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Effects of Temperature and Heavy Metals on Extractable Lipofuscin in the Blue Crab, *Callinectes sapidus*

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The potential role of environmental factors on extractable lipofuscin accumulation rate in the blue crab was studied by examining the effect of temperature on laboratory reared blue crabs and the effect of trace metals from samples collected at impacted sites (Baltimore Harbor) and a relatively pristine site (outdoor ponds at Horn Point Laboratory, Cambridge, MD, USA). Lipofuscin levels did not significantly related with sampling sites or heavy metal concentrations in the crab tissue. Heavy metal body burden was highly variable among sampling sites and tissue types but significantly higher for both impacted areas (Curtis Creek and Inner Harbor) in comparison to the reference site. Among tissue types, gills showed the highest metal concentrations with the exception of Hg, which was highest in muscle tissue. For two groups of crabs that were held at either ambient (4 to 10°C) or heated seawater (19°C) for two months, normalized-lipofuscin levels were significantly different (P=0.001). Results suggest that temperature may affect lipofuscin accumulation (≈0.25 ng-lipofuscin/mg-protein/temperature-degree day). Therefore, temperature should be considered for accurate age calibration of crab populations using lipofuscin.

Key words: Extractable lipofuscin, Temperature, Heavy metals, Blue crabs

INTRODUCTION

Age pigments known as lipofuscins (LF) accumulate in post-mitotic tissue as a consequence of peroxidation reactions and has been suggested as an alternative to conventional size-based age determination techniques in blue crabs (Ju *et al.*,1999). However, the importance of environmental factors (e.g. temperature, salinity, contaminants, etc.) on LF accumulation must be determined before LF can be applied as an ageing method for understanding demographic structure in field populations.

The generally accepted mechanism for lipofuscin formation suggests that temperature or other environmental factors (salinity and contaminants), which alter metabolic rate, might affect LF content in invertebrates (Nicol, 1987; Hole *et al.*, 1995). In particular, a major problem in predicting age based upon LF content is to disentangle temperature effects alone that increase metabolic rate from those due to increased growth. If temperature and growth are co-linear in their influence on LF, then we can pursue laboratory and field models, which predict age from LF content and size. If temperature affects metabolic rate and thus LF content in a manner independent of growth, modeling efforts may need to include environmental temperature as a covariate for predicting chronological age.

During respiration, auto-oxidation of a variety of molecules and various enzymatic reactions lead to free radical production in the mitochondria and cell membrane (reviewed by Gutteridge (1987) and Greenstock (1993)). Free radicals react with unsaturated lipids and other cellular constituents to form peroxides and ultimately form the shiff-base condensation products collectively called lipofuscin. As with other chemical species that have sufficient energy to abstract hydrogen atoms from sites of unsaturation, transition metal ions (e.g. Fe, Co, Ni, Cu, Cd, Hg, Pb) also have the ability to initiate peroxidation reactions, yielding peroxy ($LO_2\bullet$) and alkoxy ($LO\bullet$) radicals which can ultimately appear as fluorescent lipid by-products (Halliwell and Gutteridge, 1984; Guarino *et al.*, 1995; Barrier *et al.*, 1998). In addition to metal salts, metal-

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protein complexes can stimulate the rate of membrane lipid auto-oxidation, which leads to fluorescent lipid by-products (Viarengo *et al.*, 1990; Engel and Brouwer, 1993). Based on this process, some studies suggested that lipofuscin, in addition to a significant marker of the aging process, might also be used as an indicator of heavy metal pollution in aquatic environments (Totaro *et al.*, 1986; Cuomo *et al.*, 1987; Pisanti *et al.*, 1988; Viarengo and Nott, 1993). Viarengo *et al.* (1990) suggested that peroxides generated by trace metals can enhance the formation of LF in the cell (especially in the digestive gland) and LF are then sequestered or incorporated into the lysosomes. These processes may partly contribute to the detoxification of heavy metals in mussels, which, trapped both chemically and mechanically, are finally eliminated by exocytosis of residual bodies (Viarengo and Nott, 1993). However, Zn seems to be involved in the stabilization of membrane structure, thus protecting against the peroxidative damage, which means Zn may not be a contributor to LF production (Guarino *et al.*, 1995).

Because LF accumulation appears directly correlated with metabolic rate, any factor which may alter metabolic rates, i.e. temperature (Clarke *et al.*, 1990; Sheehy, 1990), contaminants (e.g. trace metals - Marzabadi and Jones, 1992; Totaro *et al.*, 1985, 1986), food quality and availability (Sohol and Donato, 1978; Castro *et al.*, 2002), must be considered for accurate age calibration using LF content in field populations.

Although toxic accumulations of heavy metals (e.g. Hg, As, Cd, Pb) in fishes have been a serious public concern during the last decade, it has not been considered a problem with blue crabs because they are migratory and frequently molt. Thus few studies have examined heavy metal bioaccumulation in blue crabs.

In order to examine the potential roles of environmental factors on LF accumulation, laboratory experiments (to examine a temperature effect) were conducted, and samples were collected from various regions, in particular highly impacted sites such as Baltimore Harbor to examine the relationship between concentrations of heavy metals and LF concentrations.

MATERIALS AND METHODS

Field sampling and laboratory rearing to examine temperature effect

To determine the temperature effects on LF accumulation, samples of adult blue crabs (size (Carapace Width: CW) range: 124.5–155.0 mm; 142.0±9.46 (mean±SD) mm, n=21) were collected in the fall of 1996, near Calvert Cliffs, MD, USA and retained for two months under either ambient (temperature decreasing from 10 to 4°C) or heated seawater (temperature maintained at 19°C). They were fed small pieces of fish (typically menhaden *Brevoortia tyrannus*) every other day. During the experimental period, salinity ranged from 10 to 15‰ and no molting occurred. After 2 months, all animals were sacrificed and analyzed for LF analysis.

To examine the role of heavy metals roles on LF content crabs were collected from Inner Harbor in the summer of 1999 (CW range: 104–124.4 mm; 115.3±8.6 mm, n=4) and Curtis Creek (CW range: 104.8–137.1 mm; 125.1±14.5 mm, n=4) both within the Baltimore Harbor area, known as a highly contaminated site in the Chesapeake Bay region (Baker *et al.*, 1997). Animals collected from Baltimore Harbor were compared to a separate group of crabs (CW range: 137–167 mm; 149±15.9 mm, n=3), which were reared for 10 months within outdoor ponds (at Horn Point Laboratory) which can be considered a pristine reference site (for details, see 'methods and materials' in Ju *et al.*, 2001). Temperature and salinity for each sampling site are relevant (difference of mean annual temperature: <2°C and salinity: <5‰).

Sample preparations

Animals collected from the field or maintained in the laboratory or pond were anesthetized on ice prior to being sacrificed. After morphological measurements were taken, eye-stalk tissues were carefully excised and transferred to a 4 ml amber vial for LF analysis. For trace metal analysis, gills, back-fin muscle, and hepatopancreas of specimens were dissected and homogenized in a plastic blender with a stainless steel blade for dry weight determination with a separate sample placed in acid cleaned glass jar for metal analysis. Homogenized tissues were kept at −70°C prior to analysis.

Analysis of extractable LF

Tissues (eye-stalks) were extracted with a 4 ml mixture of CH_2Cl_2-MeOH (2:1, v/v) with sonication using a microprobe (20 W for 2 min). The samples were then centrifuged at 800×g for 10 min. to remove cellular debris. A portion of the total extract was transferred to a 4 ml vial and dried under N_2, redis-

solved in methanol (250 μl), and transferred to a polypropylene vial (0.7 ml). Volumes of 10 μl from each extract were injected by an auto-sampler (Waters 717 Plus Autosampler) with methanol (MeOH) as carrier solvent (1 ml · min^{-1}) through the flow cell. Fluorescence intensity was measured at a maximum emission wavelength of 405 nm using a maximum excitation at 340 nm at constant temperature (ca. 10°C).

The fluorescence intensity (FI) of extractable LF was calibrated using quinine sulfate (in 0.1 N H_2SO_4) which has a maximum emission wavelength at 450 nm with excitation at 340 nm. To provide a quantitative measure of LF in tissue, FI as an arbitrary concentration (equivalent to mg-quinine sulfate/ml-H_2SO_4 (0.1 N)) was normalized to protein content of extracted tissues measured by the modified bicinchoninic acid method (BCA) (Nguyen and Harvey, 1994). Protein normalized LF content is expressed as LF index (μg-LF content/mg-protein) in this study.

Analysis of heavy metals

Samples (about 3 g of wet weight) for metal (Mn, Fe, Co, Ni, Cu, Zn, As, Ag, Cd, Hg, and Pb) analysis were digested according to EPA Methods (US EPA 1996). The digestates were analyzed using a Hewlett-Packard 4500 Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). Blanks and standards were run with each batch of samples, and samples were not analyzed if the correlation coefficient of the calibration curve was less than 0.99. Blank concentrations were significantly less than the sample concentrations for all samples. Detection limits were <5 ng g^{-1}. For analysis by ICP-MS, relative standard deviations were always less than 5%, typically around 2%. Measured metal values of samples compared well with in certified values for target metals in standard reference material (SRM) (SRM 1566a oyster tissue). Recovery of SRM by ICP-MS was a certified value ±10%.

Statistical analyses

Lipofuscin measurements as LF indices (μg/mg-protein) were $\log_e$ transformed prior to conducting statistical analyses to satisfy assumptions for homogeneity of variances and normality of residuals. Analysis of covariance (ANCOVA) was used to determine whether LF content was affected by temperature or

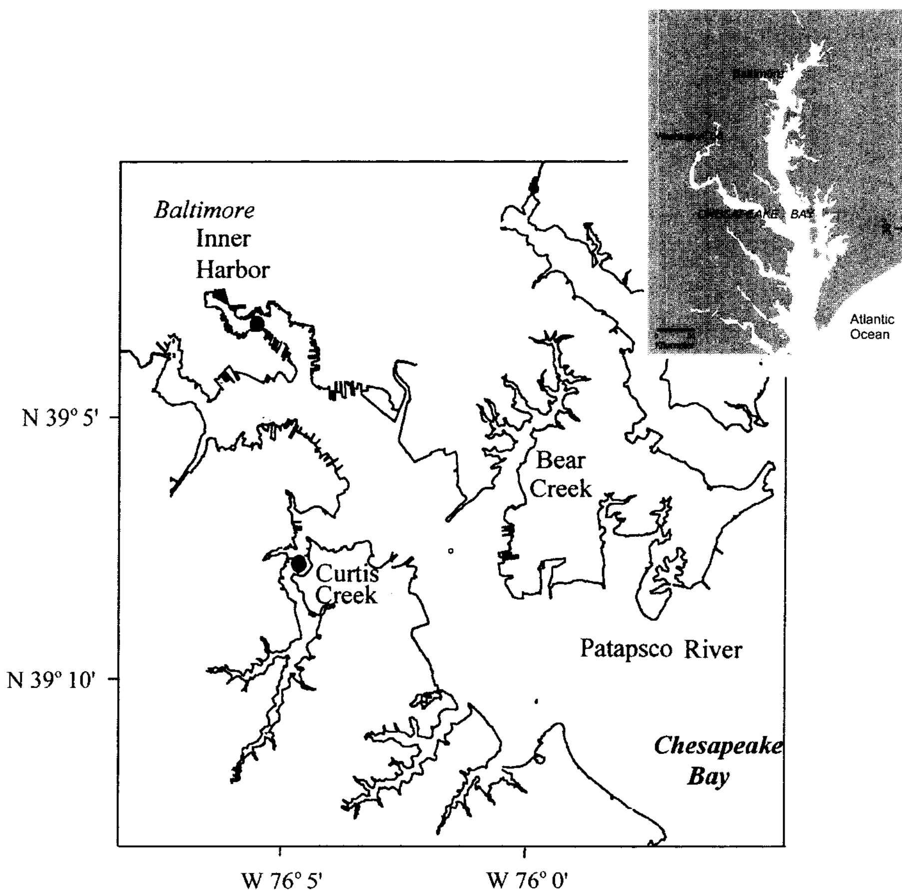

Fig. 1. Map of Baltimore Harbor with sampling sites (shown as filled circles).

sampling sites (heavy metals) with CW as a covariate. For each metal, a two-way analysis of variance (ANOVA) was used in order to test for differences among sampling sites and tissue types. All statistical analyses were performed using SAS (version 6.12).

RESULTS

The effect of temperature

Between two groups of animals held at different temperatures for two months, LF contents in eye-stalks of blue crabs were significantly different (ANCOVA; n=21; P=0.001) over a similar size range (Fig. 1). Mean LF content in animals held at heated seawater (19°C) has significantly higher (0.16 LF index higher) than that in animals held at ambient seawater. Accordingly, LF index accumulation rate affected by temperature is approximately 0.25×10^{3} μg-LF/mg-protein/temperature-degree day, which based on total accumulative temperature differences (about 600°C days) during two months. Temperature-degree days were calculated as temperature degree day (T) $=\Sigma(t-9)$; if $t \leq 9$, $t-9=0$, because no growth of the blue crab in Chesapeake Bay can occur at ≤9°C (Smith, 1997), where t is mean water temperature of each day. The value from short-term laboratory experiment is very close to our empirical LF accumulation rate (0.26×10^{3} mg-LF/mg-protein/temperature-degree day - calculated from an annual LF accumulation (0.8 mg-LF/mg-protein for adult crabs; from Ju *et al.*, 2001) divided by the mean (3000) accumulative temperature-degree day for a year in Chesapeake Bay).

The effect of heavy metals

Lipofuscin contents were not significantly different between sampling sites (impacted vs. non-impacted area) (ANCOVA; P>0.05; Fig. 3). Metal concentrations were highly varied between sampling sites and tissue types (Table 1). The two-way ANOVA test indicated no significant differences, (P>0.05) in Fe, Ni, and Cd between sampling sites or in Zn and As between tissue type (Table 2). Manganese (Mn), Co, Cu, Zn, As, Ag, Cd, Hg, and Pb contents were significantly higher in animals from both impacted areas (Curtis Creek and Inner Harbor) than in pond-reared crabs. In particular, high concentrations of Zn, As, Hg, and Pb were found in samples from Inner harbor. Among tissue types, gills showed the highest metal concentrations with the exception of Hg in muscle, particularly for samples from Inner Harbor (Table 1 and Fig. 4). LF contents in eye-stalks did not show any significant trends with different levels of trace metal content in muscle tissue of individuals from different sampling sites (Fig. 3 and 4).

DISCUSSION

Temperature is the dominant environmental variable affecting metabolic rate in poikilotherms. Therefore, temperature variation (i.e. seasonal change in the metabolic rates) can potentially alter the rate of LF accumulation based on the generally accepted

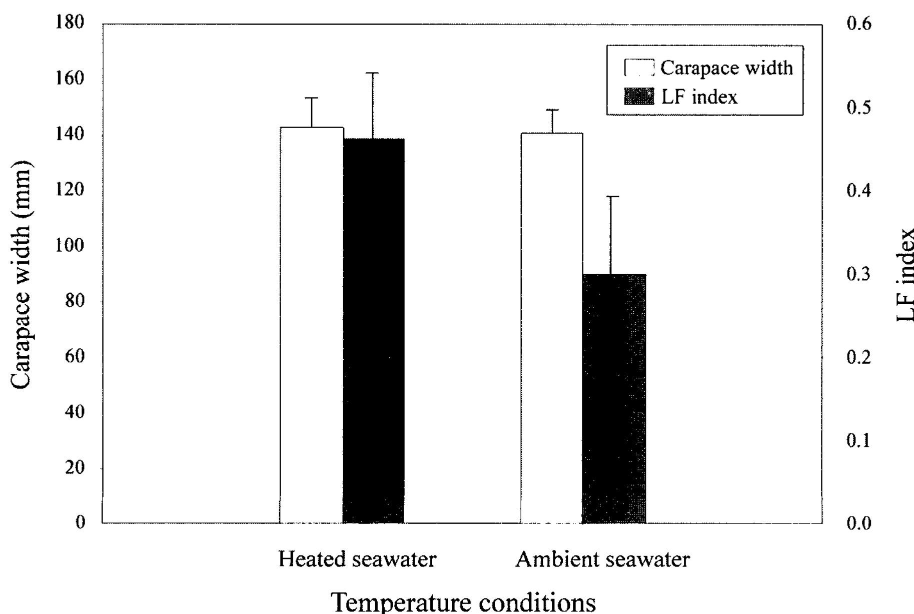

Fig. 2. Mean (+1SD) of carapace width (mm) and LF index (μg lipofuscin/mg protein) of eye-stalks of two groups of blue crabs which were held in either ambient (decreasing temperature 10 to 4°C; n=10) or heated (19°C; n=11) seawater for two months.

Table 1. Heavy metal concentrations (µg/g dry wt.) in gills, muscles, and hepatopancreas of blue crabs from outdoor ponds (control) at Horn Point and field sites at Curtis Creek and Inner Harbor.

Heavy metal	Pond (n=3)			Curtis Creek (n=4)			Inner Harbor (n=4)		
	Gill (mean±SD)	Muscle (mean±SD)	Hepatopancreas (mean±SD)	Gill (mean±SD)	Muscle (mean±SD)	Hepatopancreas (mean±SD)	Gill (mean±SD)	Muscle (mean±SD)	Hepatopancreas (mean±SD)
Mn	87.3±9.38	7.51±3.44	11.3±1.77	326±46.4	9.52±0.36	261±168	220±97.3	4.66±0.60	275±301
Fe	267±41.7	8.63±2.93	52.6±18.9	230±80.1	13.0±3.45	168±84.8	822±727	13.6±1.83	191±65.4
Co	0.34±0.054	0.04±0.003	0.64±0.13	0.41±0.11	0.03±0.006	0.47±0.28	0.63±0.25	0.05±0.01	0.40±0.16
Ni	1.00±0.187	0.09±0.032	1.82±0.36	0.77±0.21	0.13±0.20	0.68±0.65	0.94±0.14	0.03±0.05	0.52±0.38
Cu	98.0±29.3	17.9±4.35	167±77.5	428±83.1	36.8±4.63	65.9±26.2	465±176	29.2±8.59	144±122
Zn	31.7±2.77	60.2±4.33	33.7±1.88	45.6±3.71	44.6±5.08	45.0±6.70	99.5±24.0	77.1±2.46	149±54.2
As	3.44±0.526	2.85±0.078	4.75±0.564	7.44±1.39	4.20±0.439	6.70±1.61	14.6±7.76	6.40±1.41	13.8±5.82
Ag	0.56±0.23	0.11±0.04	0.79±0.34	4.33±1.47	0.43±0.20	1.01±0.68	5.24±3.37	0.38±0.07	1.85±1.29
Cd	0.21±0.11	0.01±0.003	1.10±0.36	0.17±0.051	0.01±0.009	0.97±0.45	0.32±0.12	0.07±0.03	1.39±0.73
Hg	0.005±0.001	0.039±0.007	0.009±0.001	ND	0.02±0.003	0.007±0.001	1.76±1.42	3.84±1.16	1.33±0.43
Pb	0.004±0.001	0.034±0.007	0.007±0.001	0.88±0.68	0.057±0.045	0.14±0.046	7.75±4.32	0.86±0.58	1.32±0.75

ND= Not detected

Table 2. The results of two-way analysis of variance (ANOVA) for all metal concentrations. (df, degree of freedom; F, variance ratio; P, probability).

Source		Site	Tissue	Site × Tissue
Heavy metals		(df=2)	(df=2)	(df=4)
Mn	F	4.15	5.60	1.17
	P	0.0284	0.0101	0.3485
Fe	F	2.01	5.75	1.43
	P	0.1562	0.0091	0.2537
Co	F	4.05	14.7	1.76
	P	0.0305	0.0001	0.1699
Ni	F	0.04	12.2	1.19
	P	0.9595	0.0002	0.3395
Cu	F	7.49	25.8	5.57
	P	0.0030	0.0001	0.0026
Zn	F	37.8	2.04	3.25
	P	0.0001	0.1521	0.0289
Ag	F	4.99	9.94	2.15
	P	0.0154	0.0007	0.1056
As	F	13.9	3.11	0.98
	P	0.0001	0.0630	0.4392
Cd	F	2.69	19.5	1.07
	P	0.0883	0.0001	0.3937
Hg	F	34.3	3.77	1.76
	P	0.0001	0.0378	0.0149
Pb	F	11.1	6.65	4.99
	P	0.0004	0.0050	0.0045

mechanism for LF formation (Nicol, 1987; Clarke *et al.*, 1990; Sheehy, 1990; Hole *et al.*, 1995). Because the laboratory study corresponded to relatively small fraction of the crabs life-span (loger than 3 yrs), temperature should be a significant factor on LF accumulation for adult crabs (> 120 mm CW). However, long-term (more than 6 month) laboratory and pond rearing experiments did no indication of the temperature effect on LF accumulation (Ju *et al.*, 1999 and 2001). This inconsistency of the temperature effect may come from differences in experimental design. Firstly, we did not use the same age or size among experiments; for our previous long-term pond and laboratory experiments, juveniles (≤80 mm CW) were initially used; but for this short-term laboratory experiment, only adults (CW >120 mm) were used. Therefore, these adults might have significantly different levels of LF when they were collected. Growing neural tissues during the rapid juvenile growth stage may disproportionately exceed LF accumulation rates, resulting in an apparent lack of change in LF accumulation (e.g. a growth dilution effect) as suggested by Hill & Womersley (1991) and Strauss (1999). Secondly, temperature regimes (differences less than 8°C) used for the long-term laboratory experiment (for more detail information, see Ju *et al.*, 1999) may not be enough to significantly affect metabolic rates (or LF accumulation rates). As shown by Leffler (1972), the metabolic rate of blue crabs reared under laboratory conditions was positively related to water temperature but not in a strictly linear fashion because metabolic rates did not significantly differ within the temperature range 15 to 25°C. Addition-

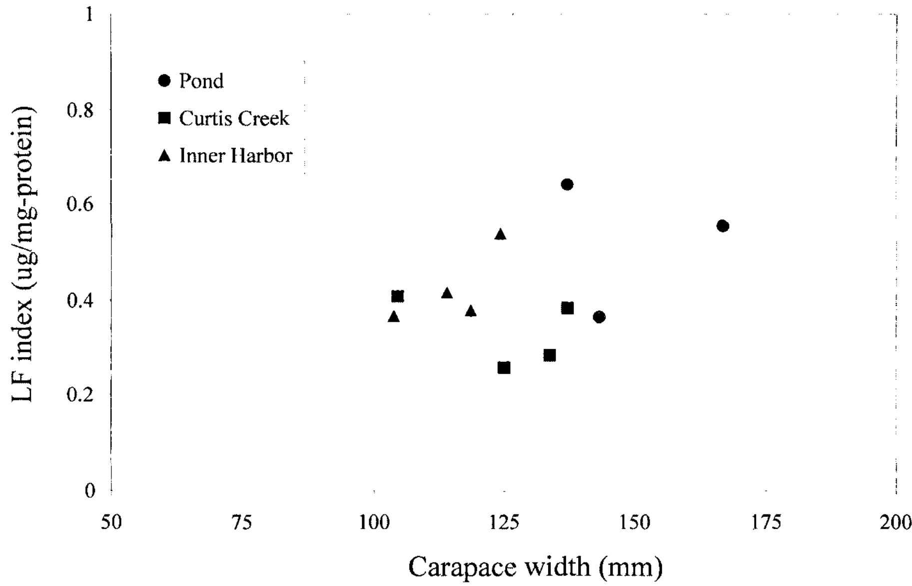

Fig. 3. Size (Carapace width: mm) versus LF index (μg/mg-protein) of crabs from non-impacted (outdoor ponds) and impacted sites (Curtis Creek and Inner Harbor).

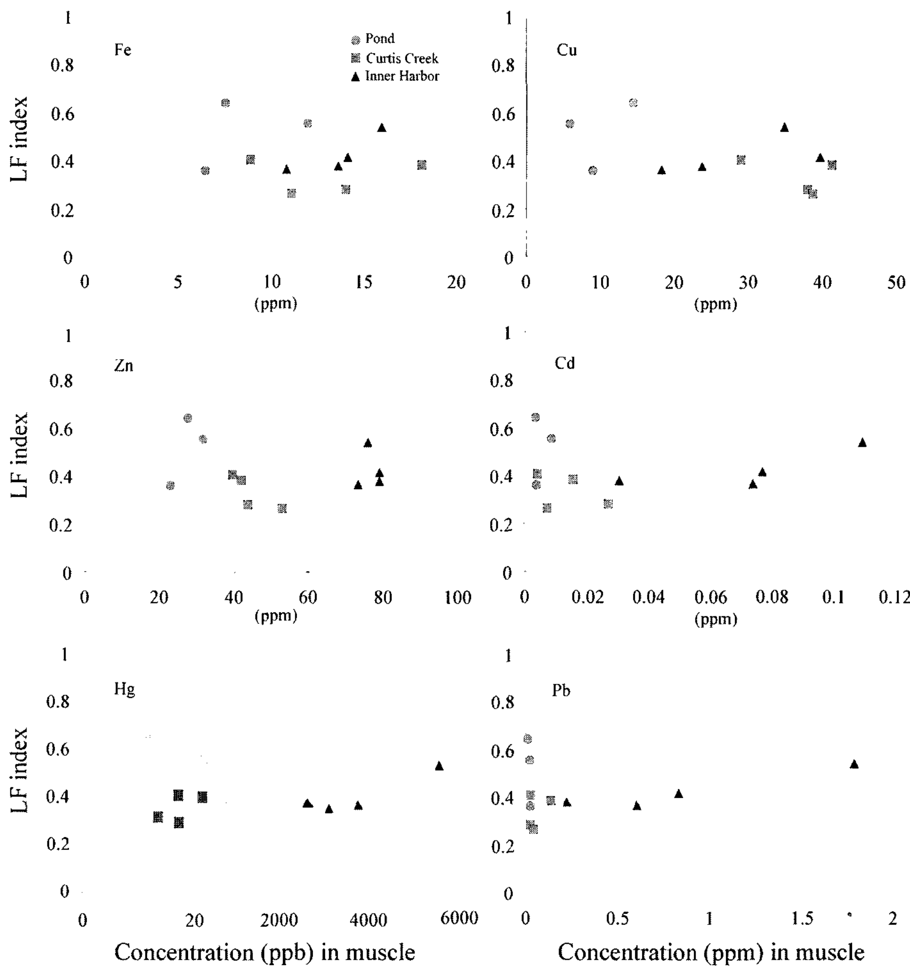

Fig. 4. The relationship between LF index (μg LF/mg protein) in eye-stalk and concentration (ppm: μg g^{-1} dry weight and ppb: ng g^{-1} dry weight for Hg) of major transition metals (Fe, Cu, Zn, Cd, Hg, and Pb) in muscle of individuals from the pond and Baltimore Harbor (Curtis Creek and Inner Harbor).

ally, other environmental factors (such as diet, density of animals, dissolved oxygen levels, etc.) that may also affect metabolic rate and consequently LF accumulation were also significantly different between laboratory and pond environments. The importance of temperature should not be ignored when applying

the empirical LF accumulation rate to natural crab populations (Ju *et al.*, 2001) given the protracted spawning seasons (from early spring to late fall) of blue crabs and temporal and spatial variation of temperature in Chesapeake Bay. For instance, crabs spawned during spring to early summer have experienced a much longer warm season than late summer and fall spawned crabs. Even though the age difference between these cohorts is less than 6 months, LF index might be significantly higher in crabs spawned early spring and summer than in crabs spawned late summer and fall. Moreover, some studies have also shown seasonal changes of LF accumulation that are related to temperature using the histological approach (Sheehy *et al.*, 1994; Vila *et al.*, 2000; O'Donovan and Tully, 1996).

Although not examined here, previous work suggests that salinity may not be significantly affect LF content of blue crab populations (Ju *et al.*, 1999). Chincoteague Bay is a polyhaline/euhaline system which experiences much less variations (<5‰) in salinity than Chesapeake Bay. Ju *et al.* (1999) found that LF index of crabs from these two systems were not significantly different.

Although the literature suggests that heavy metals can affect LF content in invertebrates (Totaro *et al.*, 1985, 1986; Cuomo *et al.*, 1987; Pisanti *et al.*, 1988; Marzabadi and Jones, 1992; Viarengo and Nott, 1993), LF levels normalized by protein content in eye-stalks of blue crabs were not significantly related with heavy metal concentrations in the muscle tissue of blue crabs (Fig. 4). This suggests enhanced LF production by exposure to contaminants (such as heavy metals) might be localized in detoxifying organs (hepartopancreas) (George, 1983; Viarengo, 1989; Viarengo *et al.*, 1990) rather than in neural tissues. Alternatively LF may not always be elevated with metal exposure because some metal(s) might prevent LF production such as Zn which can stablize the membrane structure and thus alleviate the peroxidation process within the cell membrane (Guarino *et al.*, 1995). However, the role of trace metal on LF accumulation in blue crabs is still unclear because physiological characters of crab (such as molting, migration behavior and life stage) may differentially affect tissue burdens in field-collected crabs. Therefore, rigorous empirical experimentation is required to reduce the number of variables.

The accumulation of heavy metals in the blue crab typically involves detoxification and storage to some degree, either temporarily or permanently. Essential metals include Fe, Zn, Cu, Mn, Ni, Co, etc. which play a variety of roles in biochemistry often as enzyme cofactors. For example, Cu is an essential component of hemocyanin, the respiratory pigment of certain mollusks and crustaceans. Hemocyanin is broken down releasing Cu during the molt, some of which is stored temporarily in the hepatopancreas and any resynthesis of hemocyanin may involve use of Cu from this store, or newly accumulated Cu after ecdysis (Engel and Brouwer, 1993). In this study, high variability (between individuals) of Cu concentration in the hepatopancreas, particularly samples from pond and Inner Harbor, were found. The molting cycle may have contributed to high variability; one soft crab and one peeler crab (from the Inner Harbor and the pond, respectively) showed higher Cu concentration in their hepatopancreas than all other sampled crabs. However, the role of molting stage remains unclear because the relationship between hemocyanin, Cu, blood, and hepatopancreas is complicated by changes in the water content of the hemolymph at the time of the molt.

Direct uptake of metals from solution mainly occurs through the gill. For the blue crab, Fe, Cu, Ag, and Pb appears to be strongly accumulated in the gills, suggesting that direct uptake is a major route for these metals. However, molting frequency may also influence the metal distribution in the gills. Several crabs taken from Inner Harbor, which may not have molted for a significant period of time (based upon the dark color of the gills and fouling on their hard shell), had very high Fe content in the gills. On the other hand, after ecdysis crabs may temporarily increase the uptake rate of metals into the body, presumably coincident with a temporary increase in body surface permeability prior to the tanning and/or calcification of new cuticle or filled with ambient water. Accumulation from food is the dominant uptake mechanism for some metals (such as Se, methyl Hg) into invertebrates (Mason *et al.*, 2000), with the availability of the constituent metals varying greatly with metal type and concentration, nature of food, digestive physiology of the consumer, etc. (Bryan, 1976).

Several factors (such as molting, temperature, and animal size) may interfere with the concentrations of essential metals (Cu, Mn, and Zn), but there is no correlation between their tissue concentrations and their levels in ambient seawater or sediment. By contrast, some metals (Cd, Pb, and Hg) are accumulated by crustaceans in proportion to environmental exposure (Evans *et al.*, 2000). *C. sapidus* is highly migratory within estuaries (average 130 m d^{-1}) (Wolcott

Table 3. Heavy metal concentrations (μg g^{-1} dry wt.) of Mummichog (*Fundulus heteroclitus* and *F. majalis*), which is a major diet of blue crabs in outdoor ponds at Horn Point and mean heavy metal concentration (μg g^{-1} dry wt.) in sediment from Curtis Creek and Baltimore Inner Harbor. Metal concentrations of sediments are taken from Baker *et al.* (1997) and shown for comparison.

Heavy metal	Pond (Mummichogs)	Curtis Creek (sediment)	Inner Harbor (sediment)
Mn	28	1000	800
Fe	78	7	5
Co	0.08	-	-
Ni	0.17	70	80
Cu	3.92	300	400
Zn	44	600	800
As	1.15	-	-
Ag	0.03	-	-
Cd	0.01	2	3
Hg	0.005	0.8	2
Pb	0.004	200	400

and Hines, 1990) such that metal levels in tissue might not reflect conditions at the sampling sites. This study indicates that crabs may stay and grow in sub-tributaries during the growth season because metal concentrations correspond well with those of sediments within sampling area (Table 3).

Sex-specific differences in metal concentrations have been suggested in blue crabs (Sastre *et al.*, 1999), but unfortunately, the sample size was too small to compare gender differences in heavy metal concentrations for this study. However, We expect that males have higher metal concentrations than females due to seasonal migratory behavior of adult females into the lower Chesapeake Bay, and because males generally inhabit low salinity areas (i.e. tidal tributaries, upper and mid bay), which include highly impacted areas. Crabs mainly feed on soft clams from the sediment except for pond-reared crabs whose main prey were small fish such as mummichogs (*Fundulus heteroclitus* and *F. majalis*) (Ebersole and Kennedy, 1995; Smith and Weis, 1997). High concentration of Hg was found in the muscle compared to other tissues. Particularly, crabs from Inner Harbor have 100 times higher Hg concentration although it is 5–10 times less than crabs (≤4 μg g^{-1} wet weight of muscle tissue) from the other Hg impacted area (≤1 μg g^{-1} dry weight of surface sediment) as Lavaca Bay, Texas (Blooms *et al.*, 1999; Evans *et al.*, 2000). These results agree with the belief that Hg (mainly as methyl Hg - about 60–90% of total Hg in the muscle is methyl Hg in crustacean and fish) accumulation occurs primarily from food for the crayfish (Mason *et al.*, 2000) as well as for the crab. Evans *et al.* (2000) also suggested that residence time in the impacted area and feeding habits (benthic feeding) is a major consideration of Hg accumulation in blue crabs. Mercury and lead are very toxic to humans and may cause a variety of disorders such as damage in the central nervous system (US EPA, 1984a and b).

Although total Hg concentrations (0.77±0.23 μg g^{-1} wet weight of muscle) in blue crabs from Inner Harbor were still less than the Food and Drug Administration action level of 1 μg g^{-1} wet weight as mercury, these crabs should be seriously considered for the public health. Not only did crabs contain a relatively high proportion of methyl Hg of total Hg, but also the size of crabs collected for this study were smaller than legal size (<127 mm CW) and thus legal size crabs might have higher Hg content than the FDA limit.

Across orders of magnitude of concentration of trace metals in sediments and tissue, no consistent correlation was detected between heavy metals and LF levels. Temperature may be a significant factor on LF accumulation. Seasonal changes in temperature should be considered for accurate age calibration of crab populations in the Chesapeake Bay. However, much more detailed work is needed to confirm the role of environmental factors on LF accumulation with rigorous laboratory and field experimentations.

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REFERENCES

Baker J., R. Mason, J. Cornwell, J. Ashley, J. Halka and J. Hill, 1997. Final report. Spatial mapping of sedimentary contaminants in the Baltimore Harbor/Patapsco River/Back River system. Ref No. UMCES [CBL] 97-142, CBL, Solomons, Maryland, USA, 245pp.

Barrier L., G. Page, B. Fauconneau and F. Juin, 1998. Autooxidation of rat brain homogenate: evidence for spontaneous lipid peroxidation. Comparison with the characteristics of Fe^{2+}-and ascorbic acid-stimulated lipid peroxidation. *Free Rad. Res.*, **28**: 411–422.

Bloom N.S., G.A. Gill, S. Cappellino, C. Dobbs, L. Mcshea, C. Driscoll, R. Mason and J. Rudd, 1999. Speciation and cycling of mercury in Lavaca Bay, Texas sediments. *Environ. Sci. Technol.*, **33**: 7–13.

Bryan G.W., 1976. Heavy metal contamination in the sea. In: Marine Pollution, edited by Johnston R., Academic Press, London, pp 120–145.

Castro M., P. Encarnação and O. Tully, 2002. The effect of dietary antioxidants on lipofuscin accumulation in the crustacean brain. *J. Exp. Mar. Biol. Ecol.*, **269**: 53–64.

Clarke A., M.A. Kendall and D.J. Gore, 1990. The accumulation of fluorescent age pigments in the trochid gastropod *Monodonta lineata*. *J. Exp. Mar. Biol. Ecol.*, **144**: 185–204.

Cuomo V., E.A. Totaro, F. Vanzanella, P. Russo, S. D'Antonio and M. Caramia, 1987. Age pigments in fungi as a biological marker for heavy metal pollution in aquatic environments. Pergamon Press, Oxford & New York, pp 147–154.

Ebersole E.L. and V.S. Kennedy, 1995. Prey preferences of blue crabs, *Callinectes sapidus*, feeding on 3 bivalve species. *Mar. Ecol. Prog. Ser.*, **118**: 167–177.

Engel D.W. and M. Brouwer, 1993. Crustaceans as models for metal metabolism: I. Effects of the molt cycle on blue crab metal metabolism and metallothionein. *Mar. Environ. Res.*, **35**: 1–5.

Evans D.W., R.D. Kathman and W.W. Walker, 2000. Trophic accumulation and depuration of mercury by blue crabs (*Callinectes sapidus*) and pink shrimp (*Penaeus duorarum*). *Mar. Environ. Res.*, **49**: 419–434.

George S.G., 1983. Heavy metal detoxification in the mussel *Mytilus edulis*. Composition of Cd-containing kidney granules (tertiary lysosomes). *Comp. Biochem. Physiol.*, **76C**: 53–57.

Greenstock C.L., 1993. Radiation and aging - free-radical damage, biological response and possible antioxidant intervention. *Med. Hypoth.*, **41**: 473–482.

Guarino S.M., F.M. Guarino, G. Tommonaro and M. de Nicola, 1995. Cadmium-induced lipofuscins and effect of zinc on hepatopancreas cells in *Idotea baltica*. *Experientia*, **51**: 967–969.

Gutteridge J.M.C., 1987. Oxygen radicals, transition metals and aging. In: Advances in the biosciences 64 editied by Totaro E.A., P. Glees and F.A. Pisanti , Pergamon Press, Oxford, pp 1–22.

Halliwell B. and J.M.C. Gutteridge, 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J.*, **219**: 1–14.

Hill K.T. and C. Wormsley, 1991. Critical aspects of fluorescent age-pigment methodologies: modification for accurate age assessment in aquatic organisms. *Mar. Biol.*, **109**: 1–11.

Hole L.M., M.N. Moore and D. Bellamy, 1995. Age-related cellular and physiological reactions to hypoxia and hyperthermia in marine mussels. *Mar. Ecol. Prog. Ser.*, **122**: 173–178.

Ju S-J., D.H. Secor and H.R. Harvey, 1999. Use of extractable lipofuscin for age determination of blue crab, *Callinectes sapidus*. *Mar. Ecol. Prog. Ser.*, **185**: 171–179.

Ju S-J., D.H. Secor, H.R. Harvey, 2001. Growth rate variability and lipofuscin accumulation rates in the blue crab, *Callinectes sapidus*. *Mar. Ecol. Prog. Ser.*, **224**: 197–205.

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

Mason R.P., J-M. Laporte and S. Andres, 2000. Factors controlling the bioaccumulation of mercury, methymercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. *Arch. Environ. Contam. Toxicol.*, **38**: 283–297.

Marzabadi M.R., C.B. Jones, 1992. Heavy metals and lipofuscinogenesis. A study on myocardial cells cultured under varying oxidative stress. *Mech. Ageing Dev.*, **66(2)**: 159–171.

Nguyen R.T., H.R. Harvey, 1994. A rapid micro-scale method for the extraction and analysis of protein in marine samples. *Mar. Chem.*, **45**: 1–14.

Nicol S., 1987. Some limitations on the use of the lipofuscin ageing technique. *Mar. Biol.*, **93**: 609–614.

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 *Homanus gammarus* L. *J. Exp. Mar. Biol. Ecol.*, **207**: 1–14.

Pisanti F.A., L. Lucadamo, F. Vanzanell and E.A. Totaro, 1988. Effect of heavy metal ions on microorganism aging. *Mar. Poll. Bull.*, **19**: 328–333.

Sastre M.P., P. Reyes, H. Ramos, R. Romero and J. Rivera, 1999. Heavy metal bioaccumulation in Puerto Rican blue crabs (*Callinectes sapidus*). *Bull. Mar. Sci.*, **64**: 209–217.

Sheehy M.R.J., 1990. Individual variation in, and the effect of rearing temperature and body size on, the concentration of fluorescent morphological lipofuscin in the brains of freshwater crayfish, *Cherax Cuspidatus* (Crustacea: Parastacidae). *Comp. Biochem. Physiol.*, **96A**: 281–286.

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. *Mar. Biol.*, **121**: 237–245.

Smith G.M. and J.S. Weis, 1997. Predator-prey relationships in mummichogs (*Fundulus heteroclitus* (L)): Effects of living in a polluted environment. *J. Exp. Mar. Biol. Ecol.*, **209**: 75–87.

Smith S.G., 1997. Models of crustacean growth dynamics. Ph. D. Thesis, University of Maryland, College Park, Maryland, 256 pp.

Sohal R.S. and H. Donato, 1978. Effects of experimentally altered lifespans on the accumulation of fluorescent age pigments in the housefly, *Musca domestica*. *Exp. Geront.*, **9**: 33–41.

Strauss R.E., 1999. Brain-tissue accumulation of fluorescent age pigments in four poeciliid fishes (*Cyprinodontiformes*) and the estimation of "Biological Age". *Growth Dev. Aging*, **63**: 151–170.

Totaro E.A., F.A. Pisanti, P. Glees and A. Continllo, 1985. The role of copper level in the formation of neuronal lipofuscin in spinal ganglia of *Topedo marmorata*. *Mar. Environ. Res.*, **15**: 153–160.

Totaro E.A., F.A. Pisanti, P. Glees, A. Continllo, 1986. The effect of copper pollution on mitochondrial degeneration. *Mar. Environ. Res.*, **18**: 245–253.

US EPA, 1984a. Health effects assessment for lead. US EPA, Environmental Criteria Assessment Office. Cincinnati, Ohio, U.S.A.

US EPA, 1984b. Mercury health effects update. Health issue assessment. EPA-600/8-84-019F. US EPA, Office of Health and Environmental Assessment, Washington, D.C., U.S.A.

US EPA, 1996. Compilation of analytical methods. CD-ROM. US EPA, Office of Health and Environmental Assessment, Washington, D. C., U.S.A.

Viarengo A., 1989. Heavy metals in marine invertebrates: mechanisms of regulation and toxicity at the cellular level. *C.R.C. Crit. Rev. Aquat. Sci.*, **1**: 295–317.

Viarengo A., L. Canesi, M. Pertica, G. Poli, M.N. Moore and M. Orunesu, 1990. Heavy metal effects on lipid peroxidation in

the tissues of *Mytilus Galloprovincialis* Law. *Comp. Biochem. Physiol.*, **97C**: 37–42.

Viarengo A. and J.A. Nott, 1993. Mini-Review: Mechanisms of heavy metal cation homeostasis in marine invertebrates. *Comp. Biochem. Physiol.*, **104C**: 355–372.

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

Wolcott T. G. and A. H. Hines, 1990. Ultrasonic telemetry of small-scale movements and microhabitat selection by molting blue crab (*Callinectes sapidus*). *Bull. Mar. Sci.*, **46**: 83–94.

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