.18	−3.71 (0.07) w	***0.23***	***−3.62 (0.10) w***	0.17	−3.85 (0.10) w
0.32	−2.89 (0.14) x	***0.65***	***−3.46 (0.21) w***	***0.29***	***−2.86 (0.19) x***
0.50	***−1.95 (0.09) y***	0.88	−2.08 (0.21) x	***0.73***	***−3.00 (0.19) x***
0.95	***−2.12 (0.13) y***	0.98	−2.05 (0.20) x	0.87	−1.86 (0.24) y
1.16	−1.55 (0.16) yz	1.18	−1.54 (0.34) xy	1.16	−1.34 (0.13) y
1.28	−1.78 (0.13) yz	***1.26***	***−1.92 (0.21) xy***		
1.41	−1.22 (0.20) z	***1.70***	***−0.97 (0.28) y***		
1.43	−1.27 (0.12) z	1.84	−0.84 (0.28) y		

and thus was utilized in further analyses. When data were pooled among cohorts (Figure 4B), mean $\log_e$ LF accumulation with respect to TD day was not significantly different between genders ($\chi^2 = 5.0$; df $= 3$; $P = 0.17$).

Age Prediction Error

Inverse prediction of TD day from $\log_e$ LF index and subsequent back-calculation of age for known-age, pond-reared cohorts yielded age prediction errors ranging from 0 to 1.1 years. Despite the wide range in errors, 60% of age predictions were within 1 month (0.08 years) and 80% were within 3 months (0.25 years). Over the duration of pond rearing, MAPE was 2 months (0.16 years). Mean age prediction error was not significantly affected by cohort ($F_{2,139} = 0.6$; $P = 0.6$) or gender ($F_{1,140} = 0.4$; $P = 0.5$). Age prediction errors were positively correlated with age ($r = 0.6$; $P < 0.001$). The mean age prediction error for ages 0.5, 1, 1.5, and 1.5+ was 0.05, 0.1, 0.3, and 0.5 years, respectively. Pairwise comparisons indicated that MAPE was significantly different for all age-classes ($P < 0.004$) except the two youngest (0.5 and 1; $t = -1.9$, df $= 138$, $P = 0.2$).

Age Composition and Recruitment

The range of $\log_e$ LF indices for field-collected individuals was largely (98%) within the range measured for pond-reared individuals. Lipofuscin-based age estimates of crabs collected from the Choptank and Patuxent rivers ranged from 0.1 to 4.1 years and from 0.1 to 4.2 years, respectively. Log_e LF index and CW were positively related in field-collected crabs, although there was high variability in $\log_e$ LF index at a given size ($r^2 < 0.2$; $P < 0.001$). Age-classes 0.5, 1, and 1.5 encompassed individuals ranging in size from 11 to 178 mm, 23 to 175 mm, and 47 to 189 mm, respectively. For reference, the same age-classes of known-age, pond-reared cohorts ranged in size from 11 to 155 mm, 26 to 168 mm, and 92 to 180 mm.

Annual settlement distributions were similar for individuals collected from the Choptank and Patuxent

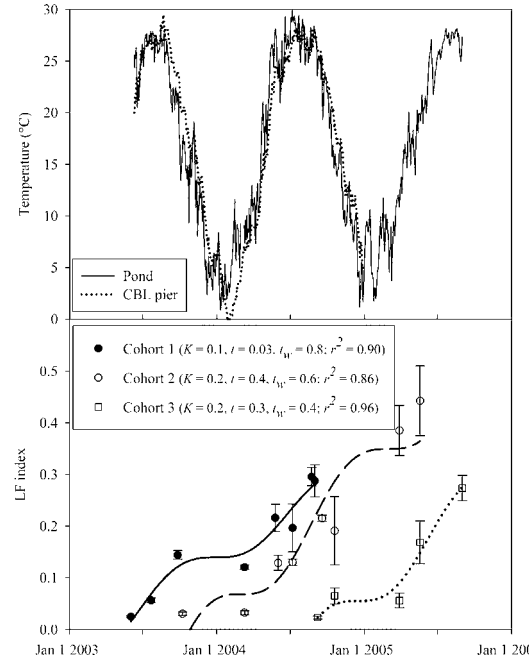

FIGURE 3.—The upper panel shows water temperatures at the earthen ponds at Horn Point Laboratory and at the Chesapeake Biological Laboratory research pier over a 3-year period. The lower panel shows the mean ± SE lipofuscin (LF) index values for three blue crab cohorts of known age (see Table 1). Mean LF index at age/date for each cohort ($n = 8$, 8, and 5 for cohorts 1–3, respectively) was fitted with a seasonalized von Bertalanffy function (see equation 2 in the text) in which the parameters for LFindex_∞ and C were constrained to 1.5 and 1.0, respectively. The shaded regions represent the dates during which water temperatures were below 10°C. The solid, dashed, and dotted lines represent the best-fitting functions for cohorts 1, 2, and 3, respectively.

TABLE 3.—Regression models of the relationship between mean $\log_e$ transformed lipofuscin (LF) index (μg LF/mg protein) and temperature degree-days for three known-age blue crab cohorts (n = 8, 8, and 5). Adjusted R^2 values and Akaike's information criteria (AIC) were used to compare model fits. Data were pooled (n = 21) to compare models among cohorts using the likelihood ratio test; model form: $y = y_0 + ax$ (linear), $y = y_0 + ax^b$ (power), and $y = y_0 + a\log_e (x - x_0$ (logarithmic).

	Model					
	Linear		Power		Logarithmic	
Cohort and test	Parameters	Adj. R^2; AIC	Parameters	Adj. R^2; AIC	Parameters	Adj. R^2; AIC
1	$y_0 = -3.5$ $a = 0.0005$	0.86; −8.8	$y_0 = -8.2$ $a = 1.6$ $b = 0.2$	0.96; −18.7	$y_0 = 1.3$ $a = -314.5$ $x_0 = 12.0$	0.95; −18.5
2	$y_0 = -3.7$ $a = 0.0007$	0.81; −3.4	$y_0 = -25.3$ $a = 15.2$ $b = 0.06$	0.87; −7.1	$y_0 = 1.1$ $a = 178.7$ $x_0 = -10.4$	0.85; −6.8
3	$y_0 = -3.7$ $a = 0.0009$	0.74; 20.7	$y_0 = -29.0$ $a = 19.1$ $b = 0.05$	0.85; 15.9	$y_0 = 1.3$ $a = -45.8$ $x_0 = -11.6$	0.84; 16.1
Pooled	$y_0 = -3.5$ $a = 0.0006$	0.79; −33.3	$y_0 = -19.6$ $a = 10.8$ $b = 0.06$	0.90; −46.0	$y_0 = 1.1$ $a = -25.6$ $x_0 = 10.5$	0.88; −43.6
Likelihood ratio test	$\chi^2 = 6.4$ df = 2 $P = 0.04$		$\chi^2 = 6.5$ df = 3 $P = 0.09$		$\chi^2 = 8.2$ df = 3 $P = 0.04$	

rivers (two-sample Kolmogorov–Smirnov statistic [D] = 0.2, P = 0.9; Figure 5). Peak monthly settlement occurred in September and October in the Patuxent (2004) and Choptank Rivers (2003), respectively. Summer and fall cohorts, as indexed by settlement distributions from June to August and from September to November, respectively, were equally represented (~40–50%) in the field collections (D = 0.3; P = 0.8). A small percentage (~10%) of back-calculated settlement dates occurred in April, May, and December.

The age composition of both peeler–soft crab and hard crab recruits collected from June to October was distributed similarly in the Choptank and Patuxent rivers ($D < 0.2$; $P > 0.9$), so the data were pooled for further analyses. During June and July, recruits to both fisheries were predominately (~60%) composed of individuals estimated to be age 1.5+ (Figure 6). Age-0.5 individuals had not recruited to either fishery by June or July. A transition occurred in August when young of the year (age 0.5) began recruiting to both commercial fisheries in small proportions (<5%; Figure 6) and the dominant age composition of fishery recruits shifted from age 1.5+ to age 1.5. By September and October, age-0.5 individuals comprised approximately 20% of fishery recruits at a time when more than 90% of recruits were age 1.5 or less (Figure 6).

Discussion

Our results verified the use of LF-based methods for the age determination of juvenile blue crabs reared in an experimental system that emulated natural conditions (e.g., seasonal temperature fluctuations). The ubiquitous cellular deposition of LF makes it an attractive and valuable aging technique with potentially widespread use (Sheehy 1990; Belchier et al. 1998). Still, as with any age determination method constrained by insufficient knowledge of the chemical composition or structural processes that underlie the aging methodology, a careful validation of LF accumulation rates is required (Harvey et al. 2008). Here, the availability of known-age material was essential for our validation study and represents one of the most robust means to test for accuracy in age determinations (Campana 2001).

The mean LF accumulation rates compared favorably with those of previous studies conducted by Ju et al. (1999, 2001; Figure 2), although the seasonal oscillations of LF accumulation observed here precluded the use of linear models. To incorporate seasonality, we developed and validated a novel LF–TD day model that was neither cohort nor gender specific. The LF-based age estimates of field-collected blue crabs generally supported our hypotheses that (1) blue crabs in Chesapeake Bay were capable of partially recruiting to both commercial fisheries by age 1 and (2) the age composition of recruits was skewed towards individuals age 1.5 or less.

Lipofuscin Accumulation

Lipofuscin has been shown to accumulate both linearly (Sheehy et al. 1996; Belchier et al. 1998; Bluhm and Brey 2001; Ju et al. 2001; Kodama et al. 2005; Uglem et al. 2005) and curvilinearly (Sheehy 1992; Tully et al. 2000; Vila et al. 2000; Sheehy

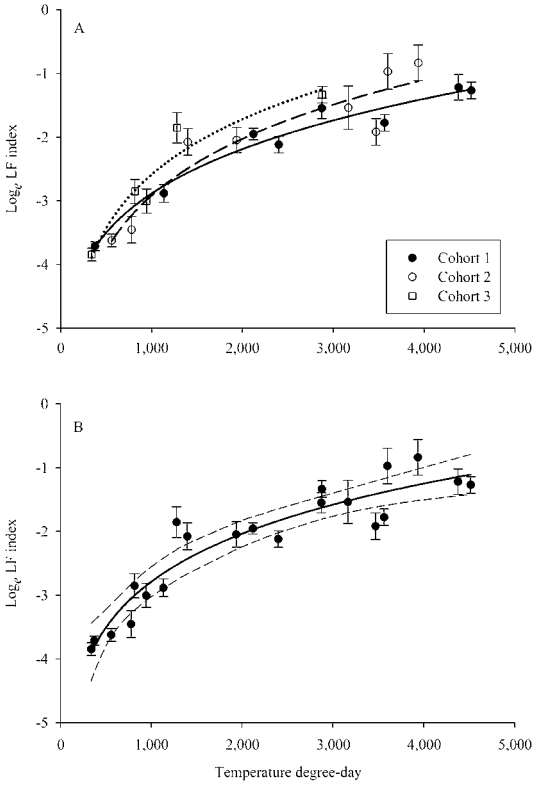

FIGURE 4.—Value of the mean log$_e$ transformed lipofuscin (LF) index as a function of temperature degree-days calculated from settlement (33 d posthatch) for **(A)** three blue crab cohorts of known age (n = 8, 8, and 5 for cohorts 1–3, respectively) and **(B)** all cohorts combined (n = 21). The error bars represent SEs. For parameter estimates and fit statistics, see Table 3. In **(A)** the solid, dashed and dotted lines represent the best-fitting function for cohorts 1, 2, and 3, respectively.

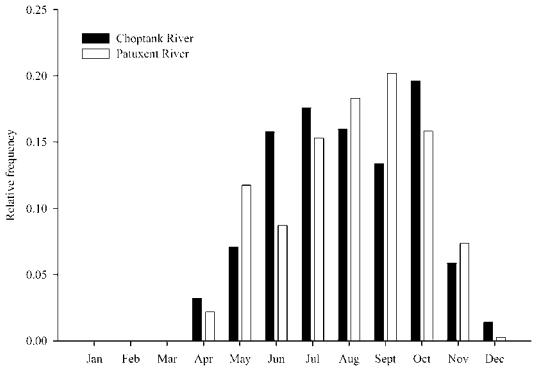

FIGURE 5.—Relative frequency of monthly back-calculated settlement dates for blue crabs collected from the Choptank River in 2003 (n = 494) and the Patuxent River in 2004 (n = 366).

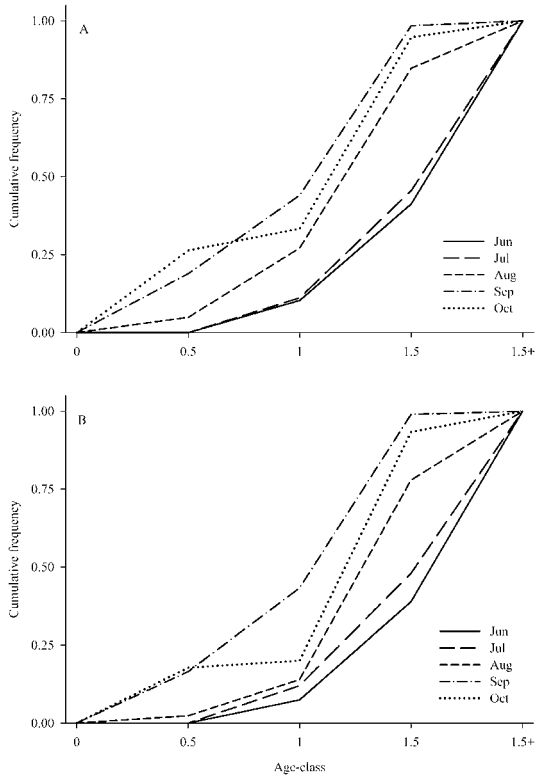

FIGURE 6.—Cumulative partial recruitment of field-collected blue crabs grouped into lipofuscin-based age-classes in **(A)** the peeler–soft crab fishery and **(B)** the hard crab fishery by month from June to October.

2002b; Kodama et al. 2006) with respect to age in crustaceans. Theoretically, a number of factors (e.g., normal senescence) could cause LF to accumulate in a nonlinear manner (Sheehy 1992; Belchier et al. 1998; Castro et al. 2002), although environmental temperature is probably the most important factor in subannual LF accumulation patterns in poikilotherms (Sheehy 2002b). To account for the oscillations in LF accumulation, seasonalized VBFs have been used (e.g., Vila et al. 2000) because these models depict the temperature-mediated metabolic production of LF, produce parameters that are biologically interpretable, and can be rearranged for prediction of chronological age. However, the application of a single seasonalized VBF becomes problematic when multiple subannual cohorts are produced. Individuals that settle from June to August (i.e., summer cohorts) are exposed to higher temperatures and are physiologically older than fall settlers at the same chronological age. Evidence for this is provided by the greater accumulation of TD days in pond cohort 1 (summer cohort) despite being chronologically younger than cohort 2 (fall cohort). Such

discrepancies could potentially lead to aging errors when using a seasonalized model.

An alternative to seasonalized models is a TD day approach, which has recently been incorporated within the framework of molt process growth models to account for phenomena such as diapause (Bunnell and Miller 2005; Brylawski and Miller 2006). Likewise, LF-based applications can benefit from incorporating TD days because the physiological effects of temperature and chronological time can be expressed simultaneously. Indeed, the LF–TD day model developed here was valid among subannual cohorts and genders, suggesting that the timing (or age) of overwintering was accounted for by this approach. The absence of a gender-specific LF accumulation rate is consistent with previous studies conducted on other crustaceans (Sheehy et al. 1994; Vila et al. 2000). Perhaps more importantly is the robustness of the model across subannual cohorts, which is further supported when considering that the model was suitable for a cohort produced outside of the spawning season (i.e., March; Table 1).

Explicit in the TD day model is a basal temperature below which accumulation of TD days ceases. We assumed a basal temperature of 10°C when growth stasis has been reported to occur (Smith 1997). Other studies reported a slightly different temperature threshold for growth stasis: 11°C (Brylawski and Miller 2006) and 15°C (Tagatz 1968). Van Engel (1958) reported that growth ceased at 15°C, but true physiological torpor occurred at 5°C. In support of our assumption, no significant accumulation of LF occurred from November to March when the mean water temperature was 8°C. If there was an error in the assigned basal temperature, the resulting bias would not change the general form of our LF–TD day model but would introduce systematic errors in predicted settlement dates and age estimates.

Changes in LF accumulation have been noted across temperature ranges and tolerance limits. Sheehy (2002b) reported reduced sensitivity of LF accumulation to temperatures in a species thermal midrange and a reduction of LF accumulation rates at high (super-optimal) temperatures, which may explain some variability in the TD day–LF index relationship. For blue crabs, growth rates are maximal from 20°C to 27°C and depressed at temperatures below 15°C and above 30°C (Leffler 1972). Future research is needed to determine the effects of optimal and super-optimal temperatures on LF accumulation in blue crabs. With such knowledge, our model could be improved by providing differing coefficients across sub-, super-, and optimal temperature ranges.

Error: Age Prediction and Composition

Lipofuscin provided moderately accurate age estimates (MAPE = 0.16 years) for three known-age cohorts of blue crabs ranging from 0.2 to 1.8 years of age. Mean age prediction error compared favorably with error estimated by Sheehy et al. (1994; 1.2 years) and Uglem et al. (2005; 0.5 years) for European lobster *Homarus gammarus*, although these studies investigated a much wider age range. Over similar ages, the MAPEs of 0.05, 0.3, and 0.5 years for ages 0.5, 1.5, and 1.5+ were comparable to those reported for signal crayfish *Pacifastacus leniusculus* (0.1, 0.2, 0.5; Belchier et al. 1998) and Australian red claw crayfish *Cherax quadricarinatus* (0.1, 0.2, 0.4; Sheehy et al. 1994). Decreased accuracy in LF-based age predictions with increasing age may be attributed to inaccuracies in assumed basal temperature, a temperature by LF interaction, individual metabolic–activity variations, the functional form of the LF–TD day model (i.e., as slope decreases, likelihood of error increases), or a combination thereof.

Errors in the age composition of field-collected individuals will also likely include the effects of the variable temperature histories experienced among individuals. The difficulties of obtaining a representative temperature record are related to the complex life history of blue crabs (recall that juvenile blue crabs disperse throughout Chesapeake Bay following settlement). Hence, the use of a single, midbay temperature record may not be representative of temperatures experienced by blue crabs throughout their ontogeny. Water temperatures can be variable at large spatial scales. For instance, daily temperatures during 2001 were, on average, 0.9°C higher at a lower bay location (VIMS 2007) than at the midbay location used in this study. Seasonal and daily temperature contrasts between the upper and lower bay are likely to be even more divergent. In most cases, utilizing a lower-bay temperature record for estimating age composition would have reduced age estimates (i.e., more TD days accumulate in lower bay), further strengthening the finding of rapid recruitment to principal fisheries.

Age prediction error cannot be calculated for field-collected individuals of unknown age, but the error in age composition may be manifested at the extremes through atypical settlement dates and growth rates. The majority of back-calculated settlement dates corresponded with what is known about blue crab cohort dynamics in Chesapeake Bay, namely, that individuals settle from June to November (van Montfrans et al. 1990). Moreover, peak settlement was predicted to occur in September and October, concurrent with reported settlement peaks in Chesapeake Bay (van

Montfrans et al. 1990). Settlement dates predicted in April, May, and December (~10%) were atypical and likely represent error in age estimates of field collections. The overall dominance of the fall cohort was not evident from the predicted settlement dates and could be construed as a misclassification of individuals into the summer cohort. We speculate that the underrepresentation of the fall cohort is a sampling artifact rather than error in age predictions. Obtaining representative samples of the smallest cohorts (<20 mm CW) is difficult when trawling because small juveniles preferentially utilize nearshore shallow habitats (Hines and Ruiz 1995). Such a bias is expected to impact both summer and fall cohort representation but would have a disproportionately large impact on the fall cohort if it is indeed dominant.

We observed very high variation in size at estimated age. Blue crabs ranged from a 50-mm CW at age 1.5 to a 180-mm CW at age 0.5. The latter scenario where a June settler grows at rates exceeding 2 mm/d to reach 140+ mm CW by August of the same year seems particularly unreasonable. Still, such cases of extreme growth were quite rare and occurred in only about 5% of age predictions. High rates of inherent growth variability for this species are common. Miller et al. (2005) conducted a meta-analysis in which the VBF growth parameters and associated variability predicted blue crabs age 0.5 and 1.5 to range in size from 21 to 109 mm and from 87 to 184 mm, respectively. In a laboratory setting, individuals reared from the same brood under the same temperature and forage regimes varied as much as threefold (50 versus 150 mm) in size at the end of 1 year (A. R. Place, Center of Marine Biotechnology, personal communication). Although it is intriguing to consider whether such extreme growth variability occurs in wild crabs, we believe possible errors associated with the LF approach dissuade us from drawing inferences on anomalous observations of size at estimated age. Nevertheless, the variability in the LF-based age–length relationship that is commonly reported for crustaceans (e.g., O'Donovan and Tully 1996; Sheehy et al. 1999; Bluhm and Brey 2001; Ju et al. 2003; Kodama et al. 2006) does not support the underlying assumptions of length frequency analysis (i.e., cohorts are represented by distinct size modes; France et al. 1991). To this end, LF-based approaches may provide an alternative for age determination and cohort designation that is truly independent of traditional size-based approaches.

Age Composition and Recruitment

Two lines of evidence presented in this study are consistent with estimates of high exploitation and observed reductions in percentage of legal-sized crabs. First, LF-based age composition was truncated, particularly if blue crab longevity is 6 (Miller et al. 2005) to 8 (Rugolo et al. 1998) years. Second, peaks in commercial landings were concurrent with pulses of new recruitment. Summer peaks in peeler–soft crab landings coincided with large-scale recruitment of individuals that settled as part of the previous year's summer cohorts. Likewise, peaks in hard crab landings in October coincided with large-scale recruitment of the previous year's fall cohorts and initial recruitment of age-0.5 summer cohorts.

Van Engel (1958) initially suggested that rapid growth during the juvenile stage led to recruitment to commercial size limits within 1.0–1.5 years of age. Ju et al. (2001) concluded that individuals settling in early summer grew to subadult size (>60-mm CW) prior to overwintering and recruited to the commercial fisheries the following summer at 1.0–1.5 years of age. Alternatively, fall settlers overwintered at small sizes (<60 mm CW) but were capable of recruiting to the commercial fisheries within their first year of life following rapid growth during the ensuing spring, summer, and fall (Ju et al. 2001). In accordance, Sharov et al. (2003) concluded that fishery removals equaled or exceeded the abundance of age 1+ crabs and, therefore, crabs designated as age 0.5 in winter (<60 mm CW) recruited to and subsidized the hard crab fishery during the ensuing summer. The growth rates observed for known-age cohorts in this study supported the prediction that field-collected crabs partially recruited to both peeler and hard crab fisheries by age 1.

Species such as the blue crab that are relatively small, fast growing, rapidly maturing, and short-lived are typically characterized by 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). The consequences of a shorter generation time are that recruitment and landings will be increasingly correlated with the environmental factors affecting growth and the annual variations in egg production, settlement, and postsettlement survival (Ju et al. 2003). Accordingly, the recent declines in spawning stock biomass, larval abundance, and postsettlement recruitment (Lipcius and Stockhausen 2002) are disconcerting for a fishery that may depend on an annual crop of new recruits. Reducing exploitation rates to build age structure should be considered as an option to buffer the blue crab population against subsequent series of poor years for juvenile production.

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References

Adams, P. B. 1980. Life history patterns in marine fishes and their consequences for fisheries management. U.S. National Marine Fisheries Service Fishery Bulletin 78:1–12.

Beamish, R. J., and G. A. McFarlane. 1983. The forgotten requirement for age validation in fisheries biology. Transactions of the American Fisheries Society 112:735–743.

Belchier, M., L. Edsman, M. R. J. Sheehy, and P. M. J. Shelton. 1998. Estimating age and growth in long-lived temperate freshwater crayfish using lipofuscin. Freshwater Biology 39:439–446.

Bluhm, B. A., and T. Brey. 2001. Age determination in the Antarctic shrimp *Notocrangon antarcticus* (Crustacea: Decapoda) using the autofluorescent pigment lipofuscin. Marine Biology 138:247–257.

Bluhm, B. A., T. Brey, and M. Klages. 2001. The autofluorescent age pigment lipofuscin: key to age, growth, and productivity of the Antarctic amphipod *Waldeckia obesa* (Chevreux, 1905). Journal of Experimental Marine Biology and Ecology 258:215–235.

Brunk, U. T., and A. Terman. 2002. Lipofuscin: mechanisms of age-related accumulation and influence on cell function. Free Radical Biology and Medicine 33(5):611–619.

Brylawski, B. J., and T. J. Miller. 2006. Temperature-dependent growth of the blue crab (*Callinectes sapidus*): a molt process approach. Canadian Journal of Fisheries and Aquatic Sciences 63:1298–1308.

Bunnell, D. B., and T. J. Miller. 2005. An individual-based modeling approach to spawning-potential-per-recruit models: an application to blue crab *Callinectes sapidus* in Chesapeake Bay. Canadian Journal of Fisheries and Aquatic Sciences 62:2560–2572.

Campana, S. E. 2001. Accuracy, precision, and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology 59:197–242.

Castro, M., P. Encarnacão, and O. Tully. 2002. The effect of dietary antioxidants on lipofuscin accumulation in the crustacean brain. Journal of Experimental Marine Biology and Ecology 269:53–64.

Chowdhury, P. K., M. Halder, G. A. Kraus, M. J. Desai, D. W. Armstrong, T. A. Casey, M. A. Rasmussen, and J. W. Petrich. 2004. Generation of fluorescent adducts of malondialdehyde and amino acids: toward an understanding of lipofuscin. Photochemistry and Photobiology 79(1):21–25.

Epifanio, C. E. 1995. Transport of blue crab (*Callinectes sapidus*) larvae in the waters off mid-Atlantic states. Bulletin of Marine Science 57(3):713–725.

Epifanio, C. E., and R. W. Garvine. 2001. Larval transport on the Atlantic continental shelf of North America: a review. Estuarine, Coastal, and Shelf Science 52:51–77.

Ettershank, G. 1983. Age structure and cyclical annual size change in the Antarctic krill, *Euphausia superba* Dana. Polar Biology 2:189–193.

France, R., J. Holmes, and A. Lynch. 1991. Use of size-frequency data to estimate age composition of crayfish populations. Canadian Journal of Fisheries and Aquatic Sciences 48:2324–2332.

Hartnoll, R. G. 2001. Growth in Crustacea—twenty years on. Hydrobiologia 449:111–122.

Harvey, R. H., D. H. Secor, and S.-J. Ju. 2008. The use of extractable lipofuscin for age determination of crustaceans: reply to Sheehy. Marine Ecology Progress Series 353:307–311, 2008.

Hilborn, R., and C. J. Walters. 1992. Quantitative fisheries stock assessment: choice dynamics and uncertainty. Chapman and Hall, New York.

Hill, K. T., and C. Womersley. 1991. Critical aspects of fluorescent age pigment methodologies: modification for accurate analysis and age assessments in aquatic organisms. Marine Biology 109:1–11.

Hill, K. T., and C. Z. Womersley. 1993. Interactive effects of some environmental and physiological variables on fluorescent age pigment accumulation in brain and heart tissues of an aquatic poikilotherm. Environmental Biology of Fishes 37:397–405.

Hines, A. H. 2003. Ecology of juvenile and adult blue crabs: summary of discussion of research themes and directions. Bulletin of Marine Science 72(2):423–433.

Hines, A. H., and G. M. Ruiz. 1995. Temporal variations in juvenile blue crab mortality: nearshore shallows and cannibalism in Chesapeake Bay. Bulletin of Marine Science 57(3):884–901.

Jennings, S., M. J. Kaiser, and J. D. Reynolds. 2001. Marine fisheries ecology. Blackwell Scientific Publications, Cambridge, Massachusetts.

Ju, S.-J. 2000. Development and application of biochemical approaches for understanding age and growth in crustaceans. Doctoral dissertation. University of Maryland, College Park.

Ju, S.-J., D. H. Secor, and H. R. Harvey. 1999. Use of extractable lipofuscin for age determination of blue crabs *Callinectes sapidus*. Marine Ecology Progress Series 185:171–179.

Ju, S.-J., D. H. Secor, and H. R. Harvey. 2001. Growth rate variability and LF accumulation rates in the blue crab *Callinectes sapidus*. Marine Ecology Progress Series 224:197–205.

Ju, S.-J., D. H. Secor, and H. R. Harvey. 2003. Demographic assessment of the blue crab (*Callinectes sapidus*) in Chesapeake Bay using extractable lipofuscins as age

markers. U.S. National Marine Fisheries Service Fishery Bulletin 101:312–320.

King, J. R., and G. A. McFarlane. 2003. Marine fish life history strategies: applications to fishery management. Fisheries Management and Ecology 10:249–264.

Kodama, K., T. Yamakawa, T. Shimizu, and I. Aoki. 2005. Age estimation of the wild population of Japanese mantis shrimp *Oratosquilla oratoria* (Crustacea: Stomatopoda) in Tokyo Bay, Japan, using lipofuscin as an age marker. Fisheries Science 71:141–150.

Kodama, K., H. Shiraishi, M. Morita, and T. Horiguchi. 2006. Verification of lipofuscin-based crustacean ageing: seasonality of lipofuscin accumulation in the stomatopod *Oratosquilla oratoria* in relation to water temperature. Marine Biology 150(1):131–140.

Leffler, C. W. 1972. Some effects of temperature on the growth and metabolic rate of juvenile blue crabs, *Callinectes sapidus*, in the laboratory. Marine Biology 14:104–110.

Lipcius, R. N., and W. T. Stockhausen. 2002. Concurrent decline in the spawning stock, recruitment, larval abundance, and size of the blue crab *Callinectes sapidus* in the Chesapeake Bay. Marine Ecology Progress Series 226:45–61.

Lomovasky, B. J., E. Morriconi, T. Brey, and J. Calvo. 2002. Individual age and connective tissue lipofuscin in the hard clam *Eurhomalea exalbida*. Journal of Experimental Marine Biology and Ecology 276:83–94.

Miller, T. J., S. J. D. Martell, D. B. Bunnell, G. Davis, L. Fegley, A. Sharov, C. Bonzek, D. Hewitt, J. Hoenig, and R. N. Lipcius. 2005. Stock assessment of blue crab in Chesapeake Bay. National Oceanic and Atmospheric Administration, Chesapeake Bay Office, Annapolis, Maryland.

Nguyen, R. T., and H. R. Harvey. 1994. A rapid microscale method for the extraction and analysis of protein in marine samples. Marine Chemistry 45:1–14.

NMFS (National Marine Fisheries Service). 2006. Annual commercial landing statistics. Available: http://www.st.nmfs.gov/st1/commercial. (October 2006).

O'Donovan, V., and O. Tully. 1996. Lipofuscin (age pigment) as an index of crustacean age: correlation with age, temperature, and body size in cultured juvenile *Homarus gammarus* L. Journal of Experimental Marine Biology and Ecology 207:1–14.

Orth, R., and J. van Montfrans. 1987. Utilization of a seagrass meadow and tidal marsh creek by blue crabs *Callinectes sapidus*, 1. Seasonal and annual variations in abundance with emphasis on postsettlement juveniles. Marine Ecology Progress Series 41:283–294.

Prager, M. H. 1996. A simple model of the blue crab, *Callinectes sapidus*, spawning migration in Chesapeake Bay. Bulletin of Marine Science 58(2):421–428.

Rugolo, L. J., K. S. Knotts, A. M. Lange, and V. A. Crecco. 1998. Stock assessment of Chesapeake Bay blue crab (*Callinectes sapidus* Rathbun). Journal of Shellfish Research 17(2):493–517.

Sharov, A. F., J. H. Volstad, G. R. Davis, B. K. Davis, R. N. Lipcius, and M. M. Montane. 2003. Abundance and exploitation rate of the blue crab (*Callinectes sapidus*) in Chesapeake Bay. Bulletin of Marine Science 72(2):543–565.

Sheehy, M., N. Caputi, C. Chubb, and M. Belchier. 1998. Use of lipofuscin for resolving cohorts of western rock lobster (*Panulirus cygnus*). Canadian Journal of Fisheries and Aquatic Sciences 55:925–936.

Sheehy, M. R. J. 1990. Widespread occurrence of fluorescent morphological lipofuscin in the crustacean brain. Journal of Crustacean Biology 10(4):613–622.

Sheehy, M. R. J. 1992. Lipofuscin age pigment accumulation in the brains of ageing field- and laboratory-reared crayfish *Cherax quadricarinatus* (von Martens) (Decapoda: Parastacidae). Journal of Experimental Marine Biology and Ecology 161:79–89.

Sheehy, M. R. J. 1996. Quantitative comparison of in situ lipofuscin concentration with soluble autofluorescence intensity in the crustacean brain. Experimental Gerontology 31(3):421–432.

Sheehy, M. R. J. 2002a. A flow-cytometric method for quantification of neurolipofuscin and comparison with existing histological and biochemical approaches. Archives of Gerontology and Geriatrics 34:233–248.

Sheehy, M. R. J. 2002b. Role of environmental temperature in aging and longevity: insights from neurolipofuscin. Archives of Gerontology and Geriatrics 34:287–310.

Sheehy, M. R. J. 2008. Questioning the use of biochemical extraction to measure lipofuscin for age determination of crabs: comment on Ju et al. (1999, 2001). Marine Ecology Progress Series 353:303–306.

Sheehy, M. R. J., R. C. A. Bannister, J. F. Wickins, and P. M. J. Shelton. 1999. New perspectives on the growth and longevity of the European lobster (*Homarus gammarus*). Canadian Journal of Fisheries and Aquatic Sciences 56:1904–1915.

Sheehy, M. R. J., J. G. Greenwood, and D. R. Fielder. 1994. More accurate chronological age determination of crustaceans from field situations using the physiological age marker lipofuscin. Marine Biology 121:237–245.

Sheehy, M. R. J., P. M. J. Shelton, J. F. Wickins, M. Belchier, and E. Gaten. 1996. Ageing the European lobster *Homarus gammarus* by the lipofuscin in its eyestalk ganglia. Marine Ecology Progress Series 143:99–111.

Smith, S. G. 1997. Models of crustacean growth dynamics. Doctoral dissertation. University of Maryland, College Park.

Smith, M. T., and J. T. Addison. 2003. Methods for stock assessment of crustacean fisheries. Fisheries Research 65:231–256.

Tagatz, M. E. 1968. Growth of juvenile blue crabs, *Callinectes sapidus* Rathbun, in the St. John's River, Florida. U.S. National Marine Fisheries Service Fishery Bulletin 67:281–288.

Tully, O., V. O'Donovan, and D. Fletcher. 2000. Metabolic rate and lipofuscin accumulation in juvenile European lobster (*Homarus gammarus*) in relation to simulated seasonal changes in temperature. Marine Biology 137:1031–1040.

Uglem, I., M. Belchier, and T. Svåsand. 2005. Age determination of European lobsters (*Homarus gammarus* L.) by histological quantification of lipofuscin. Journal of Crustacean Biology 25(1):95–99.

Van Engel, W. A. 1958. The blue crab and its fishery in Chesapeake Bay. Commercial Fisheries Review 20(6):6–17.

van Montfrans, J., C. A. Peery, and R. J. Orth. 1990. Daily, monthly, and annual settlement patterns by *Callinectes*

sapidus and *Neopanope sayi* megalopae on artificial collectors deployed in the York River, Virginia: 1985–1988. Bulletin of Marine Science 46(1):214–229.

Vernet, M., J. R. Hunter, and R. D. Vetter. 1988. Accumulation of age pigments (lipofuscin) in two coldwater fishes. U.S. National Marine Fisheries Service Fishery Bulletin 86:401–407.

Vila, Y., A. Medina, C. Megina, F. Ramos, and I. Sobrino. 2000. Quantification of the age pigment lipofuscin in brains of known-age, pond-reared prawns *Penaeus japonicus* (Crustacea, Decapoda). Journal of Experimental Zoology 286:120–130.

VIMS (Virginia Institute of Marine Science). 2007. VIMS Scientific data archive. VIMS, Gloucester Point, Virginia. Available: www.vims.edu/data_archive. (December 2007).

Wahle, R. A., O. Tully, and V. O'Donovan. 1996. Lipofuscin as an indicator of age in crustaceans: analysis of the pigment in the American lobster *Homarus americanus*. Marine Ecology Progress Series 138:117–123.

Zmora, O., A. Findiesen, J. Stubblefield, V. Frenkel, and Y. Zohar. 2005. Large-scale juvenile production of the blue crab *Callinectes sapidus*. Aquaculture 244:129–139.