Digestive toxicity in grass shrimp collected along an impact gradient

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Ingested pollutants may elicit digestive toxicity following incorporation into consumer tissues. This post-assimilatory toxicity may include tissue damage influencing synthesis of digestive enzymes, gut transit time and absorption of nutrients as well as pollutants by the gut epithelium. This study investigated impacts of chronic field exposure on gut residence time (GRT), feces elimination rate (FER), extracellular digestive protease activities and gut pH in grass shrimp Palaemonetes pugio. Adult shrimp were collected from differentially impacted sites within the New York/New Jersey Harbor Estuary and fed prepared meals containing fluorescent or near-infrared markers and analyzed for digestive toxicity. Relationships between digestive parameters and assimilation efficiencies (AE) for Cd, Hg and organic carbon reported previously were also analyzed. Minimum GRT did not vary significantly for field-collected shrimp, but was positively correlated with Cd, but not Hg or carbon, AE. FER was not impacted by field exposure. Digestive protease activities exhibited a marked decrease in grass shrimp from impacted field sites relative to reference shrimp. Relationships between the assimilation of elements and digestive physiology in field-collected shrimp suggest that digestive plasticity (increasing GRT) may be important in compensating for post-assimilatory digestive toxicity (reduced protease activities) in order to maintain nutrient assimilation. Stress-induced variability in digestive function among grass shrimp populations may, in turn, enhance the assimilation of non-essential elements, such as Cd.

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1. Introduction

Digestive plasticity may allow organisms to maximize nutrient assimilation through variability in gut function (e.g., morphology, transit time, digestive enzyme activities or gut fluid chemistry) in response to external factors such as diet composition, intraspecific competition and predation (Bock and Mayer, 1999; Jumars, 2000; Relyea and Auld, 2004; Sabat et al., 2005). For organisms exposed to chemical stressors through feeding, plasticity could potentially offset impacts on digestion and allow individuals to maintain adequate assimilation of essential elements, including organic carbon. This may be particularly important in settings (e.g., urbanized ecosystems) where the nutritional value of food organisms may also be compromised by contaminant exposure (Campbell et al., 2005). The inability to compensate for deleterious effects of ingested pollutants on assimilation by species that play key roles in nutrient cycling and maintaining ecological efficiency could have community-wide consequences, including nutrient loss or changes in community structure (Goto and Wallace, 2010; Nixon and Oviatt, 1973; Odum, 1985).

Pollutants circulating in gut fluid may interact with critical components (e.g., digestive enzymes, solubilizing agents and epithelial surfaces) involved in hydrolysis, solubilization and absorption of ingested matter during a digestive cycle (Chen et al., 2002; Mayer et al., 1996; Seebaugh, 2010). These pre-assimilatory impacts on digestion would result from exposure prior to incorporation of a pollutant into consumer tissues (Campbell et al., 2005). Digestive toxicity may also result from chronic exposure and accumulation of pollutants in tissues critical to gut function. Post-assimilatory tissue damage could potentially interfere with cellular machinery involved in synthesis of enzymes and surfactants, impact regulation of peristalsis or influence transport of nutrients as well as pollutants across epithelial surfaces from the gut lumen and to the circulatory system. Toxicity associated with assimilated pollutants may also impact tissues (e.g., musculature) that affect behaviors associated with nutrient acquisition (Perez and Wallace, 2004; Wallace et al., 2000). For organisms feeding in polluted conditions, interactions between pre- and post-assimilatory toxicity may be important in determining the extent to which ingested pollutants impact digestion and ultimately influence the assimilation of nutrients as well as pollutants.

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In previous work with the grass shrimp <i>Palaemonetes pugio</i>, assimilation efficiencies (AE) for Cd and inorganic Hg were influenced by chronic exposure to impacted field conditions, indicating that pollutant-induced digestive toxicity (i.e., post-assimilatory toxicity) may influence the subsequent assimilation of dietary metal (<i>Seebaugh and Wallace, 2009</i>). Carbon assimilation did not vary for impacted shrimp, suggesting that digestive plasticity may play a role in maintaining adequate nutrient assimilation in the field. Grass shrimp are important in nutrient cycling within estuarine food webs through conversion of algae, detritus and invertebrate tissues into fecal matter and biomass (<i>Tashiro et al., 1994; Welsh, 1975</i>). Compensation for post-assimilatory impacts of ingested pollutants on digestion and nutrient assimilation by grass shrimp may be important in distributing essential nutrients as well as maintaining growth and adequate shrimp biomass within estuarine communities (<i>Nixon and Oviatt, 1973; Stout, 2009; Welsh, 1975</i>). For the present study, toxicological endpoints related to digestion (gut residence time [GRT], feces elimination rate [FER], digestive protease activities and gut pH) were investigated for grass shrimp collected along an impact gradient within the New York/New Jersey Harbor Estuary and fed meals prepared with fluorescent or near-infrared (NIR) markers. Shrimp were assessed for digestive toxicity in vivo using microfluorometric methods (GRT/FER and gut pH) or a novel NIR imaging technique (protease activities).

2. Materials and methods

2.1. Field sampling

Impacts of chronic field exposure on digestive physiology were assessed for adult grass shrimp (∼3 cm in length), collected from three differentially impacted sites surrounding Staten Island, New York, USA and maintained for ∼3 d as described by <i>Seebaugh and Wallace (2009)</i>. Great Kills Harbor (GK) is located along the southeastern shore of Staten Island, is flushed with cleaner waters from Raritan Bay and served as the reference site for this study (Fig. 1). Main Creek (MA) and Neck Creek (NC) are located within the heavily industrialized Arthur Kill (AK) complex, which separates Staten Island from New Jersey and links Newark Bay and Raritan Bay. Organisms within this network of waterways may be impacted by discharges from industry, byproducts of petroleum processing, combined sewer overflows and landfill leachate contamination (<i>Crawford et al., 1995; Gillis et al., 1993; Gunster et al., 1993</i>) (Fig. 1). Sediment metal concentrations at these sites vary considerably (<i>Goto, 2009</i>). GRT and FER analyses were performed using the same subsample of shrimp from each collection site. Protease activity and gut pH analyses were each conducted on subsamples that were not used for other digestive toxicity assays. MA grass shrimp were not available for gut pH analysis.

2.2. Experimental meal preparation

Meals used for GRT/FER, protease activity and gut pH analyses were prepared by embedding task-specific fluorescent or NIR markers in a gelatin–oligochoaete tissue matrix readily consumed by shrimp in previous work (<i>Seebaugh and Wallace, 2009</i>). Oligochaetes <i>Tubifex tubifex</i> were obtained from a commercial supplier (Newman’s Fish Foods, Hackensack, NJ, USA) rinsed in clean seawater (2.5 ppt) and homogenized in NANOpure deionized water (Thermo Scientific Barnstead, Dubuque, IA, USA) (0.66 g worm tissue ml⁻¹) using a Polytron homogenizer (Kinematica, Lucerne, Switzerland). Meals used to estimate GRT were prepared using 0.1 ml of 0.5 μm diameter Fluoresbrite microspheres (Polysciences, Warrington, PA, USA), combined with a 0.9 ml portion of concentrated diatoms <i>Thalassiosira weissflogii</i> (CCMP 1336: ∼3.41 × 10⁶ cells ml⁻¹). 1 ml oligochaete homogenate, 0.33 ml cod liver oil to enhance palatability and 0.47 g gelatin crystals (<i>Nagano and Decamp, 2004; Seebaugh and Wallace, 2009</i>). For protease activity analysis, meal mixtures were prepared with oligochaete homogenate and gelatin in the same proportions as for GRT meals, with the remaining volume consisting of NANOpure deionized water (pH 7.8) and IRDye 800RS casein protease substrate in 50 mM Tris–HCl (preserved with 0.01% sodium azide) (Li-Cor, Lincoln, NE, USA). Hydrolysis of casein substrate releases IRDye-labeled peptides detectable by an NIR imaging system. Increases in NIR signals are proportional to the amount of casein hydrolyzed when substrate is supplied in excess. Individual meals contained ∼0.003 nmol IRDye and ∼0.012 μg casein. Meals used to estimate gut pH were prepared by suspending 2.5 mg Zymosan A BioParticles fluorescent conjugate (Invitrogen, Carlsbad, CA, USA) in 100 μl of a matrix prepared by combining 125 μl worm homogenate, 166 μl NANOpure deionized water and 0.059 g gelatin. Zymosan A consists of freeze-dried yeast <i>Sacharomyces cerevisiae</i> covalently labeled with fluorescein and has been used to estimate gut pH in deposit-feeding polychaetes based on differential sensitivities of fluorescein emission intensities to pH at specific excitation wavelengths (isosbestic at 458 nm and pH-dependent at 496 nm) (<i>Ahrens and Lopez, 2001; Molecular Probes, 2010</i>). Each meal contained ∼0.15 mg Zymosan A, which was sufficient to generate usable signals within the proventriculus. Diatoms and cod liver oil were not included in IRDye or fluorescein meals to eliminate potential background signals from these components. Fluorescent/NIR meal mixtures were sealed in a microcentrifuge tubes, warmed with tap water and vortexed to uniform consistency. Individual (6 μl) meals were dispensed onto Nucleopore filters (Whatman, Florham Park, NJ, USA) and stored frozen (∼−20 °C) −2 h prior to feeding experiments.

2.3. GRT/FER

Following clearance of gut contents, ∼15 shrimp were transferred to aquaria containing clean, filtered seawater (1.0 μm filter, 10 ppt, 18–19 °C) and allowed to feed on Fluoresbrite microsphere-labeled meals for 30 min. Shrimp were then rinsed with seawater.
and the proventriculus of individuals inspected using a dissecting microscope to determine whether or not labeled food was consumed. Shrimp that consumed meals (n = 10–11) were transferred to individual defection chambers housed within 381 aquarium containing clean, aerated seawater and allowed to feed ad libitum on commercial fish food. Fecal strands were collected from individual shrimp on paper filters for 1.5 h after feeding and every 30 min thereafter for up to 13 h. Filters were air-dried and scanned for microspheres using an Axio Observer.Z1 inverted microscope (Zeiss Microimaging, Jena, Germany) equipped with a fluorescent light source. Median minimum GRT for individual shrimp was estimated as time between introduction of fluorescent meals and first detection of microspheres in feces, to the nearest 30 min. Comparison of median values was necessary as some individual GK and MA shrimp did not produce feces during the 13 h assay. To estimate FER, lengths of dried fecal strands produced by shrimp used for GRT analysis (n = 7–11) were measured using a dissecting microscope (Olympus SZ40, Tokyo, Japan) equipped with a Moticam 1000 digital camera and Images Plus 2.0 (Motic, Hong Kong, China). Software measurement tools were calibrated with a 1.5 mm diameter circle. As a quality control check, one fecal strand per filter was measured three times and individual measurements were within 2% of mean length. Mean FER was calculated for individual shrimp for 2 h following minimum GRT.

2.4. Protease activities

After clearing their guts, ~15 shrimp were transferred to clean, filtered seawater (1.0 μm, 10 ppt, 18–19 °C) and allowed to feed on IRDye-labeled casein meals for 14 min. Shrimp were then immobilized, dorsal-side-up, within 30 mm diameter glass tubes (0.6 mm wall-thickness; 5.3 mm inner diameter) cut from Pasteur pipettes and mounted to 100 mm diameter glass Petri dishes with cyanoacrylate adhesive. Shrimp that consumed meals (n = 6–9) were scanned every 90 s with an Odyssey infrared imaging system (Li-Cor, Lincoln, NE, USA) (800 nm channel; 3.9 nm focus offset; 169 μm resolution; 10 ppt seawater in Petri dishes; 18–19 °C within the scanning chamber) beginning at time (t) = 20 min from the introduction of IRDye-labeled meals. Whole shrimp were scanned during assays and NIR signals were only detectable within the gut. Background signals from unlabeled shrimp tissues were characteristic of NIR imaging (i.e., negligible). Casein hydrolysis within the proventriculus and hepatopancreas of individual shrimp was measured as the increase in integrated intensity over time, relative to t = 20 min. Shrimp that repositioned themselves during scanning could be identified through drastic changes in integrated intensity or visual inspection and were excluded from further analysis. A regression was fit to the linear portion of the curve (i.e., before substrate was exhausted), representing the mean increase in integrated intensity. Corresponding slopes were used to compare rates of casein hydrolysis for shrimp from the study sites.

2.5. Gut pH

Following clearance of gut contents, ~15 shrimp were transferred to a 9.51 aquarium containing clean, filtered seawater (1.0 μm, 10 ppt, 18–19 °C). One fluorescein meal was administered at a time. Shrimp that acquired a meal (n = 4–8) were isolated and allowed to feed for ~5 min, which was sufficient time for ingestion, and then immobilized within 30 mm diameter glass tubes mounted to 20 mm × 40 mm chamber slides containing clean seawater (10 ppt). pH within the lumen of the anterior and posterior regions of the cardiac chamber of the proventriculus (~100 μm from the dorsal wall) was estimated using the 496 nm:458 nm intensity ratio (emissions at 530 ± 25 nm) method of Ahrens and Lopez (2001). Shrimp were scanned with a Leica DM IRE2 (inverted) or Leica DM RXA2 (upright) microscope (10× objective) attached to a Leica SP2 confocal microscope equipped with an argon/krypton laser (Leica Microsystems, Wetzlar, Germany). Fluorescent images for each excitation wavelength were captured and analyzed using Leica Confocal Software (LCS). Peristaltic contractions of the proventriculus typically subsided within 2–3 min after shrimp were removed from the feeding aquarium and did not interfere with image capture. The mean baseline correction feature of LCS was used to correct for background fluorescence and was sufficient to correct for minimal autofluorescence generated within the proventriculus. Intensity ratios within the proventriculus were calibrated to standards prepared by adding 15 μl reconstituted Zymosan A (2.5 mg in 600 μl 50 mM Tris–HCl; 0.01% sodium azide) to 200 μl of 100 mM MES (pK_a = 6.16), MOPS (pK_a = 7.28) or HEPES (pK_a = 7.55) buffers dissolved in NANOPure deionized water (Ahrens and Lopez, 2001). Buffers were adjusted over the range of pH 4.5–8.3 with 0.1 N HCl or 0.1 N NaOH. Calibration standards were drawn into glass capillary tubes and images acquired and analyzed as described for live shrimp. Borosilicate glassware was used for preparation of calibration standards to minimize effects of glass composition on pH. Intensity ratios obtained for the pH standards were regressed onto pH using a third-degree polynomial fit (Ahrens and Lopez, 2001). Standard calibration plots were generated for each set of analyses to control for fluctuations in instrumentation.

2.6. Statistical analyses

Effects of field site on median minimum GRT in shrimp were tested using Kruskal–Wallis analysis of variance (K–W ANOVA) (Zar, 1999). Curves showing percentages of individuals with fluorescent microspheres in feces over time were compared using the Mantel–Cox test (Altman, 1991). Linear trends between treatments and median GRT were analyzed with the logrank test for trend. Normality of log_{10} transformed FER data was tested with Shapiro–Wilk’s W test and homoscedasticity tested using Levene’s test. Effects of field site on FER were analyzed using one-way ANOVA (Sokal and Rohlf, 1995). Homogeneity of IRDye-labeled food ingestion (integrated intensity at t = 20 min) was tested using ANOVA, followed by the Tukey–Kramer multiple comparisons test. Casein hydrolysis rates were compared through unplanned testing of regression coefficients (slopes: Tukey–Kramer method) (Sokal and Rohlf, 1995). Correlations between casein hydrolysis rates and initial NIR signals were analyzed using Pearson product–moment correlation (r) or Spearman rank correlation for non-normal data. Gut pH data were converted to free H^+ concentrations, tested for normality and analyzed for differences among study sites as well as variability within the proventriculus using the Mann–Whitney U test (Murphy, 1981). Gut pH data are reported as –log_{10}[mean [H^+]], which can result in asymmetrical standard errors resulting from transformation following analyses for study sites effects (Hu et al., 2007). After testing data for normality, r was used to evaluate relationships among Cd, Hg and carbon AE by field-collected shrimp reported in related work and digestive physiology in the present study (Seebaugh and Wallace, 2009). pH data were not included in correlation analyses since data were available for only two study sites. Analyses were conducted using STATISTICA 7.1 (Statsoft, Tulsa, OK, USA), GraphPad Instat 3.10 and GraphPad Prism 5.03 (GraphPad, La Jolla, CA, USA).

3. Results

3.1. GRT/FER

Shrimp typically consumed fish food throughout the course of the GRT assay and fluorescent microspheres were easily detectable
in feces (Fig. 2). Although individual markers could not be identified within the proventriculus or hepatopancreatic tubules of live shrimp due to interference from surrounding tissues, fluorescence within these organs could be easily distinguished from background (scans of abdominal segments; images not shown) following ingestion. Median minimum GRT did not vary significantly for shrimp from the Staten Island study sites (Fig. 3). Analysis of percentages of shrimp with microspheres in fecal strands over time did not reveal variability among sites or a linear trend between impact gradient and GRT (Fig. 4). FER did not vary for shrimp from the study sites (Fig. 5).

3.2. Digestive protease activities

Casein hydrolysis rates for shrimp were approximately linear from the time between initial scan and substrate exhaustion (Fig. 6). Exhaustion was not reached for NC shrimp and regression lines for all sites were plotted from \( t = 20 \) min to \( 30.5 \) min, the time point that GK and MA shrimp reached plateaus in enzyme activity (Fig. 7). Shrimp did not exhibit homogeneity of IRDye-labeled meal ingestion (Table 1). Shrimp collected from MA and NC exhibited a marked decrease in digestive protease activities relative to GK shrimp (Fig. 7). Although rates of casein hydrolysis were positively correlated with initial IRDye signal in shrimp from the study sites, hydrolysis in GK shrimp was \( \sim 3.14 \times \) the rate for MA shrimp, suggesting greater than linear enzyme activity with respect to ingestion \( (\sim 2.09 \times) \) (Table 1 and Fig. 7). Protease activity in GK shrimp was \( \sim 1.46 \times \) greater than would be expected had activity been proportional to the difference in initial IRDye signal observed for NC shrimp.
Table 1
Effects of chronic field exposure on initial IRDye signal in grass shrimp *P. pugio*. Collection sites included Great Kills Harbor (GK), Main Creek (MA) and Neck Creek (NC). Initial IRDye signal is integrated intensity at t = 20 min from the introduction of IRDye-labeled casein meals. Correlations between casein hydrolysis rate and initial IRDye signal were analyzed for individual treatments and treatment groups using Pearson product-moment (r, except where indicated). Data that did not fit a normal distribution were analyzed using Spearman rank correlation (rₛ).

<table>
<thead>
<tr>
<th>Study site or dietary treatment (n)</th>
<th>Initial IRDye signal</th>
<th>Casein hydrolysis rate vs. initial IRDye signal (r or rₛ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GK [9]</td>
<td>246.6 ± 31.5</td>
<td>-0.0824 (rₛ; ns)</td>
</tr>
<tr>
<td>MA [6]</td>
<td>164.2 ± 29.2</td>
<td>0.0003 (ns)</td>
</tr>
<tr>
<td>NC [6]</td>
<td>43.2 ± 7.1</td>
<td>0.5244 (ns)</td>
</tr>
<tr>
<td>All study sites</td>
<td>164.9 ± 24.3</td>
<td>0.6143 (rₛ; p &lt; 0.05)</td>
</tr>
</tbody>
</table>

Ingested IRDye ANOVA: p < 0.05 (GK = MA > NC*)

* or rₛ: not significant.

* Multiple comparisons conducted using Tukey–Kramer multiple comparisons test.

3.4. Relationships between AE and digestive physiology

Significant correlations among Cd, Hg and carbon AE were not established for shrimp from three Staten Island study sites (Table 2) (Seebaugh and Wallace, 2009). Cd assimilation was positively correlated with GRT. A negative relationship between Cd assimilation and digestive protease activities is also suggested (r = 0.059). Correlations between Hg or carbon AE and digestive physiology were not apparent. GRT and protease activities were negatively correlated for field-collected shrimp (Table 2).

4. Discussion

In the present study, grass shrimp collected across an impact gradient were evaluated for digestive toxicity after clearing their guts for ~3 d and then feeding on meals containing fluorescent or NIR markers. Previous work indicates that the assimilation of elements by this species is complete by 48 h following ingestion (Seebaugh and Wallace, 2009). Loss of elements beyond this point is related to physiological turnover and possibly loss of metal stored by resorptive (R) cells of the hepatopancreas (Al-Mohanna and Nott, 1987; Wang and Fisher, 1999). Although it is presumed that any impacts of previous dietary exposure on digestion are related to post-assimilatory toxicity, it is possible that residual metal or organic pollutants from a previous digestive cycle (i.e., pollutants ingested in the field just prior to collection) may remain in the gut.
lumen and are potentially available to interact with enzymes and other components of gut fluid (Icely and Nott, 1992). Estimates for other decapods (crabs and penaeid shrimp) indicate that ingested elements clear the hepatopancreas 12–24 h after feeding (Icely and Nott, 1992). Blister (B) cell extrusion and increased mitotic activity of embryonic (E) cells take place during the excretory phase of digestion (24–48 h following ingestion) and the hepatopancreas is typically inactive by 48 h (Al-Mohanna and Nott, 1986; Hopkins and Nott, 1980). Considering patterns of radioisotope depuration observed for grass shrimp, it seems probable that a clearance time of ~3 d would be sufficient to remove ingested pollutants from the gut prior to assays for digestive toxicity (Seebaugh and Wallace, 2009).

GRT was estimated following ingestion of meals containing 0.5 μm diameter microspheres, which are small enough to pass through setal screens within the cardiac chamber of the proventriculus as well as the gland filter along the floor of the pyloric chamber (Dall and Moriarty, 1983; Icely and Nott, 1992). Materials that pass through the gland filter then enter the hepatopancreas. Based on the size of fluorescent markers and fluorescence detected within the hepatopancreas following ingestion, it is presumed that GRT values in this study represent the influence of hepatopancreatic processes that may ultimately determine timing of the decapod digestive cycle (Al-Mohanna and Nott, 1987). Hoyt et al. (2000) observed GRT from 0.5 to 2 h for P. pugio following ingestion of 2–4 μm diameter latex beads, which could not pass from the proventriculus to the hepatopancreas. GRT values for ingested particles >1 μm may represent ‘bulk’ transit times and not have any direct bearing on the actual time required for digestion (Al-Mohanna and Nott, 1987; Hoyt et al., 2000; Icely and Nott, 1992).

From the results of the present study, it does not appear that chronic field exposure exerted significant post-assimilatory effects on the epithelium or musculature of the hepatopancreas or perhaps nervous tissues involved in regulation of gut peristalsis (Maynard and Dando, 1974; Meiss and Norman, 1977; Shuranova et al., 2006).

In related work, FER was impacted by a pulse of ingested Cd which may have been related to pre-assimilatory effects on midgut epithelial secretions (peritrophic membranes or mucopolysaccharides) (Forster, 1953; Seebaugh, 2010). Any post-assimilatory effects of previous exposure on midgut secretions, gut musculature or abdominal ganglia in shrimp from impacted field sites may not have reached the level of severity necessary to impact feces packaging, compaction and transport (Lovett and Felder, 1990; Shuranova et al., 2006). For shrimp subjected to chronic exposure, acclimatisation of midgut and hindgut functions may be necessary to sustain anal drinking and generate peristaltic/antiperistaltic contractions to maintain hydraulic pressure necessary for hepatopancreatic tubule expansion (Lovett and Felder, 1990). Prolonged exposure may also require that shrimp compensate for pre-assimilatory impacts of circulating pollutants on midgut secretions in order to maintain intestinal function over successive digestive cycles. In other decapods (penaeid shrimp), feces production was not related to timing of the digestive cycle (Al-Mohanna and Nott, 1986).

Extracellular digestive enzymes, including carbohydrases, proteases and lipases, have been characterized for a wide variety of decapod taxa (see reviews by Gibson and Barker, 1979; van Weel, 1970). Specific enzymes are typically identified and activities characterized in vitro using gut (e.g., hepatopancreas) homogenates or gut fluid extracts (De La Ruelle et al., 1992; Glass and Stark, 1994; Muhlia-Almazán and García-Carreño, 2002). Recently, few studies have characterized enzymes using non-invasive methods (e.g., extracted from fecal strands) and very little is known about enzyme activities within the confines of a functional proventriculus or hepatopancreas (Campbell et al., 2005; Córdova-Muruetu et al., 2003).

Extracellular digestive protease activities were characterized for grass shrimp in vivo, in real time, using non-invasive methods. Since the time required to feed and prepare shrimp for NIR scanning was 20 min, initial casein hydrolysis rates (from r = 0 to 20 min) could not be monitored for linearity. Ahrens and Lopez (2001) reported that protease activities in guts of deposit-feeding polychaetes monitored in vivo were approximately linear for 5–10 min following ingestion until available substrate (gelatin-embedded casein) was hydrolyzed. Protease activities in shrimp reached saturation for two study sites within 30.5 min, indicating that casein substrate was exhausted. NIR imaging techniques in the present study required longer scan times (90 s) than methods utilizing fluorescently labeled casein (3.5 s), resulting in reduced resolution over the time scale axis (Ahrens and Lopez, 2001). NIR imaging does, however, allow for estimation of extracellular protease activities in several shrimp simultaneously with negligible background signal interference.

Functions of epithelial cell types that line decapod hepatopancreatic tubules have been the subject of significant controversy (particularly for B cells), however, it is widely recognized that fibrillar (F) cells synthesize and secrete enzymes that catalyze extracellular hydrolysis of macromolecules in the proventriculus and lumen of the hepatopancreas (Al-Mohanna et al., 1985; Al-Mohanna and Nott, 1986; Gibson and Barker, 1979; Vogt, 1993; Vogt et al., 1989). Vogt et al. (1989) determined that Astacus protease in crayfish is synthesized in F cells, transported to brush border surfaces and exocytosed into the hepatopancreatic tubules. Enzyme activity is then accumulated and stored in the anterior region of the cardiac chamber of the proventriculus for the next feeding cycle (Vogt et al., 1989). Extracellular digestive enzyme activities in decapods can be impacted by molt cycle and feeding condition prior to analysis in vitro (Muhlia-Almazán and García-Carreño, 2002; Van Wormhoudt, 1974). De La Ruelle et al. (1992) reported that metal exposure reduced activity of aminopeptidase isolated from crayfish.

Protease activities in shrimp from the AK complex were reduced considerably, relative to reference shrimp. Casein hydrolysis rates were also disproportionately lower for AK shrimp with respect to initial IRDye signal, suggesting that any impacts of reduced food ingestion may be compounded by impaired digestive enzyme function. Since field-collected shrimp were allowed to clear their guts for ~3 d prior to analysis, variability in protease activities may be related to post-assimilatory impacts on F cell machinery (ribosomes, endoplasmic reticulum, Golgi apparatus, vesicle transport or mechanisms of exocytosis) involved in synthesis and discharge of digestive enzymes (Vogt et al., 1989). Shrimp from the study sites investigated in the present study also exhibited variability in 14C-labeled meal ingestion in related work (GK × NC > MA; unpublished analysis of data related to Seebaugh and Wallace, 2009). Interestingly, homogeneity of radiolabeled amphipod ingestion was observed for shrimp from study sites used in metal AE analysis, suggesting that dietary considerations (e.g., palatability of field-available prey vs. prepared meals) may be important in determining effects of dietary pollutant exposure on ingestion and digestive enzyme activities (unpublished analysis of data from Seebaugh and Wallace, 2009). Post-assimilatory impacts on tissues and processes related to ingestion (e.g., mandibular mastication in carideans with reduced gastric armature) may also be important and require additional study (Felgengauer and Abele, 1983; Sousa and Petriella, 2006). Perez and Wallace (2004) reported that impaired prey capture by AK shrimp was related to behavioral efficiency, suggesting that effects of pollutant exposure on the assimilation of elements by shrimp in the field may involve interactions between nutrient acquisition, ingestion and gut physiology.

Gut pH for grass shrimp was estimated in vivo, using a non-invasive technique. Although direct measurements of gut juice with microelectrodes may provide for greater accuracy in estimating pH (e.g., in polychaetes), microfluorometric methods allow for
characterization of gut chemistry without risk of gut wall perforation (Ahrens and Lopez, 2001). Gut juice pH (typically determined in vitro) is weakly acidic in many decapods, but neutral in some species (e.g., fiddler crabs) (Gibson and Barker, 1979; Johnston and Yellowlees, 1998; van Weel, 1970). Mechanisms for regulation of gut pH in decapods are poorly understood (Gibson and Barker, 1979). Once digestive enzymes are stored in active form within the cardiac proventriculus, the possibility that enzyme vacuoles released to the lumen of the hepatopancreas by F cells contain components that influence extracellular pH requires additional study (Vogt et al., 1989; Zwilling and Neurath, 1981). Dall and Moriarty (1983) suggested that factors regulating gut pH in decapods may be produced in anterior midgut caeca. The structures have not yet been described for palaeomonids (Sousa and Petrella, 2006).

Bioavailability and assimilation of ingested elements depends upon the extent to which they are desorbed from ligands in food, sediment particles or gut fluid, which may be related to internal pH (Griscom et al., 2002a). Changes in pH within the decapod hepatopancreas could potentially influence extracellular digestive processes, including activities of digestive enzymes. Divakaran and Ostrowski (1998) found that trypsin activity was not influenced by changes in pH (5.5–8.0) in hepatopancreatic extracts from peneid shrimp. In many cases, digestive enzymes appear to have pH optima, which can vary considerably among species (van Weel, 1970 and references therein; Glass and Stark, 1994). pH within the anterior and posterior regions of the cardiac chamber did not vary for shrimp from individual collection sites and peristalsis subdivided shortly after ingestion, indicating that mixing of food, enzymes and other components of gut fluid was rapid and complete (King and Alexander, 1994; Powell, 1974; Vogt et al., 1989). Storage of enzymes in preparation for the next feeding cycle, thorough mixing of gut fluid and well-defined timing of events within the hepatopancreas are consistent with a batch reactor model for extracellular digestion (Al-Mohanna and Nott, 1986; Penry and Jumars, 1986; Vogt, 1993).

Although GRT did not vary significantly for shrimp collected along an impact gradient, this digestive parameter was positively correlated with Cd AE, but not carbon or Hg AE, reported previously (Seebaugh and Wallace, 2009). Extracellular digestive protease activities were negatively correlated with GRT and a negative relationship between casein hydrolysis rate and Cd AE was indicated for Staten Island shrimp. Digestive enzyme synthesis and activities in decapods can be influenced by diet composition (e.g., protein content) (Le Moullac et al., 1996; Rodriguez et al., 1994). Interactions between post-assimilatory impacts of field exposure on F cell machinery and the nutritional content of food organisms may, therefore, be important in determining enzyme availability and assimilation (Campbell et al., 2005). Assuming that shrimp inhabiting the AK complex do not possess the enzyme plasticity required to compensate for effects of diet or ingested pollutants, increased contact time between nutrients, gut fluid and absorptive surfaces of the gut epithelium may be necessary to maintain nutrient assimilation (Bock and Mayer, 1999; Relyea and Auld, 2004). AK shrimp assimilated nearly identical percentages of carbon from radiolabeled meals as shrimp collected from GK, suggesting that digestive plasticity (i.e., changes in hepatopancreatic processes that influence GRT) may be important in the assimilation of essential elements (e.g., organic carbon) (Al-Mohanna and Nott, 1986, 1987; Seebaugh and Wallace, 2009). For impacted shrimp populations, a consequence of longer GRT may be enhanced assimilation of non-essential elements, including pollutants, such as Cd. Lack of correspondence between GRT and Hg AE by field-collected shrimp may be related to metal-specific transport pathways across gut epithelial surfaces that may not be influenced by transit time (Zhang and Wang, 2006).

It is not known whether or not grass shrimp inhabiting the AK complex (MM and NC) or sites flushed with cleaner waters from Raritan Bay (GK) represent the same source populations (Fig. 1). Compensation for pollutant-impaired digestive physiology (variable GRT to offset reduced enzyme function) among impacted shrimp populations may represent a phenotypic response to differential exposure to contaminant (e.g., metal and organic) loads in the field or may have an underlying genetic component (Janssens et al., 2009; Klersk and Weis, 1987). Rapid evolution and subsequent loss of genetic resistance was reported for benthic oligochaetes exposed to high concentrations of metals in sediments, which were later removed during site remediation (Klersk and Levinton, 1989; Levinton et al., 2003). Adaptation of oligochaetes to metal–impacted conditions may be attributable to genetic control of metal detoxification (Klersk and Bartholomew, 1991; Martinez and Levinton, 1996). The hepatopancreas plays a key role in regulation and storage of divalent cations as well as metal detoxification in decapods (Al-Mohanna and Nott, 1987; Chavez-Crooker et al., 2003; Hopkins and Nott, 1979). Adaptation of digestive physiology to dietary pollutant exposure may be manifested as variation in grass shrimp gut function over successive generations. Transplant and selection studies would be required to determine whether population differences in digestive function are related to phenotypic or genotypic plasticity. Gene flow may also be important in determining population responses to changes in environmental stress (e.g., metal pollutants) (Mackie et al., 2009). The possibility that migration and gene flow between AK and Raritan Bay shrimp populations may be restricted by long water residence times resulting from concurrent tidal surges from Newark Bay and Raritan Bay also requires investigation (Fig. 1) (Mathews, 2007; Oey et al., 1985).

5. Conclusion

In addition to routes of environmental pollutant exposure (e.g., dissolved vs. diet), it is important to consider the mode in which tissues are exposed once a pollutant is internalized (Campbell et al., 2005; Griscom et al., 2002b; Hook and Fisher, 2001). For dietary pollutants, toxicological impacts may vary depending on whether exposure is through circulating gut fluid or follows assimilation by tissues. In the present study, we have demonstrated post-assimilatory impacts on digestion in grass shrimp collected along an impact gradient. These effects are presumed to be related to incorporation of metal or organic pollutants into cells of the hepatopancreatic epithelium that synthesize or secrete extracellular digestive enzymes. Impacts of field exposure on shrimp digestion may also have resulted from interactions between ingested pollutants. Previous studies have demonstrated digestive plasticity (enzyme activities and surfactant secretion) in benthic invertebrates in response to variation in diet (Bock and Mayer, 1999). Digestive plasticity may also be important in maintaining nutrient assimilation in spite of post-assimilatory impacts of dietary pollutants on consumer digestion.

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