The Ecology of Contaminant Exposure in Uca pugnax (Smith):

Physiological, Reproductive, and Behavioral Sublethal Effects

by

DALE MARIE HAROSKI

A Dissertation submitted to the

Graduate School-New Brunswick

Rutgers, The State University of New Jersey

in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Graduate Program in Ecology, Evolution and Natural Resources

written under the direction of

Dr. Gary L. Taghon

and approved by

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New Brunswick, New Jersey

[October, 2008]

ABSTRACT OF THE DISSERTATION

The Ecology of Contaminant Exposure in Uca pugnax (Smith): Physiological, Reproductive, and Behavioral Sublethal Effects

By DALE MARIE HAROSKI

Dissertation Director:
Dr. Gary L. Taghon

Physiological, reproductive, and behavioral studies were conducted to determine the sublethal effects of contaminant exposure on Uca pugnax in two New Jersey marshes. Total lipids and lipid classes were examined in male crabs to examine the relationship between contaminant exposure and lipid variability. Lipids were examined seasonally, during various molt cycle stages, and after a 28-day reciprocal transplant exposure study. Seasonally, lipids were significantly different between months and although differences in total lipids and classes occurred between sites, results were not statistically different. During the molt cycle, total lipids and lipid classes differed between sites and R-categories. For the exposure study, total lipids were similar between sites and treatments while phospholipids increased with exposure to clean sediment. It is not clear whether contaminants influenced lipid composition but natural lipid fluctuations occur seasonally and during the molt cycle.

Fecundity and larval morphology were examined to determine the effects of contaminant exposure on reproductive endpoints of field-collected organisms. Mean fecundity was higher at the contaminated site but crabs were slightly larger at this site

which may have contributed to fecundity differences. Abnormal larvae were observed at both sites although the proportion of abnormal larvae was higher at the contaminated site. Hypopigmented eyes and hydropsy were the most common abnormalities at the contaminated site while hydropsy was the most common at the non-contaminated site. These morphological abnormalities were unspecific pathologies likely manifested as a general response to pesticides and metals.

Oophagy was quantitatively documented for U. pugnax from both sites in a site comparison study and a feeding study. For the site comparison study, egg ingestion was typically greater at the non-contaminated site, although statistically the sites did not differ. Similarities in oophagy between sites indicate that contaminants do not appear to influence oophagy. For the feeding study, crabs from both sites still ingested eggs even when food pellets were offered although pellet ingestion was higher than egg ingestion for both sites. Egg ingestion in the site comparison study was similar to egg ingestion in the feeding study. This similarity in egg ingestion between the two studies indicates that the presence of food does not decrease or stop egg ingestion.

Acknowledgments

I wish to thank the U.S. Environmental Protection Agency's Environmental Response Team for funding this research and for supplying the ideas and initial data behind this study. My advisor, Dr. Gary L. Taghon, is deserving of my gratitude for his patience, advice, and real-world perspective. Additional thanks to my committee members for their insights and improvements to my manuscripts. Special thanks to my field sampling partner, Jennifer Badner, for putting up with heat, humidity, mud, and insidious swarms of greenhead flies. Additional thanks to the staff of Lockheed Martin's Response, Engineering and Analytical Contract for their assistance with statistics, chemical analyses, and sample processing.

I offer my deepest thanks and love to my parents, family members, and friends for their unwavering support, love, and belief in me. Much love and thanks to Michael and Zoe Perry for tolerating my moods, work schedule, and dissertation-related distractedness. There is a special place in my heart for Abbey and Louis who provided company, welcomed distractions, and unconditional love. Finally, endless gratitude and vibes to my retreaters and friends at PhinisheD. This never would have happened without their cheerleading, words of wisdom, humor, and friendship.

Table of Contents

List of Tables

List of Figures

Introduction to the Dissertation

The research described in this dissertation investigates basic and applied aspects of the ecology of contaminant exposure in the Atlantic marsh fiddler crab, Uca pugnax (Smith). The inspiration for this study partially derives from a previous United States Environmental Protection Agency (U.S. EPA) study examining total lipids in contaminant-exposed (primarily Hg and PCBs) U. pugnax in a Georgia salt marsh. As part of a Superfund site investigation, relationships between contaminant body burden, sediment concentrations, and total lipids were discerned. Overall, there was a 36 to 48% reduction in whole body lipid content at the most highly contaminated areas.

These results instilled a desire in U.S. EPA to examine this relationship more closely for several reasons. First, a single data set collected in a short time period may not reveal the true nature of the lipid-contaminant relationship. Second, total lipid analyses may mask fluctuations occurring within individual lipid classes or proportions of lipids (Bergen 1999). Third, the natural variations of lipids in fiddler crabs are not well documented, and therefore the degree to which lipids respond to contaminants is difficult to interpret. Although the initial goal of this study was to study the relationship between contaminants and lipids in U. pugnax, I expanded the study to include an examination of contaminant effects on reproductive endpoints and behavior. Specifically, I examined sublethal effects on crab physiology, reproduction, and behavior by studying: 1) variation in lipid class composition in response to season, molt cycle and contaminants; 2) contaminant effects on fecundity and larval morphology; and 3) the roles of food and contaminants in filial cannibalism.

The first study examining lipid class variability attempts to answer the question of whether lipid classes in U. pugnax fluctuate predictably in response to contaminants and, if so, to determine their potential utility for biomonitoring. In addition to contaminant-driven responses and sensitivity, however, seasonal variation in lipid content in Brachyuran crabs may naturally result from lipid storage during the reproductive cycle (Pillay and Nair 1973), from changes during molting (Passano 1960), and through changes in food and temperature conditions (Leavitt et al. 1990, Parrish et al. 1998). For example, Pillay and Nair (1973) examined fluctuations in the ovaries and testis of U. annulipes, Portunus pelagicus, and Metapenaeus affinis. The authors found that total lipids fluctuate greatly in the ovaries during the reproductive cycle while biochemical changes in the testis are less pronounced as the testicular cycle is almost constant. Lipid levels are also known to fluctuate during chitin production and ecdysis (Passano 1960) as associated with the molt process. In an attempt to track seasonal lipid fluctuations, my study establishes the first seasonal lipid profile for U. pugnax and also considers lipid variability during the molting process. A 28-day reciprocal transplant exposure study offers additional insights into possible contaminant-driven lipid fluctuations.

The second study examines fecundity and larval morphology in contaminated and non-contaminated crabs. Mating in U. pugnax typically occurs bi-weekly and usually four or five days before a spring tide (Christy 1978). Prior to mating, females search for males and breeding burrows while males attempt to attract females by waving their large cheliped or perhaps via tactile stimulation (Christy 2007). Once mate choice has been decided, the female enters the burrow of the male where mating, ovulation, fertilization, egg extrusion occurs (Christy 2007). The female oviposits the eggs onto the pleopods of

the abdomen and remains in the enlarged terminal chamber of the plugged burrow for approximately two weeks until she emerges to release larvae on the extremely high spring tide for transport out of the estuary (Christy 2007). It is not known whether females ingest sediment while underground though it is hypothesized that feeding is limited (Christy and Salmon 1984)yet no observational data appears to exist to support these claims.

The newly hatched larvae typically develop in the costal ocean and molt into five zoeal stages before molting into megalopae (Bergey and Weis 2008). The postlarvae (megalopae) then return to the estuary on nocturnal flood tides where they settle and molt into adult crabs (Epifanio et al. 1988, Christy 2007)). The timing of the larval return to the estuary, as well as the timing of larval release, allows for predator avoidance during these critical life stages (Christy 2007).

Contaminants can impact fiddler crab reproduction in several ways and throughout several stages during the reproductive process. The ingestion of or direct contact with contaminated sediment by ovigerous females could impact both batch fecundity and developing eggs. Direct contact with contaminated water or sediment could impact newly hatched and settling larvae. Most work examining the effects of contaminants on reproduction focus on laboratory toxicity studies and not field-collected data. This study thus attempts to answer the following two questions: 1) do contaminants reduce fecundity in field-collected crabs (i.e., crabs that have not been exposed to pre-determined levels of contaminants in the laboratory); and 2) do field-collected crabs from the contaminated site exhibit abnormal larval morphology as typically manifested in laboratory water column exposures. While the identification of sublethal effects from

laboratory manipulations is clearly useful information, it is important to interpret these results against field-collected crabs to test the reality of laboratory-derived results. If the aforementioned sublethal effects on fecundity and larvae are exhibited, these results would be significant on both the individual and population levels, potentially affecting both fitness and population dynamics.

Finally, the goal of the third study was to quantify egg ingestion or oophagy in U. pugnax. This phenomenon has not been previously documented in the scientific literature but initially was observed in crabs collected from the contaminated site. As ovigerous U. pugnax primarily remain in their burrows while carrying eggs (Crane 1975), it is possible that oophagy results as a response to limited foraging capability. Egg ingestion may supplement nutrition and provide reproductive benefits for the current or future broods.

Whether or not ovigerous Ocypodids feed in the field appears to depend on clutch size and the species. Early work by Christy and Salmon (1984) suggests that Uca females who spend most of the incubation period underground feed little if at all yet no data is provided to support these claims. Essentially, there appears to be a trade-off between feeding and clutch size. For example, species that carry relatively large clutches and incubate in burrows, such as U. pugilator, must choose between feeding and strong selection pressures to remain underground thus protecting their clutch from stresses such as temperature and desiccation (Christy and Salmon 1984). In a species that produces many small broods, such as U. vocans, it is more advantageous to spend time actively feeding above ground (Christy and Salmon, 1984). My initial observations revealed that

U. pugnax (a large clutch species) clearly eat in the laboratory while carrying eggs but it was not clear why this behavior occurs or whether it was simply a laboratory artifact.

As oophagy has not previously been document for Ocypodids, the following three questions were addressed: 1) do contaminants in sediment cause oophagy; 2) does oophagy occur to provide a nutritional benefit to non-fed ovigerous females; and 3) does egg ingestion reduce fecundity in oophagous crabs? The answer to the third question will be relevant to researchers quantifying fecundity in laboratory-held crustaceans.

Often, single-species laboratory toxicity tests are used to determine the effects of contaminants on estuarine organisms (Hall and Giddings 2000). These tests yield information regarding potential toxic effects under controlled laboratory conditions but provide limited information about actual field responses to contaminants. By examining the effects of contaminants on fiddler crab physiology, reproduction and potentially on behavior in field-collected organisms, these studies provide a more holistic picture of contaminant exposure in U. pugnax.

CHAPTER ONE

Variation in lipid class composition in Uca pugnax (Smith) in response to season, molt cycle, and contaminants in two New Jersey marshes

ABSTRACT

Total lipids and lipid classes were examined in male Uca pugnax from a contaminated and non-contaminated site in New Jersey to examine the relationship between contaminant exposure and lipid variability. Lipids were examined seasonally, during various molt cycle stages, and after a 28-day reciprocal transplant exposure study. Three lipid classes (phospholipids (PL), free fatty acids (FFA) and triacylglycerols (TG)) comprised approximately 80 percent of total lipid content. Seasonally, total lipids were similar between sites but differed between months. For the three main lipids classes there were no statistically significant differences between sites but significant differences between months. These results suggest that lipids fluctuate seasonally and that total lipids mask variations occurring on the class level but it is not clear if contaminants affect lipid levels and, if so, to what level.

For the molt cycle study, total lipids and lipid classes were evaluated per R-category (length of regenerating limb bud/carapace width × 100). Lipids fluctuated during limb regeneration and mean total lipids were greater at the contaminated site for each R-category. Phospholipids were not significantly different between sites or R-categories while FFA were significantly different between R-categories but not between sites. TG was significantly different between sites and R-categories.

For the 28-day reciprocal transplant exposure study, total lipids were similar between all four treatments and sites. Crabs from the contaminated site showed increased

PL after exposure to clean sediment while mean FFA and TG were similar between treatments. Future studies should include lipid class data and utilize long-term data sets to provide the necessary temporal and/or biological context for the observed lipid fluctuations as it is not clear what impact contaminants had on lipids in this study.

INTRODUCTION

Early in the study of fish biology, a theory originated proposing that organic contaminant uptake was linked to total lipid content (Reinert 1969). Today it is virtually a paradigm that lipophilic organic contaminants bioaccumulate in lipids (Stow et al. 1997). This relationship has resulted in the calculation and expression of bioaccumulation factors on a per-gram lipid basis (i.e., lipid normalization) as most researchers generally accept that hydrophobic contaminants accumulate in lipids in aquatic organisms (Newman 1998, Bergen 1999). Today, altered lipid concentrations in aquatic organisms are often used as evidence of contaminant-driven sublethal effects.

Studies examining the effects of contaminants on lipids often compare lipid concentrations along a contaminant gradient and presume that differences between sites are contaminant-related without evaluating the effects of season, physiological state, or other variables. Also, many studies focus only on total lipid composition, which often masks fluctuations occurring in individual lipid classes or proportions of lipids (Bergen et al. 2001). To address these limitations, this study determined how lipid classes in male Atlantic marsh fiddler crab (Uca pugnax) responded to contaminants with respect to season and the physiological effects of molting. I evaluate the use of lipid class fluctuations in fiddler crabs as indicators of sublethal environmental stress in estuarine systems.

Fiddler crabs were chosen for this study because they are ubiquitous marsh organisms with a relatively sedentary lifestyle and intimate association with the sediment. As such, they are an important component of the trophic and community dynamics of coastal ecosystems. Studies have shown that fiddler crabs affect smooth cordgrass (Spartina alterniflora) production at different tidal heights, suggesting the existence of a facultative mutualism between the plants and crabs (Bertness 1985). On a community level, fiddler crabs are an abundant food resource for birds and other aquatic predators as they comprise the greatest invertebrate biomass in the salt marsh intertidal zones (Grimes et al. 1989). Ingestion of contaminated crabs by upper trophic level estuarine species can lead to transfer of contaminants through the food chain through bioaccumulation and biomagnification. Given this important role, sublethal effects of contaminants on fiddler crabs could influence other aspects of marsh function.

While contaminant-driven lipid fluctuations have been observed and studied in a number of different invertebrate and vertebrate aquatic organisms, limited studies examine this response in fiddler crabs. One three-month field study, examining lipid class response to a mosquito-control pesticide (fenthion), determined that changes in lipid composition in fiddler crab hepatopancreas correlated with high tissue fenthion concentration (McKenney et al. 1996). More specifically, phospholipids were consistently reduced after pesticide applications while the triacylglycerol:phospholipid ratio increased. Total lipids also decreased at two of the three study locations after a final fenthion application but it is not clear whether seasonal or physiological drivers were considered.

Other fiddler crab lipid studies examine natural lipid fluctuations in Uca spp. during maturation and reproductive cycles (Pillay and Nair 1973, Mourente et al. 1994). Pillay and Nair (1973) examined fluctuations in the ovaries and testis of U. annulipes, Portunus pelagicus, and Metapenaeus affinis. The authors found that total lipids fluctuate greatly in the ovaries during the reproductive cycle while biochemical changes in the testis are less pronounced as the testicular cycle is almost constant. Mourente et al. (1994) also observed changes in total lipids and lipid classes in ovaries of female U. tangeri where non-polar lipids tended to increase throughout maturation while total polar lipids in the ovaries only increase during the final stage of maturation.

This study examined seasonal changes in total lipids and lipid class composition in male Uca pugnax at a contaminated and non-contaminated site in New Jersey. Lipids were also examined during various molt cycle stages as lipid levels are known to fluctuate during chitin production and ecdysis (Passano 1960). To further examine the relationship between contaminant exposure and lipid class variability, a reciprocal transplant study was performed to determine whether lipids in contaminated and non-contaminated crabs would change after 28 days of exposure to non-contaminated and contaminated sediment.

RESEARCH LOCATIONS

Sheepshead Meadows (SHM)

The reference or "non-contaminated" site was Sheepshead Meadows (SHM) located within the Barnegat Bay-Little Egg Harbor estuarine system in southern New Jersey (39° 18' N, 74° 12' W). The SHM site is located on the southern tip of a salt marsh peninsula removed from any major industries or point contaminant sources. The system

is one of the least disturbed in the northeast United States (Psuty et al. 1993) and, partly as a result, was included in the National Oceanic and Atmospheric Administration (NOAA)-operated National Estuarine Research Reserve System on October 20, 1997 (Able et al. 1996). The estuary is classified as a highly eutrophic estuary based on NOAA's National Estuarine Eutrophication Assessment model (Kennish et al. 2007). The lower estuary is polyhaline with a salinity range of 23.6 to 34.5 at the nearby Rutgers University Marine Field Station (Able et al. 1992). At the nearby Schooners Creek, mean C is 45.7 mg/g, mean N is 4.48 mg/g (Horng and Taghon 1999) and C:N ratios range from 12.1 to 13.6 (G. Taghon, unpublished data). Seasonal water temperatures range from -2º C to 28º C (Able et al. 1992) with a tidal range of 1.1 m near the mouth of Great Bay (Martino and Able 2003). Some low levels of contaminants have been measured at near the site and mean metal levels (µg/g) were: Hg 0.19, Cu 43.8, Pb 73.2, Zn 141, Cd 2.1 (Weis et al. 2001a, Weis et al. 2001b).

Contaminated Site

Piles Creek (PC) (40° 36.5' N, 74° 13.6'W) in Linden, New Jersey is a tributary of the Arthur Kill, the navigational channel between New Jersey and Staten Island, New York. Piles Creek served as the "contaminated" site as the creek is surrounded by industrial sites such as petroleum refineries, oil storage tanks, a sewage treatment plant, and a major highway. The site has likely received contaminants from nearby industries and oil spills in the Arthur Kill (Bergey and Weis 2008). Site contaminants include metals, pesticides, polycyclic aromatic hydrocarbons (PAHs), and some polychlorinated biphenyls (PCBs). Mean metal levels (µg/g) have previously been measured for Hg 6.3, Cu 485, Pb 107, Zn 525, Cd 7.1 (Weis et al. 2001a, Weis et al. 2001b). Salinity

measurements at the site consistently ranged from 20 to 23 during the sampling period. Mean C at the site is 44.6 mg/g and N is 3.32 mg/g (Horng and Taghon 1999) while C:N range from 16.3 to 19.6 (G. Taghon, unpublished data).

MATERIALS AND METHODS

Crab Collection and Handling

Crabs were collected at low tide from the intertidal zone and marsh surface at both sites from April 2001 to October 2001. Crabs were removed from their burrows by gently inserting a trowel next to the burrow until the crab emerged for collection. Size and color differences were used to isolate U. pugnax from other Uca species at the site. Crabs were not collected after October as the colder weather drove the crabs to burrow deeply in the sediment, requiring extensive digging and marsh destruction for collection. Only male crabs were collected and analyzed for lipids because the reproductive state of female crabs could often not be determined upon physical inspection (Appendix C).

Immediately upon arrival to the laboratory, crabs were rinsed to remove extraneous sediment and placed into plastic storage containers (approximately 42.5 × 31.5 × 15 cm; 17 L) and arranged on top of laboratory benches. Room temperature (24º C) 0.5 µm filtered sea water obtained from Corson's Inlet, New Jersey (a non-contaminated site) was then added to each container, which were propped up at an angle, until the water covered approximately 75% bottom. Sea water added to PC containers was diluted with deionized water to a salinity of 21 to approximate site salinity conditions while water for SHM crabs was unadjusted at salinity 33. An air stone was submerged in the deepest part of the water, approximately 3 to 4 cm deep. Natural lighting was provided via a window in the laboratory (approximately 14:10 light:dark period). Crabs

being held for tissue and lipid class analyses were held under these conditions without food for 24 h to allow for the depuration of sediment from the gut (see Appendix B: Depuration Study Results).

Tissue and Sediment Analyses

Sediment and tissue data were collected from June to November in 2000 and in April, May, and July in 2001. Tissue data was collected in April and July in 2001. As concentrations of contaminants were found to not vary temporally in 2000, it was determined that three months of sediment data and two months of tissue data in 2001 were adequate for characterizing the sediment and tissue. Sediment was collected from the marsh surface and sampling locations were co-located with the fiddler crab collection area. Sediment was collected with a stainless steel trowel, homogenized in-situ, and placed into 8 oz. glass containers for transport to the laboratory. Surficial sediment (approximately zero to 15 cm) was collected and analyzed as fiddler crabs feed primarily on the marsh surface.

For tissue analyses (both for lipid class and body burden analyses), entire crabs were homogenized using a Waring blender with dry ice added to facilitate a thorough homogenization. Whole crab bodies were analyzed, as opposed to the hepatopancreas or other organs, to follow the crab collection methods often used on Superfund sites by the U.S. EPA (personal observation). After homogenization, the dry ice was allowed to sublimate overnight and aliquots of tissue were removed either for contaminant or lipid analyses.

Following homogenization, fiddler crab tissue samples underwent microwave digestion prior to graphite furnace atomic absorption spectroscopy. For each tissue

sample undergoing digestion, a representative portion (0.5 to 1.2g) of tissue sample was placed into a pre-cleaned digestion vessel and 10mL of 6N HNO_3 was added to all sample vessels. The samples were then heated on a hot plate at 80° C for approximately one hour or until the sample appeared to be partially digested by the acid. The samples were then microwaved and allowed to cool overnight or immediately filtered to remove suspended particles.

Tissue and sediment samples from both PC and SHM were analyzed for metals using graphite furnace atomic absorption spectroscopy. Prior to analysis, sediment samples were digested and microwaved following the process outlined above for tissue samples. Microwave digested samples were then placed into clean sample bottles and a representative aliquot of sample was injected into the graphite tube in the furnace, evaporated to dryness, charred and atomized. The metal atoms were then measured using an atomic absorption spectrophotometer.

Tissue and sediment samples were analyzed for pesticides using a gas chromatograph (GC) electron capture detector (ECD). For tissue samples, ten gram aliquots of homogenized tissue sample were dried with anhydrous sodium sulfate and Soxhlet extracted with methylene chloride solvent. The methylene chloride extract was cleaned up by Gel Permeation Chromatography (GPC); solvent exchanged to hexane and then concentrated to 1-mL final extract volume. The extracts were analyzed using GC/ECD.

For analyzing pesticides in sediment samples, approximately 30 g of sediment sample mixed with 30 g of anhydrous sodium sulfate was extracted with 140 mL of 1:1 acetone/hexane using a Soctherm extractor for 2 h or 300 mL of 1:1 acetone/hexane using

a Soxhlet extractor for 16 h. The extract was concentrated to 10 mL, 60 mL of hexane was added as an exchange solvent and the extract was concentrated to a final volume of 5 mL. The extracts were analyzed for pesticides using GC/ECD. A second column was used for confirmation whether the pesticide was tentatively identified or not.

Lipid Analyses

For lipid analyses, similarly sized crabs were analyzed from PC (mean = 17.9, min = 13.0, max = 20.7) and SHM (mean = 18.2, min = 14.6, max = 21.4). The Ocean Sciences Centre at the Memorial University of Newfoundland performed the lipid analyses. Extraction of lipids followed a variation of the Folch (1957) and Bligh and Dyer (1959) methodologies as detailed by Parrish (1998). Briefly, three 100 µL samples were taken for weight determinations and several 1-2 mL samples were taken for lipid analysis and placed in clean centrifuge tubes. One mL of ice cold methanol was added to each sample and ground with a metal-tipped rod. The rod was then washed with 1 mL of chloroform-methanol (2:1) and 0.5 mL of chloroform extracted water. The tube was then sonicated in an ice bath for 4 minutes and then centrifuged for 2-3 minutes. The bottom organic layer was then removed using a double pipetting technique, which involves placing a long Pasteur pipette inside a short one, and transferred into pre-rinsed vials. The long pipette was washed to remove the organic layer with three, 1mL rinses of ice cold chloroform. The short pipette was washed into the tube containing the aqueous layer with three, 1mL ice cold chloroform. The sample was then sonicated and centrifuged again and double pipetted each time (using new pipettes) until no color remained in the organic layer. The organic layers were then pooled for each subsample.

Total lipids were determined gravimetrically; lipid class composition was determined using the Iatroscan TLC-FID (thin-layer chromatography-flame ionization detector)(Ackman et al. 1990, Shantha 1992, Parrish 1998). The Iatroscan was used to quantify lipid class composition as methods such as high performance liquid chromatography are best suited for the separations of molecular species within classes, a level of detail that is unnecessary for this study. The TLC-FID method used was a double development/double burn method in which the Chromarods are first scanned for sterol ester, triacylglycerol, free fatty acid and sterol while the second burn quantifies monoglycerides and phospholipids (Sasaki and Capuzzo 1984, Bergen 1999). Prior to the second scan, the second development involves placing the rack of rods into a development tank of 70ml hexane/diethyl ether/acetic or formic acid (55ml/15ml/0.15ml). This development assists in differentiating the presence of lipid molecular species that have widely different degrees of unsaturation which results in chromatographic peaks that are difficult to interpret if only one development is used. As most marine lipids are heavily polyunsaturated (Parrish et al. 1992), this method, in addition to using a proper standard, offers a higher degree of confidence in lipid class detection and interpretation.

Molt Cycle Study

Similarly sized, small crabs (approximately 10 mm carapace width) were selected for use in the molting study as larger crabs (> 15 to 20 mm carapace width) may reach a terminal plateau and never undergo ecdysis in the laboratory (J. Weis, personal communication). One chela and 6 walking legs were autotomized by pinching the merus of the leg using forceps. This method was used as brachyuran crabs will autotomize

limbs along a pre-formed breakage plane (Weis 1978). Following autotomy, each crab was placed into an individual plastic cup (473 mL) and sea water (salinity 33 for SHM, 21 for PC) was added until the crab was just covered with water. One pellet of "Fiddler Crab Food" (Carolina Biological Supply) was added to each container twice a week following a change in water. The length of the limb bud of the first walking leg was measured using a calibrated ocular micrometer twice a week and the crab was removed and frozen when it reached its assigned R-value. Crabs were randomly assigned to a pre-labeled cup with an R-value of 5, 10, 15, or 20 where R is calculated as (length of regenerating limb bud/carapace width) × 100 (Bliss 1956). Twenty individual male crabs were assigned to each R-value from PC and SHM and were submitted for lipid class analyses.

28-Day Reciprocal Transplant Exposure Study

The reciprocal transplant study was initiated on August 18, 2001 and lasted 28 days. Four different treatments were used: SHM crabs on SHM sediment, SHM crabs on PC sediment, PC crabs on PC sediment, and PC crabs on SHM sediment. Sediment was collected from SHM and PC using a clean stainless steel trowel, homogenized in situ, and transported back to the lab. The sediment was then added to four plastic containers (60.6 × 44.8 × 15.2 cm). The sediment was placed into the containers is such a way that it was approximately 5 cm thick at the rear of the container and then sloped, until it met an area of added sea water at the front of the container. Ten randomly chosen male fiddler crabs were then added to each of the four containers. Mean crabs carapace widths (mm) were similar per treatment: SHM crabs on SHM sediment: 15.7 (min = 13.5, max = 18.5); SHM crabs on PC sediment: 15.9 (min = 13.6, max = 18.3); PC crabs on PC sediment:

16.0 (min = 14.2, max = 18.7); and PC crabs on SHM sediment: 15.6 (min = 14.1, max = 17.9). The water was aerated using an air stone and salinity was adjusted to reflect site salinity conditions. Water was changed twice a week. The crabs were not fed to encourage ingestion of sediment and all crabs were observed to ingest sediment throughout the exposure period. After 28 days, the crabs were, measured, weighed, homogenized, and submitted for lipid analysis.

Statistical Analyses

Lipid data were initially recorded in micrograms/gram wet weight but were converted to milligrams/gram dry weight for ease of comparison to other studies and to remove variability introduced by fluctuating water contents. Percent solids were assumed to be 11% based on the average of 10 samples of fiddler crab homogenate that were oven dried. All results are presented in dry weight.

Statistical analyses were performed using SAS version 8 and SigmaStat version 2.03. Graphs were created in SigmaPlot 2001. Descriptive statistics were used to examine basic trends in total lipids for each site, month, molting category and exposure treatment. For use in a two-factor Analysis of Variance (ANOVA), the data were natural log (ln) transformed or square root transformed to normally distribute the data.

For lipid class data, a two-factor ANOVA would be appropriate to test for interactions of effects between sampling locations and other parameters. In some cases, however, none of the parameters of concern met the required assumptions for a two-factor ANOVA. As such, the interactions were broken down into individual components (e.g., comparison of lipid class results among R-categories within each sampling location) and analyzed utilizing one-way and non-parametric tests, as appropriate

RESULTS

Sediment and Tissue Concentrations

The following elements and compounds were detected in both sediment (Table 1-1) and tissue samples (Table 1-2): aluminum, antimony, arsenic, barium, beryllium, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, mercury, nickel, potassium, selenium, silver, sodium, thallium, vanadium, zinc, p,p'-dichlorodiphenyl dichloroethane (DDD), p,p'-dichlorodiphenyl dichloroethylene (DDE), p,p'-dichlorodiphenyl trichloroethane (DDT). The mean concentrations of all compounds were greater at the contaminated site, PC, than at SHM. Individual concentrations per month and site can be found in Appendix A (Tables A1-1a-d, Tables A1-2a-d). One-factor ANOVAs or Kruskal-Wallis ANOVA on ranks were used to compare differences between sites for Cd, Cu, Pb, Hg and Zn. These five metals were highlighted as they have been previously measured and studied at SHM and PC (Weis et al. 2001b). Sediment concentrations at PC were significantly greater than concentrations at SHM for all five elements ($p<0.001$) (Table 1-1; Appendix A, Fig. A1). Depending on the normality of the data, either Pearson or Spearman correlation analyses (table below) were performed for sediment and tissue concentrations for both sites but no significant relationship were found with the exception of copper at PC.

Element	PC	SHM
Cadmium	r = -0.045, p = 0.923*	r = 0.171, p = 0.687*
Copper	r = -0.911, p = 0.004	r = 0.713, p = 0.047
Lead	r = 0.122, p = 0.795	r = -0.163, p = 0.700*
Mercury	r = 0.652, p = 0.113	r = -0.032, p = 0.940
Zinc	r = - 0.144, p = 0.758*	r = 0.133, p = 0.754**

* = Spearman Correlation for non-parametric data,

all other correlations are Pearson Correlations
** = data natural log transformed

Lipid Analyses

Seasonal Data – Total Lipids

In general, seasonal total lipid concentrations increased from April to June (Fig. 1-1) with seeming differences in concentrations between sites as PC concentrations often appear higher than total lipids at SHM. Results for a two-factor ANOVA, however, found no difference in mean total lipid concentrations between sites ($p=0.176$) (Table 1-3). For SHM crabs, there was a sharp drop from June to July followed by essentially constant levels. PC crabs showed a similar trend although there was a more gradual decline from June to July. The greatest variability in seasonal total lipid concentrations occurred in June for SHM crabs and in August for PC crabs. While it is not clear what caused the high variability in June at SHM, it should be noted that August results might be lower than other months as these samples were delayed in the mail and subsequently thawed allowing for degradation to occur (Parrish, pers. comm.).

Although there were no differences between sites, ANOVA results revealed a statistical difference in mean total lipid dry weight concentrations between sampling months ($p<0.001$) (Table 1-3). Tukey test results revealed that the month of June was marginally different from July ($p = 0.003$), August ($p = 0.005$) and September ($p = 0.008$) and significantly different from October ($p<0.001$). There were no interactions between the sampling site and the month of collection for fiddler crab total lipid dry weight concentration ($p=0.080$) (Table 1-4).

Seasonal Data – Lipid Classes

Eleven individual lipid classes were identified in each sample: acetone mobile polar lipids (AMPL), alcohols, diacylglycerols, ethyl ketones, free fatty acids (FFA), glycerol ethers, hydrocarbons/methyl esters (HC/ME), methyl ketones, phospholipids (Hebel et al.), sterols, and triacylglycerols (TG). HC/MEs were defined as the sum of hydrocarbons, steryl esters, wax esters, and methyl esters. Three lipid classes (PL, FFA and TG) comprised approximately 80 percent of total lipid content although concentrations varied between months. Phospholipids were approximately 35 to 40 percent of total lipids, FFA were approximately four to seven percent of total lipids, and TG approximately 45 to 50 percent of total lipid content. The remaining lipid classes combined typically comprised approximately 15 to 20 percent of total lipid composition. Statistical comparisons were generated for the three main lipid classes.

Phospholipid concentrations were fairly constant between months with the exception of high PL at SHM in June and lower PL at both sites in August (Fig. 1-2). The high variability in PL in June contributed to the high variability in total lipids during this month (Fig. 1-1). Results of a two-factor ANOVA using data from both sites found no significant differences between sites but significant differences in PL between sampling months ($p<0.001$) (Table 1-3). Tukey test results revealed that overall PL concentrations in August were significantly lower than in April, May, July, and September (Table 1-4) and that June was significantly different from September (Table 1-4). Reduced PL in August (Fig 1-2.) could have resulted from the samples thawing in the mail thus increasing FFA through lipid degradation. There were no statistically significant differences in PL between sites ($p=0.28$) and no interaction between site and month ($p=0.06$) (Table 1-3).

Free fatty acid concentrations slightly increased from April to June, decreased in July and were greatly increased in August (Fig. 1-3). Results of a two-factor ANOVA using data from both sites revealed that differences in FFAs between sampling months were greater than would be expected by chance ($p<0.001$) (Table 1-3). There were no significant differences between sites ($p=0.74$) and no interaction between site and month ($p=0.71$) (Table 1-3). Tukey test results revealed that the month of August was significantly greater than all other sampling months (Table 1-4). This increase in FFA in August likely resulted from the degradation of lipids caused by the accidental thawing of samples when lost in the mail. When FFA data from August is removed from the ANOVA there are still differences between months ($p=0.001$) (Table 1-3). Tukey test results excluding the month of August revealed that the month of June was marginally higher then April and July while the month of October was marginally higher than April and July (Table 1-4).

Seasonal fluctuations in TG were similar to total lipid fluctuations (Fig. 1-4). Results of a two-factor ANOVA using data from both sites showed that differences in TG between sampling months were significantly different ($p<0.001$) with a marginal difference between sites ($p=0.03$) (Table 1-3). There were no interactions between site and month (Table 1-3). Tukey test results revealed that TG concentrations in May and June were greater than TG concentrations in September and October (Table 1-4).

Molt Cycle Study Total Lipids and Lipid Class Results

Overall, mean total lipids were greater at PC than SHM for each R-category (Fig. 1-5). Differences in total lipids between sites and R-categories were evaluated using a two-factor ANOVA following ln transformation to normalize the data. Total lipids were

significantly different between sites ($p<0.001$) and among R-categories ($p=0.026$) (Table 1-5). Tukey Test results revealed significant differences between R-categories 5 and 15 where mean total lipids were lower for R-category 5 than R-category 15 (Table 1-6). There were no significant interactions between site and R-categories for total lipids ($p=0.202$) (Table 1-5).

Phospholipid concentrations appeared to be similar between sites and R-categories (Fig. 1-6.). Phospholipid data did not pass the test for normality regardless of data transformation attempts so the data were broken into two data sets according to site. Results of a one-factor ANOVA for PC found no significant differences between R-categories ($p=0.918$) (Table 1-7a) and results of a Kruskal-Wallis test for SHM also found no significant difference between R-categories ($p=0.282$) (Table 1-7b). To compare R-categories between sites, T-test comparisons were performed for each R-category between sites and no significant differences were found between R-categories (Table 1-7c).

Free Fatty Acid concentrations were lower than all lipid classes for all R-categories and both sites (Fig. 1-6). Differences in FFA were evaluated for each R-category using a two-factor ANOVA following square root transformation of the data. No significant differences were found between sites ($p=0.282$) (Table 1-8,) but significant differences were found between R-categories ($p<0.001$) (Table 1-8). Overall, when both sites were combined, a Tukey test revealed that FFA for R-category 5 was significantly greater than all other R-categories (Table 1-6). When R-categories were examined for each site individually, there were no significant differences between R-categories at SHM. For PC, R-category 5 was significantly greater than all other R-

categories (Table 1-6). There were significant interactions between site and R-category (p=0.008) (Table 1-8) where Tukey Test results revealed R-categories 5 and 15 to be significantly different between PC and SHM (Table 1-6).

Triaclyglycerol concentrations were greater at PC than at SHM for all R-categories (Fig. 1-6). Triaclyglycerol was evaluated for each R-category using a two-factor ANOVA following square root transformation of the data. Significant differences were found between sites (p=0.002) (Table 1-9) and between R-categories (p=0.007) where R-category 5 was found to be significantly lower than R-categories 15 and 20 (Table 1-9). Significant differences were found between R-categories within a site but difference could not be isolated using multiple comparison tests. Between sites, Tukey tests revealed R-category 5 to be significantly different from R-categories 15 and 20 (Table 1-6). There were no significant interactions between site and R-category (p=0.073) (Table 1-9).

28-Day Reciprocal Transplant Exposure Study Results for Total Lipids and Lipid Classes

Total lipids for crab tissue appeared to be similar between all four treatments at the conclusion of the exposure experiment (Fig. 1-7). Total lipid data failed the initial test for normality so the data were natural log transformed to perform a two-factor ANOVA. Differences in total lipids between fiddler crabs collected at PC and those collected at SHM were not great enough to exclude the possibility that the differences were attributable to random sampling variability, after allowing for the effects of sediment exposure. There was no statistically significant difference (p=0.940) between total lipids in crabs collected from either location (Table 1-10). Additionally, there was no significant difference (p=0.930) between fiddler crabs exposed to PC sediment and

those exposed to SHM sediment (Table 1-10). There was no significant interaction ($p=0.247$) between the site the fiddler crabs were collected from and the sediment treatment (Table 1-10). In summary, total lipids did not vary between PC and SHM, nor did exposure to sediment collected from either site affect total lipid concentrations in the fiddler crabs (Fig. 1-7).

Mean PL were highest for PC crabs exposed to SHM sediment (Fig. 1-8). Phospholipids did not pass the test for normality regardless of attempts at data transformation so a Kruskal-Wallis ANOVA on ranks was used to compare differences between treatments. For fiddler crabs collected at PC, there was a statistically significant difference ($p=0.002$) in PL between treatments (Table 1-11). Results of Dunn's Method multiple comparison test found PL in PC crabs exposed to PC sediment (median PL = 20.86 mg/g) to be significantly lower than PL in PC crabs exposed to SHM sediment (median PL = 32.09 mg/g) (Table 1-11). That is, contaminated PC crabs exposed to SHM sediment showed increased PL after exposure to cleaner sediment (Fig. 1-8).

Mean FFA and TG concentrations were similar between treatments (Fig. 1-8). For both FFAs and TGs, the results of a two-factor ANOVA on natural log transformed data revealed that there were no significant differences based on fiddler crab collection site (p-value of 0.232 and 0.656 respectively) (Tables 1-12 and 1-13). In addition, there were no significant differences based on sediment exposure (p-values of 0.361 and 0.701 respectively), and no significant interaction between sediment exposure and fiddler crab collection site (p-values of 0.313 and 0.342 respectively) (Tables 1-12 and 1-13). In summary, exposure to clean or contaminated sediment had no effect on FFA or TG concentrations.

DISCUSSION

Although contaminants were present in sediment at both sites, with statistically higher concentrations at PC, there was no relationship between sediment and fiddler crab tissue concentrations except for copper at PC. The lack of a statistically significant relationship between sediment and tissue contaminant concentrations in fiddler crabs is a potential example of exposure modification. Contaminant-driven changes in lipid composition may be modified by the contamination gradient, bioavailability, the spatial variation of contaminants, the ability of a given species to accumulate and metabolize lipophilic contaminants and the fate of these metabolites, and altered metabolic processes resulting from exposure (Capuzzo and Leavitt 1988). The presence of sediment contaminants in tissue indicates that contaminant uptake is clearly occurring but the lack of a statistical relationship between the two could result from contaminant regulation, degradation, mediation, or a low concentration gradient.

In this study, total lipids and lipid classes in fiddler crabs fluctuated seasonally, during limb regeneration, and between the contaminated and non-contaminated site. Little is known about seasonal or contaminant-driven lipid variation in fiddler crabs, making it difficult to assign direct causes to lipid variability between months and sites. The observed differences in lipids between sites may, however, provide some preliminary evidence of lipids fluctuating in response to contaminant exposure and some useful building blocks for future studies.

Seasonally, total lipids and lipid classes were significantly different among months but not significantly different between sites. Also, lipid classes did not fluctuate in the same pattern as total lipids with differences between months and sites for each class. These differences in lipid class fluctuations compared to total lipids support the

hypothesis that total lipid analyses may mask variations occurring on the class level (Bergen 1999). Total lipid analyses alone may therefore not be the best indicators of sub-lethal effects or for generating a seasonal profile of lipid fluctuations. It should be noted, however, that while not statistically different, total lipids were higher at the contaminated site in five out of seven months (May, July, August, September and October). Ribbed mussels (Geukensia demissa) exposed to PCBs exhibit a similar trend where total lipids were higher at the contaminated station (Bergen et al. 2001).

Contaminant exposure may have affected fiddler crab lipid class concentrations in this study. Although no statistically significant differences occurred between sites, mean TG concentrations were often higher at the contaminated site while PL concentrations were relatively similar and static between sites and months. The constancy of PL pools in American lobsters Homarus americanus exposed to petroleum hydrocarbons is attributed to the tendency to preserve membrane structure regardless of exposure (Capuzzo et al. 1984). Callinectes sapidus exhibited similar decreases in energy storage lipids and stability in structural lipids when exposed to the water-soluble fraction of crude oil (Wang and Stickle 1988). Microscopic examination of tubules in the hepatopancreas of red-jointed fiddler crabs (U. minax) exposed to naphthalene revealed that cells with storage lipids (such as TG) are the most altered following contaminant exposure while membrane lipids (such as PL) are the least altered (Robinson and Dillaman 1985). Larval mud crabs Rhithropanopeus harrisii exposed to the pesticide Fenoxycarb® also exhibit decreased TG content with increasing contaminant concentrations while PL concentrations remain the same (Nates and McKenney 2000). While these differences in classes in this study cannot definitively be attributed to

contaminant exposure, they are deserving of attention in future studies. At the very least, the observed lipid class fluctuations reinforce the need to examine polar and non-polar lipid pools, in addition to total lipids, when attempting to determine the effects of contaminant exposure (Bergen 1999).

As in this study, multiple authors have also reported increasing TG with increasing contamination in various marine and freshwater organisms (Capuzzo and Leavitt 1988, Leavitt et al. 1990, Chetty and Indira 1994, Bergen et al. 2001). Capuzzo and Leavitt (1988) suggest that increased TG may translate into decreased mobilization of TG into PL pools potentially affecting membrane structure and function. In this study, PL in contaminated crabs were fairly constant (Fig. 1-2) and do not appear to decrease with increasing TG which does not support Capuzzo and Leavitt's (1988) hypothesis. Decreasing TG and stable PL may also be a defense against incorporating lipophilic contaminants into metabolic pathways (Capuzzo et al. 1984).

Mean TG concentrations were noticeably lower at SHM during July, August, September and October and it is not clear what caused this decrease in TG. Bergey and Weis (2008) found that SHM crabs have a longer reproductive period than PC crabs. It is possible that male SHM crabs are drawing on TG reserves during July, August, September and October for energy for reproduction. Ultimately, yearly seasonal lipid profiles are necessary to determine why TG is decreased at the non-contaminated site as variability around TG was high for all months sampled.

In addition to contaminant-driven responses and sensitivity, seasonal variation in lipid content may naturally result from lipid storage during the reproductive cycle (Pillay and Nair 1973), from changes during molting (Pillay and Nair 1973), and through

changes in food and temperature conditions (Leavitt et al. 1990, Parrish et al. 1998). Total lipids and lipid classes may have fluctuated during reproductive periods for male crabs in this study. June, July, and August are the height of the reproductive period for fiddler crabs in New Jersey. During this period, male fiddler crabs wave the large cheliped as part a courtship display (Crane 1975). Extensive waving clearly requires energy that could impact lipid stores. Although lipid concentrations were not specifically measured, Yamaguchi (2001) examined the hepatopancreas index (H.I.) in male U. lactea as this organ is the center for the storage of carbohydrate and lipid reserves. (The H.I. equals the hepatopancreas dry weight/body weight excluding the large cheliped × 100.) H.I. dropped significantly from May to June with the onset of mating and extensive waving. The author notes, however, that males begin molting in June which also affects energy stores (Yamaguchi and 2001).

Physiological events, such as limb regeneration, also affected lipid stores in this study as evidenced by differences in total lipids, FFA, and TG between sites and among R-categories. Many studies have documented the effects of environmental factors (e.g., light, temperature, presence of other crabs) and various contaminants on limb regeneration and molting in fiddler crabs (Weis 1976, Weis. 1977, Weis 1978, Callahan and Weis 1983, Weis et al. 1986, Weis et al. 1987, Weis et al. 1992). Metals, such as tributyltin and methylmercury, retard regeneration and molting in fiddler crabs (Weis. 1977, Callahan and Weis 1983, Weis et al. 1987) and horseshoe crabs (Itow et al. 1998). While lipid concentrations were not examined in the studies cited above, metals have been shown to affect lipid biosynthesis in scallops (Chelomin and Belcheva 1991) and

cause lipid peroxidation (Reddy 1994) in mussels which could contribute to slowed regeneration and molting.

As stated previously, increased TG in contaminated crabs may imply decreased TG mobilization, which could also impede limb regeneration. Further studies of lipid fluctuations in response to the molt cycle and contaminant-exposure are necessary to determine modes of action and biochemical responses. Also, given the changes in lipid class observed during limb regeneration in this study, researchers attempting to document lipid fluctuations in intermolt crabs should take care to exclude crabs undergoing limb regeneration so as to not inadvertently influence their results.

The exposure of contaminated crabs to clean sediment and vice versa for a 28-day exposure period generally did not result in statistically significant changes to total lipids or lipid classes. One exception to this was an increase in PL in contaminated crabs exposed to clean sediment. Capuzzo and Leavitt (1988) found that mussels from low and medium dose treatments had higher PL than mussels from the highest dose treatment. They suggest these differences reflect the nutritional status of the organisms combined with exposure conditions. It is also possible that food quality differences between the sites contributed to differences in lipid pools as nutrient supply directly impacts lipid composition (Leavitt et al. 1990).

Given that metal concentrations were lower in the "non-contaminated" sediment in this study, it is possible that decreased metals resulted in increased PL levels in the reciprocal transplant study. Reducing metal exposure has been shown to improve lipid functioning. For example, copper-induced lipid peroxidation in gill and muscle tissue in the mussel (Perna viridis) decreases after a 30-day exposure to clean seawater (Reddy

1994). In addition, a study of gill cells in scallops (Mizuhopeten yessoensis) exposed to Cd found that high concentrations disturb the metabolic processes responsible for membrane lipid biosynthesis (Chelomin and Belcheva 1991). Additional exposure and dosing studies are required to more accurately characterize lipid class response to various metals and concentrations.

In conclusion, total lipids and lipid classes of male U. pugnax were observed to fluctuate seasonally and during the molt cycle. Such fluctuations indicate that researchers desiring to use lipid data should include lipid class data or, at a minimum, the sum of polar and non-polar lipids. Fractionation clearly reveals fluctuations in classes which would be masked by employing only total lipid analysis (Bergen 1999). Researchers should also utilize long-term data sets to provide the necessary temporal and/or biological context for the observed lipid concentrations. If long-term data collection is not feasible, lipids should be sampled during periods when lipid concentrations for the species are known.

A goal of this study was to clarify the potential utility of using lipid class fluctuations in fiddler crabs as indicators of sublethal effects at contaminated sites. Given the lack of statistical significance for the null hypotheses tested in this study, it cannot be strictly concluded that contaminant exposure resulted in lipid fluctuations. As Nakagawa and Cuthill (2007) point out, however, a null hypothesis in natural systems can rarely be true and can only be exactly true for categorical data. In a broader context, many of the studies cited here have shown contaminant-driven lipid responses for crustaceans. Also, the lipid concentrations in this study did often differ between the contaminated and non-contaminated site in ways similar to other cited results. Without more data, however,

identifying the influence of abiotic and biotic factors on lipid class fluctuations in U. pugnax, it would be premature to attribute differences in lipids between sites to contaminants. While one can cautiously hypothesize the existence of a biologically relevant, contaminant-driven difference in lipids between sites or this study, longer term studies, in-depth analyses of biotic and abiotic factors, and larger data sets are clearly required.

Results of this study indicate that lipid sampling could be useful when applied to routine (i.e., repetitive) monitoring studies of contaminated areas especially at sites with high concentrations of contaminants that are known to impact lipid stores and functioning. Lipids may be less useful when attempting to screen or quickly identify contaminant effects at less contaminated sites, as results are best understood within the context of a longer-term seasonal data set. When combined with other analyses, lipid data could be useful for determining toxic modes of action.

Table 1-1.
Mean seasonal concentrations (± 95% Confidence Intervals) of contaminants in sediment from Piles Creek (PC) and Sheepshead Meadows (SHM). Means reflect a range of sampling dates from June to October, 2000 and April, May, and July 2001. One-factor ANOVAs or Kruskal-Wallis ANOVA on ranks) were performed for Cd, Cu, Pb, Hg, and Zn between PC and SHM and all concentrations were significantly different between sites (* = $p<0.001$).

Element (mg/g)	**PC**	**95% CI**	**SHM**	**95% CI**
Al	1622.220	2491.590	8311.110	1311.500
An	6.890	0.917	5.900	1.690
As	118.440	52.240	8.790	3.890
Ba	1696.670	616.900	25.330	4.530
Be	0.670	0.160	0.444	0.100
Cd*	1.710	0.690	0.444	0.100
Ca	3205.560	904.300	2744.440	422.960
Cr	208.330	130.920	36.440	5.390
Co	14.720	2.230	5.570	1.050
Cu*	373.890	172.840	18.560	3.130
Fe	38111.000	4587.030	14411.110	3814.110
Pb*	325.300	141.000	24.670	4.010
Ms	7055.560	993.420	5388.890	1047.220
Mn	268.890	60.000	117.780	31.620
Hg*	16.940	10.430	0.180	0.043
Ni	50.940	13.140	15.110	3.270
K	3383.330	391.000	2533.330	434.820
Se	2.100	1.100	1.290	0.940
Ag	2.380	1.350	0.444	0.100
Na	12266.670	2528.440	13955.560	4539.070
Th	0.801	0.242	0.580	0.310
V	67.830	20.370	28.780	4.720
Zn*	347.220	95.370	99.000	62.450
Compound (µ/g)				
DDD	1771.670	1747.820	3.700	0.720
DDE	232.940	117.140	3.360	0.880
DDT	79.310	49.430	3.700	0.720

Table 1-2.

Mean concentrations (± 95% Confidence Intervals) of contaminants in tissue from Piles Creek (PC) and Sheepshead Meadows (SHM). Means reflect a range of sampling dates from June to October, 2000 and April, May, and July 2001.

Element (mg/g)	**PC**	**95% CI**	**SHM**	**95% CI**
Al	250.42	65.35	211.98	40.65
An	0.33	0.14	0.33	0.14
As	9.18	1.60	18.80	3.11
Ba	105.15	10.70	18.69	3.11
Be	0.23	0.26	0.23	0.32
Cd	0.65	0.16	0.56	0.75
Ca	137187.50	10203.78	152604.17	23901.26
Cr	3.64	1.22	1.80	0.52
Co	1.14	0.13	1.11	0.14
Cu	179.69	19.04	108.00	8.36
Fe	670.31	151.25	459.38	90.61
Pb	13.82	2.49	1.51	0.91
Ms	9342.71	975.75	11427.08	1757.90
Mn	25.12	5.50	22.71	4.54
Hg	0.59	0.08	0.19	0.02
Ni	1.12	0.13	9.18	19.01
K	7089.58	399.00	7319.80	608.56
Se	2.21	0.39	1.12	0.18
Ag	0.64	0.16	1.01	0.24
Na	15697.92	1211.23	18645.83	2426.49
Th	0.22	0.16	0.23	0.50
V	1.50	0.43	1.11	0.14
Zn	95.94	8.66	85.48	7.37
Compound (µ/g)				
DDD	1095.31	493.04	7.09	0.95
DDE	624.38	262.20	6.35	1.38
DDT	20.93	14.03	7.11	3.12

Table 1-3.

Two-Factor Analysis of Variance (ANOVA) ($\alpha = 0.05$) results for differences in total lipids, phospholipids (PL), free fatty acids (FFA) and triacylglycerols (TG) between sites, months, and site ×month. Significant differences were only found between months for total lipids and all three classes although there was a significant difference between sites for TG. Results for FFA are calculated with and without the month of August as lost samples likely increased FFA for this month.

Total Lipids					
Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Site	1	2887.42	2887.42	1.85	0.176
Month	6	3846.78	6410.3	4.11	<0.001
Site x Month	6	18208.97	3034.83	1.95	0.08
Residual	106	165287.1	1559.31		
Total	119	231174.6	1942.64		
PL					
Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Site	1	349.1	349.1	1.16	0.28
Month	6	9550.03	1591.67	5.29	<0.001
Site x Month	6	3853.53	642.26	2.13	0.06
Residual	105	31614.55	301.09		
Total	118	45064.01	381.9		
FFA with August					
Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Site	1	3.57	3.57	0.11	0.74
Month	6	3935.87	655.98	20.83	<0.001
Site x Month	6	118.16	19.7	0.63	0.71
Residual	105	3307.12	31.5		
Total	118	7367.22	62.43		
FFA without August					
Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Site	1	22.01	22.01	1.65	0.2
Month	5	290.46	58.1	4.36	0.001
Site x Month	5	48.05	9.61	0.72	0.609
Residual	87	1158.34	13.31		
Total	98	1501.98	15.33		
TG					
Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Site	1	3262.48	4362.48	5.1	0.03
Month	6	18499.18	3083.2	4.82	<0.001
Site x Month	6	6757.52	1126.25	1.76	0.12
Residual	105	67232.82	640.31		
Total	118	98971.75	838.74		

Table 1-4.

Tukey test results for differences in total lipids, phospholipids (PL), free fatty acids (FFA), and triacylglycerols (TG) between months for both sites combined. Results for FFA are calculated with and without the month of August as lost samples likely increased FFA for this month.

Lipids	**Months**
Total Lipids	June > July, Aug., Sept., Oct.
Phospholipids	June > September
Phospholipids	Aug. < April, May, Sept.
Free Fatty Acids (w/ Aug.)	Aug. > April, May, June, July, Sept., Oct.
Free Fatty Acids (w/out Aug.)	June > April, July
Free Fatty Acids (w/out Aug.)	Oct. > April, July
Triacylglycerols	June > Sept., Oct.
Triacylglycerols	May > Sept., Oct.

Table 1-5.
Two-Factor Analysis of Variance (ANOVA) (α = 0.05) results using natural log transformed data for differences in total lipids between sites and R-categories. Significant differences were found between sites and between R-categories. Each R-category was calculated as (length of the regenerating limb bud/carapace width) × 100.

Source of Variation	DF	SS	MS	F	P
Site	1	1.162	1.162	18.04	<0.001
R-Category	3	0.612	0.204	3.169	0.026
Site x R-Category	3	0.301	0.1	1.588	0.202
Residual	153	9.853	0.0644		
Total	160	11.923	0.0745		

Table 1-6.
Tukey test results for differences in total lipids, free fatty acids (FFA), and triacylglycerols (TG) for Piles Creek (PC) or Sheepshead Meadows (SHM). Differences are shown between R-categories for both sites combined for total lipids and FFA, for differences between R-categories within a site for FFA at PC, and between sites for TG.

Lipid Class	**Differences bet. R-Categories**
Total lipids both sites combined	R5 < R15
FFA both sites combined	R5 > R10, R15, R20
FFA at SHM	No difference bet. R-categories
FFA at PC	R5 > R10, R15, R20
FFA Site × R-Category	R5 ≠ R15
TG between sites (PC vs. SHM)	R5 < R15, R20

Table 1-7a.
Results for a one-factor Analysis of Variance (ANOVA) (α = 0.05) for Piles Creek (PC) for differences in phospholipids between R-categories. No significant differences were found between sites or R-categories.

Source of Variation	DF	SS	MS	F	P
Between Groups	3	90.14	30.04	0.168	0.918
Residual	76	13594.15	178.87		
Total	13684.29				

Table 1-7b.

Results for a Kruskal-Wallis one-factor ANOVA on ranks (α = 0.05) for Sheepshead Meadows (SHM) for differences in phospholipids between R-categories. No significant differences were found between R-categories.

Group	N	Median	25%	75%	P
R-category 5	20	27.53	23.08	30.96	0.282
R-category 10	20	24.25	14.81	27.34	
R-category 15	20	29.55	22.26	32.96	
R-category 20	20	26.00	20.50	34.68	

Table 7-c.

T-test results for comparison of each R-category between Piles Creek (PC) and Sheepshead Meadows (SHM) for phospholipids. No significant differences were found between R-categories.

Site & R-Category	N	Mean	Std. Dev.	SEM	P
SHM R5	20	26.52	7.53	1.68	0.878
PC R5	20	26.92	8.75	1.96	
SHM R10	20	22.65	9.13	2.04	0.138
PC R10	20	28.19	13.57	3.04	
SHM R15	20	28.59	12.95	2.90	0.981
PC R15	20	28.70	15.96	3.57	
SHM R20	20	29.95	16.40	3.67	0.990
PC R20	20	29.88	14.15	3.16	

Table 1-8.
Results for a two-factor Analysis of Variance (ANOVA) (α = 0.05) using square root transformed data for free fatty acids (FFA) between sites and R-categories. No significant differences were found between sites but significant differences were found between R-categories and a significant interaction was found between site and R-category.

Source of Variation	DF	SS	MS	F	P
Site	1	1.09	1.09	1.17	0.282
R-Category	3	17.48	5.83	6.26	<0.001
Site x R-Category	3	11.35	3.78	4.07	0.008
Residual	152	141.43	0.93		
Total	159	171.35	4.07		

Table 1-9.
Results for a two-factor Analysis of Variance (ANOVA) (α = 0.05) using square root transformed data for triacylglycerols (TG) between sites and R-categories. Significant differences were found between sites and between R-categories.

Source of Variation	DF	SS	MS	F	P
Site	1	65.1	65.1	9.5	0.002
R-Category	3	86.97	28.99	4.23	0.007
Site x R-Category	3	48.65	16.22	2.37	0.073
Residual	152	1041.14	6.85		
Total	159	1241.86	7.81		

Table 1-10.
Results for a two-factor Analysis of Variance (ANOVA) (α = 0.05) using natural log transformed data for total lipids between treatments for the 28-day reciprocal transplant exposure study. Sample site was either Piles Creek (PC) or Sheepshead Meadows (SHM) and exposure treatments were as follows: PC crabs on PC sediment; PC crabs on SHM sediment; SHM crabs on SHM sediment; and SHM crabs on PC sediment. There were no significant differences between sites or treatments and no interaction between site and treatment.

Source of Variation	DF	SS	MS	F	P
Sample Site	1	0.000595	0.000595	0.0057	0.94
Sediment Site	1	0.00081	0.00081	0.00776	0.93
Sample Site x Sediment Site	1	0.144	0.144	1.383	0.247
Residual	36	3.758	0.104		
Total	39	3.902	0.1		

Table 1-11.

Results for a Kruskal-Wallis Analysis of Variance (ANOVA) on ranks (α = 0.05) for phospholipids (PL) between treatments for the 28-day reciprocal transplant exposure study. Sample site was either Piles Creek (PC) or Sheepshead Meadows (SHM) and exposure treatments were: PC crabs on PC sediment; PC crabs on SHM sediment; SHM crabs on SHM sediment; and SHM crabs on PC sediment. Significant differences were found between treatments as PL increased for PC crabs exposed to SHM sediment.

Treatment	**N**	**Median**	**25%**	**75%**	**P**
PC on SHM	10	32.09	28.71	38.33	0.002
SHM on PC	10	17.44	15.75	22.65	
SHM on SHM	10	28.63	21.78	32.57	
PC on PC	10	20.86	19.31	24.54	

Table 1-12.

Two-Factor Analysis of Variance (ANOVA) (α = 0.05) results for free fatty acids (FFA) between treatments for the 28-day reciprocal transplant exposure study. Sample site was either Piles Creek (PC) or Sheepshead Meadows (SHM) and exposure treatments were: PC crabs on PC sediment; PC crabs on SHM sediment; SHM crabs on SHM sediment; and SHM crabs on PC sediment. No significant differences were found between treatments.

Source of Variation	DF	SS	MS	F	P
Sample Site	1	0.711	0.711	1.48	0.232
Sediment Site	1	4.12	0.412	0.86	0.361
Sample Site x Sediment Site	1	0.502	0.502	1.05	0.313
Residual	36	17.3	0.48		
Total	39	19.03	0.49		

Table 1-13.

Two-Factor Analysis of Variance (ANOVA) (α = 0.05) results for triacylglycerols (TG) between treatments for the 28-day reciprocal transplant exposure study. Sample site was either Piles Creek (PC) or Sheepshead Meadows (SHM) and exposure treatments were: PC crabs on PC sediment; PC crabs on SHM sediment; SHM crabs on SHM sediment; and SHM crabs on PC sediment. No significant differences were found between treatments.

Source of Variation	DF	SS	MS	F	P
Sample Site	1	646.89	646.89	0.202	0.656
Sediment Site	1	479.83	479.83	0.15	0.701
Sample Site x Sediment Site	1	2968.61	2968.61	0.925	0.342
Residual	36	115493	3208.15		
Total	39	119589	3066.41		

Fig. 1-1

Mean total lipids (mg/g dry weight) (± 95% confidence intervals) for whole body Uca pugnax tissue per month for Piles Creek (PC) and Sheepshead Meadows (SHM). Total lipids were not significantly different between sites but were significantly different between months. Multiple comparison test results for both sites combined revealed concentrations in June to be significantly greater than concentrations in July, August, September and October. Any two means with the same single-letter code cannot be said to be significantly different.

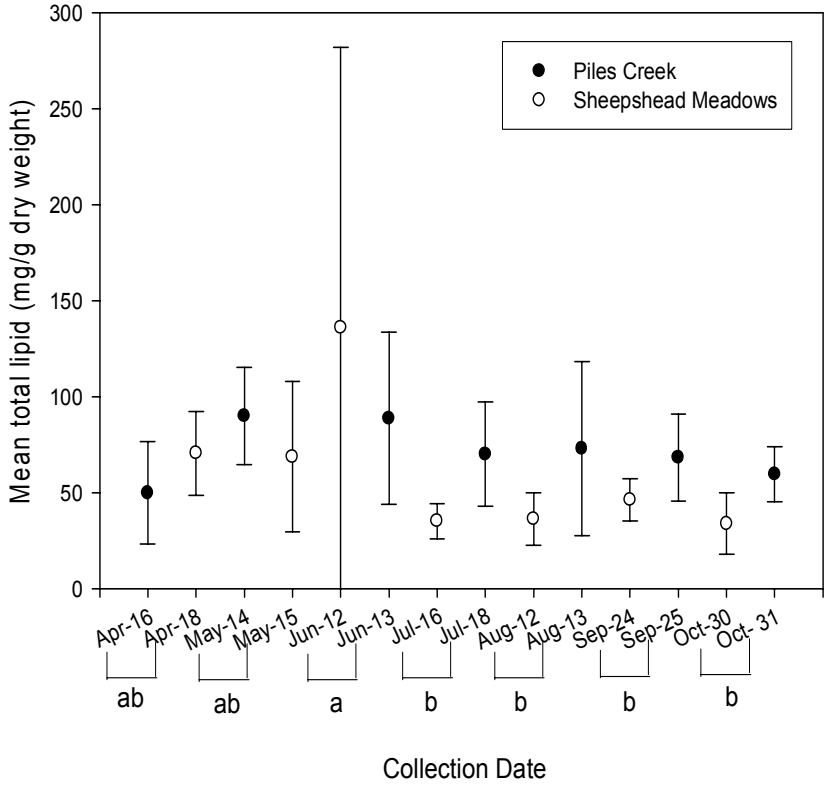


Fig. 1-2

Mean phospholipids (PL) (mg/g dry weight) (± 95% confidence intervals) for whole body Uca pugnax tissue per month for Piles Creek (PC) and Sheepshead Meadows (SHM). Phospholipids were not significantly different between sites but were significantly different between months. Multiple comparison test results for both sites combined revealed PL concentrations in June to be significantly greater than September while PL in August was significantly lower than concentrations in April, May, July, and September. Any two means with the same single-letter code cannot be said to be significantly different.

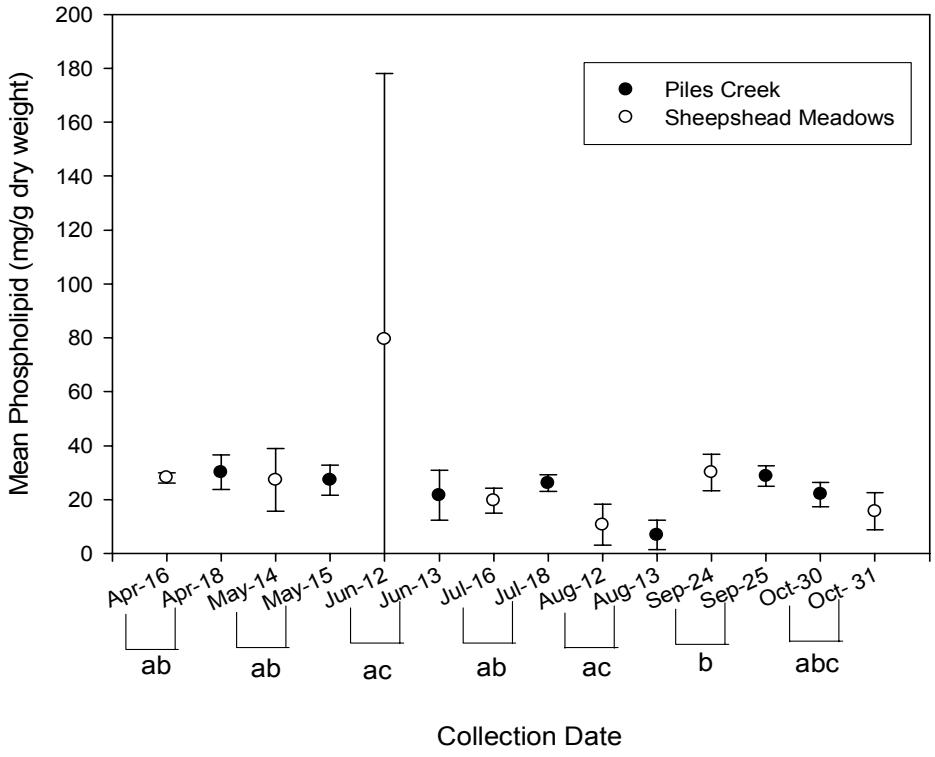


Fig. 1-3

Mean free fatty acids (FFA) (mg/g dry weight) (± 95% confidence intervals) for whole body Uca pugnax tissue per month for Piles Creek (PC) and Sheepshead Meadows (SHM). Free fatty acids were not significantly different between sites but were significantly different between months. Multiple comparison test results revealed FFA concentrations in August to be significantly greater than FFA for all other months likely resulting from samples thawing in the mail. When the month of August is removed from the analyses FFA in June were significantly greater than FFA in April and July. Any two means with the same single-letter code cannot be said to be significantly different. Letters a, b and c are for comparisons including August and x, y and z are for comparisons without the month of August.

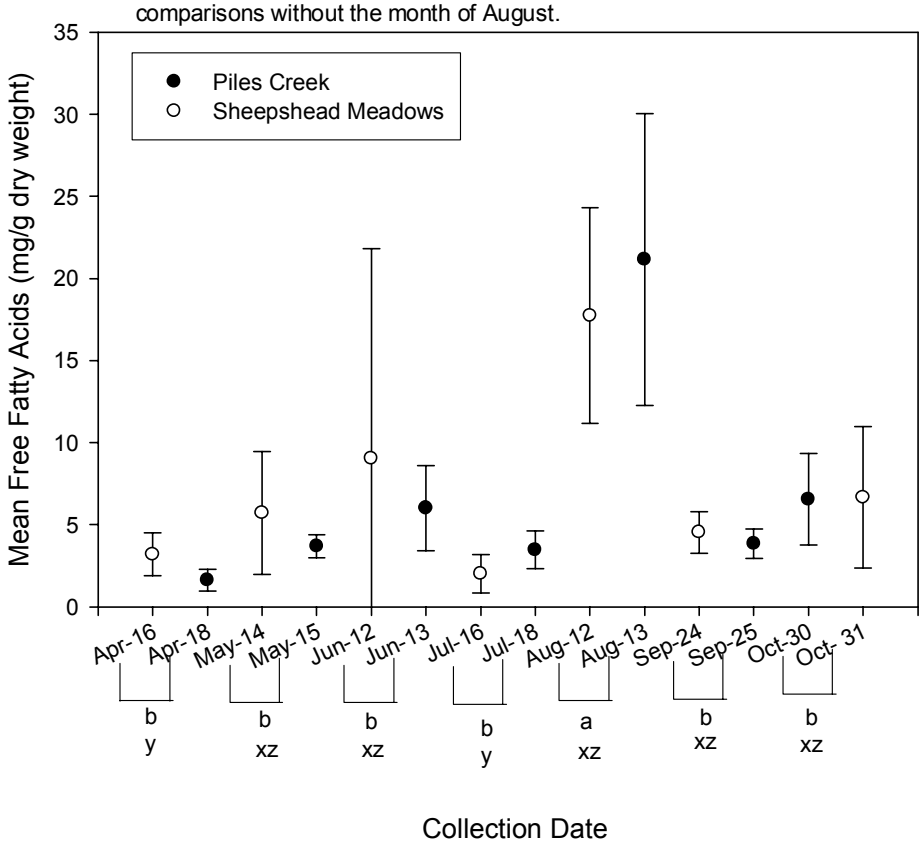


Fig. 1-4

Mean triacylglycerol (TG) (mg/g dry weight) (± 95% confidence intervals) for whole body Uca pugnax tissue per month for Piles Creek (PC) and Sheepshead Meadows (SHM). Concentrations of TG were significantly different between months and sites. Multiple comparison test results revealed TG concentrations in May and June to be significantly greater than concentrations in September and October. Any two means with the same single-letter code cannot be said to be significantly different.

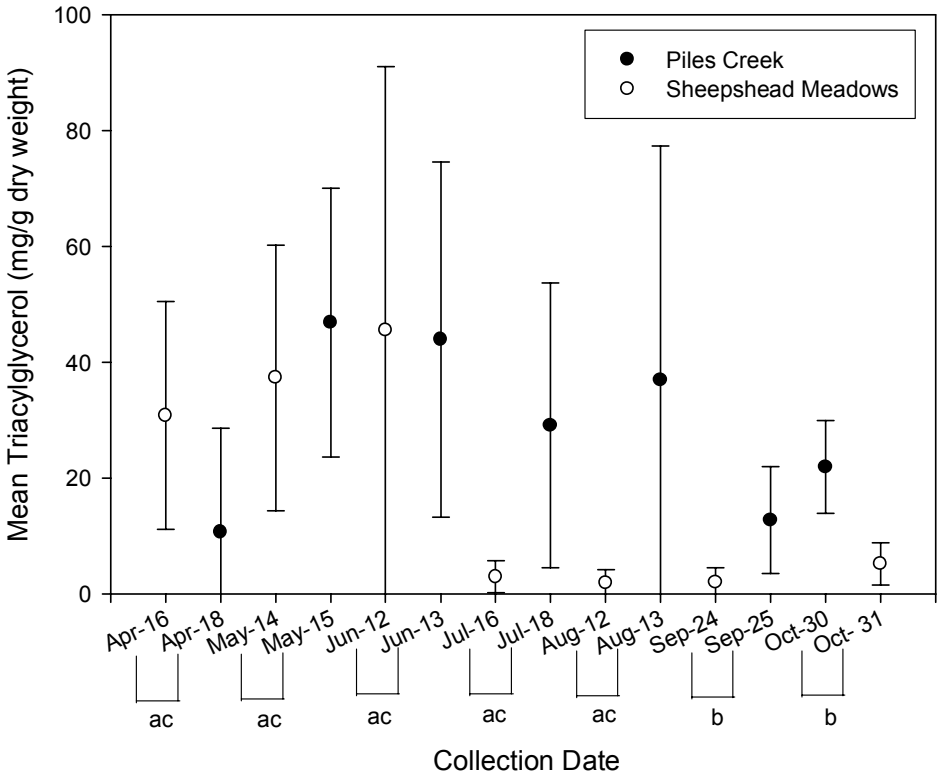


Fig. 1-5

Mean total lipids (mg/g dry weight) per R-category where total lipids were significantly different between sites and R-categories. Multiple comparison test results revealed total lipids for R-category 5 to be lower than R-category 15. Any two means with the same single-letter code cannot be said to be significantly different.

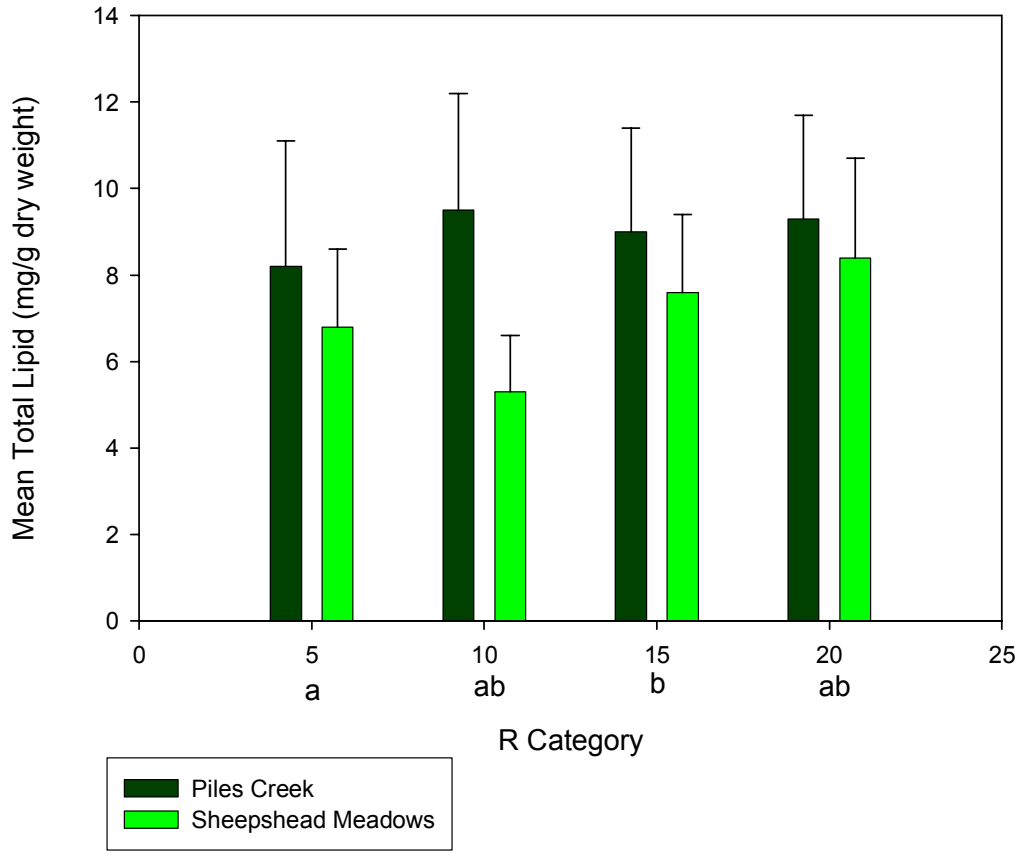


Fig. 1-6

Mean lipid classes (mg/g dry weight) for Piles Creek (PC) and Sheepshead Meadows (SHM) per R-category. For phospholipids (PL), there were no significant differences between R-categories at PC or at SHM. There were also no differences between R-categories between sites. For free fatty acids (FFA), there were no significant differences were found between sites but significant differences were found between R-categories. For triacylglycerols (TG), there were significant differences between sites and R-categories.

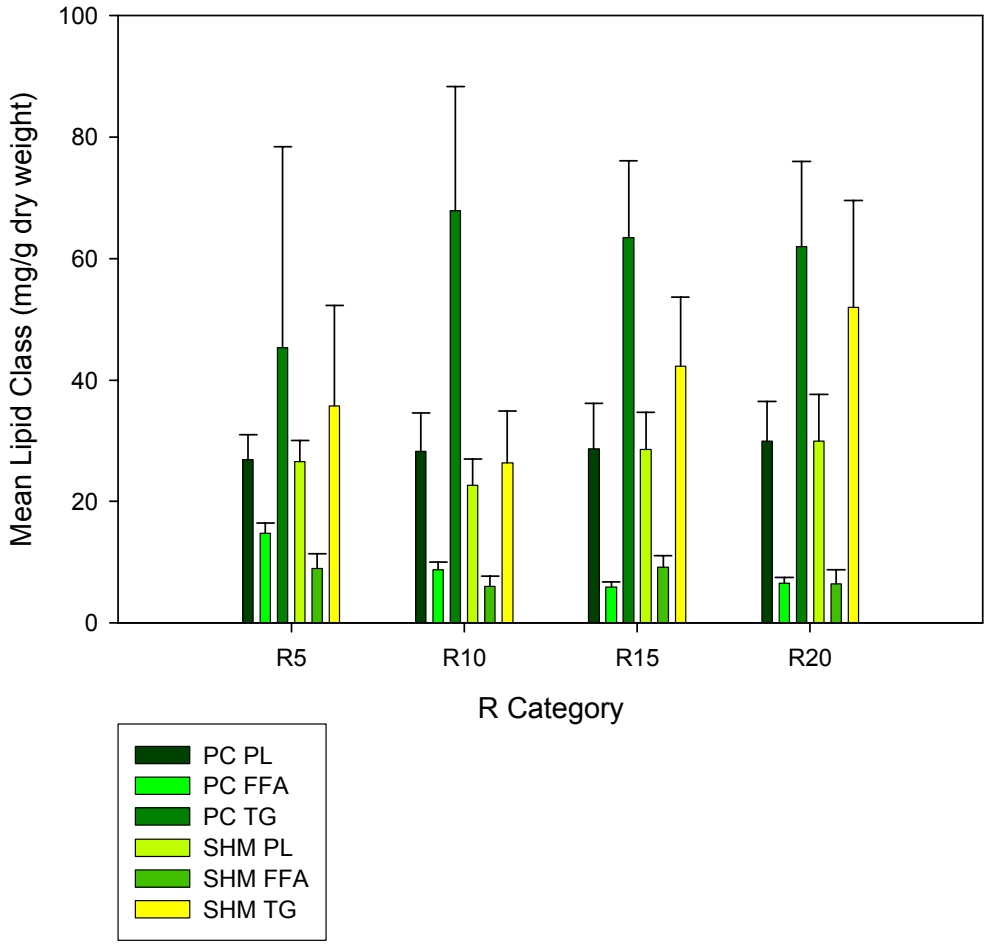


Fig. 1-7

Mean total lipids between treatments for the 28-day reciprocal transplant exposure study. Sample site was either Piles Creek (PC) or Sheepshead Meadows (SHM) and exposure treatments were as follows: PC crabs on PC sediment; PC crabs on SHM sediment; SHM crabs on SHM sediment; and SHM crabs on PC sediment. There were no significant differences between sites or treatments and no interaction between site and treatment.

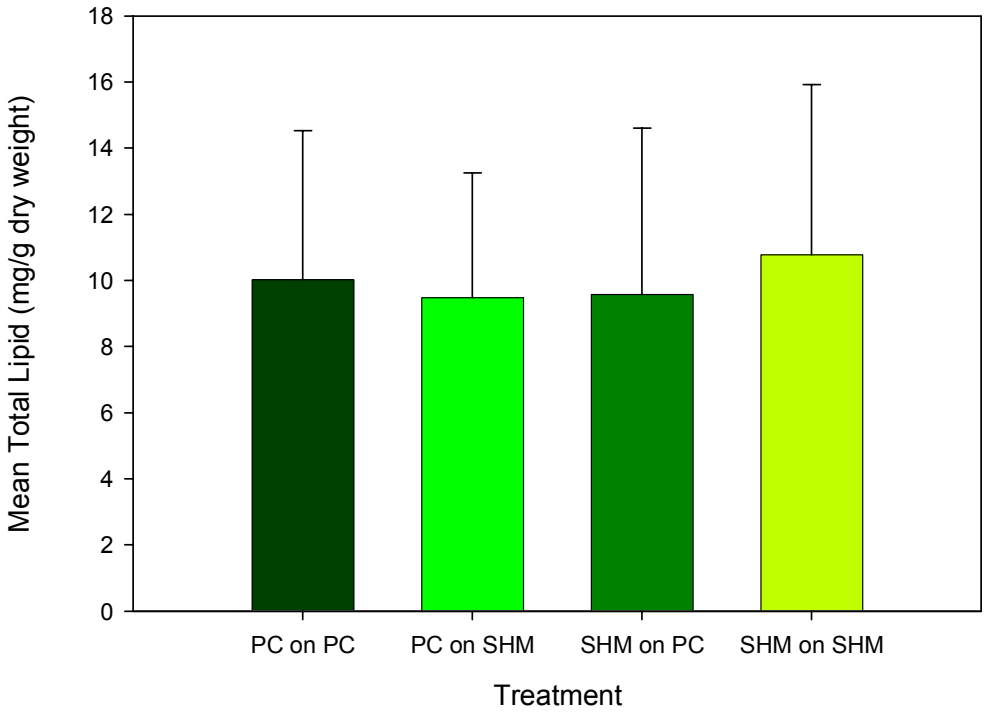
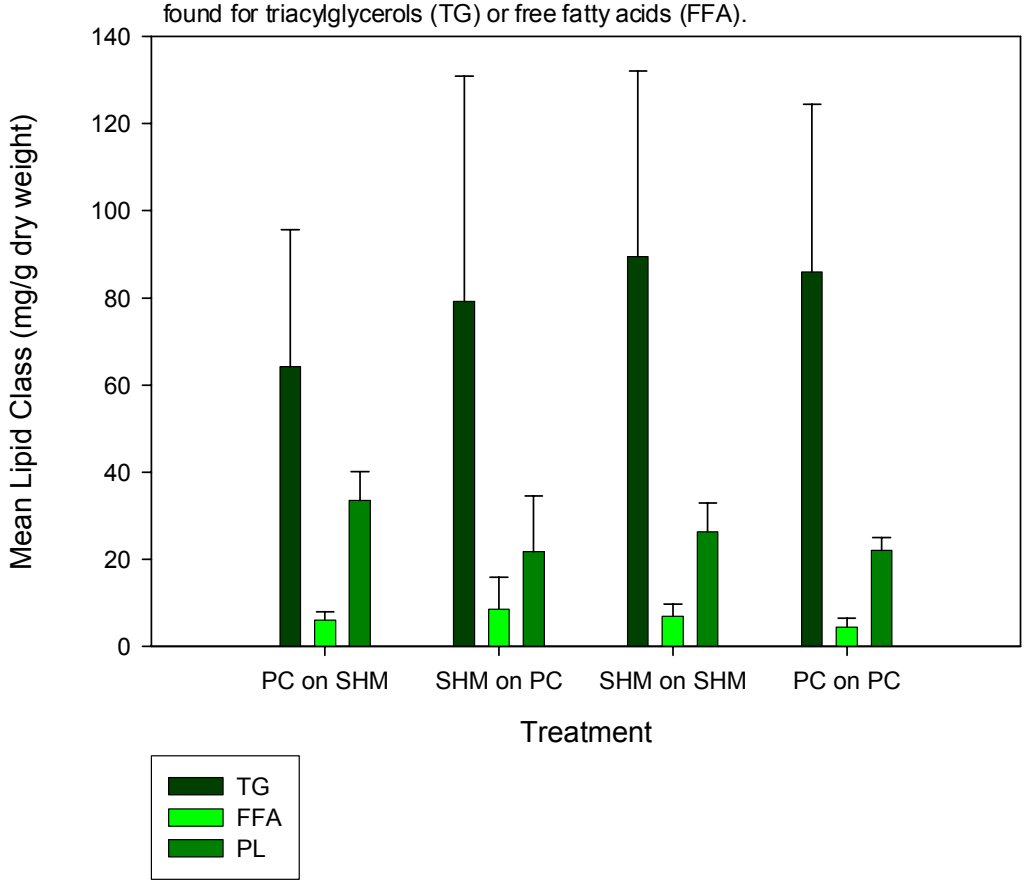


Fig. 1-8

Mean lipid classes lipids between treatments for the 28-day reciprocal transplant exposure study. Sample site was either Piles Creek (PC) or Sheepshead Meadows (SHM) and exposure treatments were: PC crabs on PC sediment; PC crabs on SHM sediment; SHM crabs on SHM sediment; and SHM crabs on PC sediment. Significant differences were found between treatments for phospholipids (PL) as PL increased for PC crabs exposed to SHM sediment. No significant differences between treatments were found for triacylglycerols (TG) or free fatty acids (FFA).



CHAPTER TWO

Fecundity and larval morphology in Uca pugnax from a contaminated and non-contaminated site in New Jersey

ABSTRACT

Fecundity and larval morphology were examined in U. pugnax to determine the effects of contaminant exposure on field-collected organisms from a contaminated and non-contaminated site. Fecundity was determined gravimetrically and larvae were examined for eye and body morphological abnormalities. A site comparison study and a reciprocal transplant exposure study were used to compare both fecundity and larval morphology between sites and exposure treatments.

Mean fecundity was higher at the contaminated site for both the site comparison and exposure studies. Crabs were slightly larger at the contaminated site which may have contributed to the differences in fecundity between sites. Variation in environmental parameters and low levels of potentially bioavailable contaminants at the non-contaminated site may also have influenced fecundity.

Newly hatched stage I zoeae were collected from field-collected U. pugnax from the contaminated and non-contaminated site and abnormal eye and body morphology were recorded for both the site comparison and reciprocal transplant exposure studies. Abnormal larvae were observed at both sites although the proportion of abnormal larvae was higher at the contaminated site. Hypopigmented eyes and hydropsy were the most common abnormalities at the contaminated site, while hydropsy was most common at the non-contaminated site. The most common morphological abnormalities observed at both sites were unspecific pathologies likely manifested as a general response to pollutants and

metals. Supernumerary eyes and eye atrophy, both of which occurred in low numbers at the contaminated site, have been found to correspond to specific metals like Pb and Cu, which are both present at the contaminated site.

INTRODUCTION

Estuaries are dynamic ecosystems exhibiting spatial and temporal gradients in many chemical, physical, and biological processes. Contaminants are also often present in estuaries adding to the complexity of the system and our understanding of it (Chapman and Wang 2001). These contaminants can be acutely toxic but more often they result in chronic or sublethal effects (Day 1989). Many toxicity studies are concerned with the acute effects of contaminants on organisms but sublethal effects, while subtle, may have far-reaching effects with respect to reproduction, development, and the ultimate survival of an organism (Rosenthal and Alderdice 1976). Reduced fitness resulting from reproductive impairment is a commonly measured sublethal effect (Sprague 1971, 1976, Newman 1998), yet most studies focus on laboratory concentration-response data and not real-world exposure conditions.

Laboratory studies of contaminant effects on fiddler crab reproduction are limited. Most are 24-48 h exposure studies on released larvae (Vernberg et al. 1973) and focus on dose-response relationships. One 24 h study generated Lethal Concentration (LC) 50 values for U. pugilator zoeae exposed to four trimethyltin concentrations at varying temperatures and toxicities, documenting increased toxicity to zoeae with increasing temperature and decreasing salinity (Wright and Rosenburg 1982). A 96 h study by Vernberg et al. (1973) examined the synergistic effects of mercury, temperature and salinity on mortality in U. pugilator zoeae.

Other studies of reproductive effects in marine crustaceans have looked for larval morphological abnormalities after exposing ovigerous females to varying contaminant concentrations. Toxicological bioassays were performed on the burrowing crab Chasmagnathus granulata to examine the nature and extent of teratogenic effects of Cd on egg incubation in ovigerous females and on released larvae (Rodriguez and Medesani 1994). Exposure of ovigerous females did not appear to affect egg incubation or hatching, but did cause hydropsy and atrophy of exoskeletal structures in hatched zoeae. Similar morphological abnormalities were observed in zoeae of southern king crab (Lithodes santolla) after Cd and Pb exposure, although Pb decreased the proportion of hatched larvae while Cd significantly increased hatching rate (Amin et al. 1998). Other bioassays have been performed with similar abnormalities and mortality observed in adults and larvae of the horseshoe crab (Limulus polyphemus) (Botton et al. 1998, Itow et al. 1998, Botton 2000), pea crab larvae (Tunicotheres moseri) (Lopez Greco et al. 2001), and in the semi-terrestrial estuarine crab C. granulata (Zapata et al. 2001).

Single-species laboratory toxicity tests are frequently used to determine the effects of contaminants on estuarine organisms (Hall and Giddings 2000). These tests yield information regarding potential toxic effects under controlled laboratory conditions but provide limited information about actual field responses to contaminants. Ambient tests (using water or sediment, or both, from the field) and *in situ* tests more closely attempt to replicate field conditions yet problems still arise when trying to use these data to predict ecological condition (Hall and Giddings 2000). While the identification of sublethal effects from laboratory manipulations is useful information, it is important and

necessary to interpret these results against field-collected species to test the reality of laboratory-derived results.

No studies for Uca spp. could be identified that compare fecundity or larval morphological abnormalities in field-collected organisms to laboratory exposed individuals yet the reproductive process is well understood for Ocypodid crabs like U. pugnax. Mating in U. pugnax typically occurs bi-weekly and usually four or five days before a spring tide (Christy 1978). Once a female chooses a mate, the female enters the burrow of the male where mating, ovulation, fertilization, egg extrusion occurs (Christy 2007). The female remains in the plugged burrow for approximately two weeks until she emerges to release larvae on the extremely high spring tide for transport out of the estuary (Christy 2007). It is not known whether females ingest sediment while underground though it is hypothesized that feeding is limited (Christy and Salmon 1984) yet no observational data appears to exist to support these claims. The newly hatched larvae typically develop in the costal ocean and molt into five zoeal stages before molting into megalopae (Bergey and Weis 2008). The postlarvae (megalopae) then return to the estuary on nocturnal flood tides where they settle and molt into adult crabs (Epifanio et al. 1988, Christy 2007).

Contaminant exposure can occur in several ways and throughout several stages during the reproductive process. The ingestion of or direct contact with contaminated sediment by ovigerous females could impact both batch fecundity and developing eggs while direct contact with contaminated water or sediment could impact newly hatched and settling larvae. Most work examining the effects of contaminants on reproduction focus on laboratory toxicity studies and not field-collected data. As such, this study seeks

to determine whether the sublethal reproductive effects typically observed in laboratory exposures are seen in field-collected U. pugnax. To that end, fecundity was determined and compared for U. pugnax from both a contaminated and non-contaminated estuarine system. In addition, morphological larval abnormalities in newly hatched stage I zoeae from field-collected crabs were examined to determine if sediment exposure can result in the abnormalities typically observed from laboratory water column toxicity tests. Laboratory sediment exposure experiments were also performed using reciprocal transplant studies.

RESEARCH LOCATIONS

Sheepshead Meadows (SHM)

The reference or "non-contaminated" site was Sheepshead Meadows (SHM) located within the Barnegat Bay-Little Egg Harbor estuarine system in southern New Jersey (39° 18' N, 74° 12' W). The SHM site is located on the southern tip of a salt marsh peninsula removed from any major industries or point contaminant sources. The system is one of the least disturbed in the northeast United States (Psuty et al. 1993) and, partly as a result, was included in the National Oceanic and Atmospheric Administration (NOAA)-operated National Estuarine Research Reserve System on October 20, 1997 (Able et al. 1996). The estuary is classified as a highly eutrophic estuary based on NOAA's National Estuarine Eutrophication Assessment model (Kennish et al. 2007). The lower estuary is polyhaline with a salinity range of 23.6 to 34.5 at the nearby Rutgers University Marine Field Station (Able et al. 1992). At the nearby Schooners Creek, mean C is 45.7 mg/g, mean N is 4.48 mg/g (Horng and Taghon 1999) and C:N ratios range from 12.1 to 13.6 (G. Taghon, unpublished data). Seasonal water temperatures range

from -2º C to 28º C (Able et al. 1992) with a tidal range of 1.1 m near the mouth of Great Bay (Martino and Able 2003). Some low levels of contaminants have been measured at near the site and mean metal levels (µg/g) were: Hg 0.19, Cu 43.8, Pb 73.2, Zn 141, Cd 2.1 (Weis et al. 2001a, Weis et al. 2001b).

Contaminated Site

Piles Creek (PC) (40° 36.5' N, 74° 13.6'W) in Linden, New Jersey is a tributary of the Arthur Kill, the navigational channel between New Jersey and Staten Island, New York. Piles Creek served as the "contaminated" site as the creek is surrounded by industrial sites such as petroleum refineries, oil storage tanks, a sewage treatment plant, and a major highway. The site has likely received contaminants from nearby industries and oil spills in the Arthur Kill (Bergey and Weis 2008). Site contaminants include metals, pesticides, polycyclic aromatic hydrocarbons (PAHs), and some polychlorinated biphenyls (PCBs). Mean metal levels (µg/g) have previously been measured for Hg 6.3, Cu 485, Pb 107, Zn 525, Cd 7.1 (Weis et al. 2001a, Weis et al. 2001b). Salinity measurements at the site consistently ranged from 20 to 23 during the sampling period. Mean C at the site is 44.6 mg/g and N is 3.32 mg/g (Horng and Taghon 1999) while C:N range from 16.3 to 19.6 (G. Taghon, unpublished data).

MATERIALS AND METHODS

Crab Collection and Handling

Crabs were collected at low tide from the intertidal zone and marsh surface at both sites. Crabs were removed from their burrows by gently inserting a trowel next to the burrow until the crab emerged for collection. Size and color differences were used to isolate U. pugnax from other Uca species at the site. Immediately upon arrival to the

laboratory, crabs were rinsed to remove extraneous sediment and placed into plastic storage containers (approximately 42.5 × 31.5 × 15 cm; 17 L) and arranged on top of laboratory benches. Room temperature (24º C) 0.5 µm filtered sea water obtained from Corson's Inlet, New Jersey was then added to each container until the water covered approximately 75% of the angled bottom. Sea water added to PC containers was diluted with deionized water to a salinity of 21 to approximate site salinity conditions while water for SHM crabs was unadjusted at salinity 33. An air stone was submerged in the deepest part of the water, approximately 3 to 4 cm deep.

Ovigerous females were placed into individual glass bowls with enough sea water to cover approximately half of their bodies. Females not observed to be carrying eggs were held in the plastic storage containers and checked for eggs bi-weekly. Once identified as bearing eggs, these females were then removed and placed into individual bowls. The populations of crabs held in the large containers were fed while the ovigerous females were not fed in keeping with the standard practice observed by researchers working with ovigerous fiddler crabs (Nancy O'Connor, pers. comm.).

Batch Fecundity Estimates

Female Atlantic marsh fiddler crabs extrude eggs several times during the breeding season but the fecundity estimated in this study only examined one batch of eggs. Batch fecundity is hereafter referred to as fecundity throughout this paper. Eggs were removed with forceps under a dissecting scope (12×) to ensure that all eggs were removed from the pleopods. The eggs were then placed into a small container of sea water and gently agitated to facilitate separation of the eggs.

Given the large number of eggs carried by a female, I used a gravimetric method commonly used in aquaculture (Case 2001) to estimate the number of eggs per female. Aliquots of known numbers of eggs (three aliquots of ten) were used to calculate the average mass of an egg, and a conversion factor was used to estimate the number of eggs in the total egg mass removed from each crab. All egg samples were oven-dried (60° C) for 24 h and the weight of the dried eggs was calculated for each set of three eggs. The weight of the total egg mass was determined in a similar manner and included the weights from the three sets of 10 eggs. Each of the three weights of 10 eggs were divided by 10 (e.g., Weight #1 /10, Weight #2/10 and Weight #3/10) and the results from these three calculated weights were added together and averaged yielding milligrams per egg (MGPE). Eggs per milligram (EPMG) was then calculated by dividing one by the MGPE (1/MGPE). Finally, the total egg weight was multiplied by the EPMG to yield fecundity. Fecundity estimates were calculated for 50 crabs from PC and 50 crabs from SHM. Carapace width was also measured for each crab to determine if a relationship existed between carapace width and fecundity.

Larval Collection and Observations

Ovigerous females were checked every morning until larval hatching. Upon hatching, a subsample of newly hatched first stage zoea were immediately removed via pipette and fixed in a 1% glutaraldehyde solution after stirring the water for homogenization. Random sub-samples of 50 larvae each were examined from 50 SHM and 50 PC crabs for a site comparison study. Morphological abnormalities in larvae were examined qualitatively using a stereoscope (25×). Eye and body morphological

abnormalities were characterized as either present or absent using Lavolpe's et al. (2004) definitions (Fig. 2-1):

Hypertrophied eyes (HYPR-E) - eyes have a consistently higher number of ommatidia. Hypertrophied sections of eyes appeared either much darker than non-pigmented areas and/or made the eye irregularly shaped in appearance.

Hypopigmented eye (HYPO-E) - the quantity of screening pigments is completely reduced leading to a lighter aspect or a heterogeneous distribution of pigments among ommatidia. Hypopigmented eyes are clearly lighter than darker parts of the eye or in some cases were completely lacking in pigmentation.

Atrophied Eyes - bilateral or unilateral absence of eyes.

Supernumerary eyes, though rare, were also documented if observed.

Hydropsy - this abnormality is a deformity caused by increased tissue volume in the cephalothoracic region. Larvae with hydropsy have a clearly enlarged cephalothoracic region that appears almost balloon-like in appearance.

Atrophied Dorsal Spine (ADS) - the dorsal spine is shorter because of incomplete evagination. Atrophied dorsal spines appear much shorter than unaffected spines and often appear to be truncated or, in some cases, almost non-existent.

Atrophied Maxilliped Setae - setae are invaginated or scarcely evaginated in the distal segments of the maxillipeds. Atrophied setae appear much shorter than unaffected setae and often appear to be truncated, non-existent or withered.

Reciprocal Transplant Exposure Study

A reciprocal transplant exposure study was performed in the laboratory to examine the effects of contaminants in sediment from direct contact or sediment

ingestion on fecundity and larval morphology. The following four treatments were used: SHM crabs on SHM sediment (SHM on SHM), PC crabs on PC sediment (PC on PC), SHM crabs on PC sediment (SHM on PC), and PC crabs on SHM sediment (PC on SHM). Approximately 30 to 40 field-collected fertilized females from SHM and PC who had not yet extruded their eggs were placed onto the sediment in the appropriate exposure treatment. Initial exposure time varied per individual as the time to egg extrusion was unknown upon collection although most crabs were fertile when placed into each treatment as almost all extruded eggs. Crabs were then checked twice weekly for eggs. Once a crab had extruded its eggs, the crab was marked with a water-proof marker to identify it and removed from the sediment after 5 to 7 days. Once removed the crab was either held until larval release for sampling of larvae or the eggs were removed for fecundity estimates. Ten crabs were sampled for fecundity and ten crabs were sampled for larval morphology from each of the four exposure treatments.

RESULTS

Fecundity Estimates: Site Comparison Study

When the two sites were compared, mean fecundity was lower at SHM (500.92 ± 57.7 95% CI) than at PC (1334.12 ± 220.14 95% CI) and a Mann-Whitney rank sum test found this difference to be statistically significant (Table 2-1a). To determine if differences in fecundity could be driven by differences in crab size, a Kruskal-Wallis ANOVA on ranks was performed and revealed that PC crabs were marginally larger than SHM crabs (Table 2-1b). The mean carapace width of PC crabs was 16.0 mm (± 2.15 SD, min = 12.4, max = 20.3) versus 14.7mm (± 2.5 SD, min = 11.7, max = 20.1) at SHM. Multiple linear regression was used to determine the effect of site and carapace width on

fecundity. Regression results show that carapace width explains very little of the variability for fecundity while site explains a highly significant amount (Table 2-1C, Fig 2-2).

Fecundity Estimates: Reciprocal Transplant Exposure Study

Fecundity was slightly greater for PC crabs regardless of sediment treatment (Table 2-2) but results of a two-factor ANOVA revealed that exposure did not affect fecundity in crabs as no significant differences occurred between sites, treatments, or between the interaction of sites and treatments (Table 2-3). Results of a one-factor ANOVA comparing carapace width between PC and SHM crabs found no differences in crab size between sites ($p = 0.053$). Mean crab size for PC crabs was 14.9 mm (± 1.5 SD) and 14.0 mm (± 1.4 SD) for SHM crabs. A two-factor ANOVA was used for comparisons of fecundity between crab site and sediment site. Though not statistically significant, fecundity for SHM crabs on PC sediment was greater than SHM crabs on SHM sediment though an increase in fecundity cannot be attributed to sediment exposure (See Discussion).

Larval Morphology Observations: Site Comparison Study

For the site comparison study, 32% of SHM larvae and 70.5% of PC larvae exhibited eye, body, or both eye and body morphological abnormalities. Abnormalities were greater in PC larvae with the exception of hypertrophied eyes which were similar between the two sites (Table 2-4). Body morphological abnormalities were greater than eye abnormalities at PC with the exception of hypopigmented eyes (Table 2-4). The proportion of eye and body abnormalities was similar at SHM with the exception of

hydropsy which was the most commonly observed abnormality in SHM larvae (Table 2-4).

Site comparison study data did not pass the test for normality regardless of attempts at data transformation so Mann-Whitney Rank Sum tests were performed to compare abnormalities between sites. For eye abnormalities, HYPR-E and AE were not significantly different between sites but the proportion of HYPO-E was significantly greater at PC (Table 2-5). For body abnormalities, hydropsy and AMS were marginally significantly greater at PC (Table 2-5).

Larval Morphology Observations: Reciprocal Transplant Exposure Study

Out of the four exposure treatments, the proportion of body morphological abnormalities was greater for PC crabs while eye abnormalities were similar between the four treatments (Table 2-4). Exposure study data did not pass the test for normality regardless of attempts at data transformation so a Kruskal-Wallis ANOVA on ranks was used to compare differences between sites. For the eye abnormality HYPR-E there was a significant difference between treatments (Table 2-6) and although multiple comparison tests did not reveal differences this abnormality was slightly greater for SHM crabs on SHM sediment (Table 2-4). HYPO-E was also significantly different between sites (Table 2-6) and Student-Newman-Keuls (SNK) results found HYPO-E to be significantly greater for PC crabs on SHM sediment versus PC crabs on PC sediment. Hydropsy was significantly different between treatments (Table 2-6) but SNK results that hydropsy was different between all treatments with the exception of SHM crabs on PC sediment versus SHM crabs on SHM sediment. The abnormalities ADS and AMS were also significantly different between treatments (Table 2-6). For both ADS and AMS, SNK results revealed

significant differences between all treatment combinations excluding PC crabs on SHM sediment versus PC crabs on PC sediment and SHM crabs on PC sediment versus SHM crabs on SHM sediment.

DISCUSSION

Both laboratory and field data examining the effects of contaminants on fiddler crab fecundity and larval morphology are limited. More specifically, the few laboratory toxicity tests performed have no supplemental field data which would create a more holistic picture of the reproductive effects of contaminant exposure. To advocate the field validation of laboratory toxicity tests is unrealistic (Chapman 1995). I instead suggest that the field data collected here provide important information about the real-world reproductive conditions of U. pugnax from a contaminated and non-contaminated site. This field data, when combined with and compared to peer-reviewed laboratory toxicity data, will help determine whether correlations exist between field and controlled conditions and, if present, the nature of these relationships.

Fecundity

In this study, fecundity was higher at the contaminated site (PC) for both the site comparison and reciprocal transplant exposure studies. Larger crabs and higher fecundity at PC when compared to SHM was also discovered by Bergey and Weis (2008). While site was more relevant to fecundity than carapace width in this study, most studies of Uca spp. and other crustaceans find that fecundity increases with body size (Haddon 1994, Pinheiro and Terceiro 2000, Nakata and Goshima 2004, Hamasaki et al. 2006). It is possible that the relationship between fecundity and carapace width would be stronger in this study by estimating fecundity for a wider range of size classes. Hines (1982)

examined the allometry of reproductive effort in 20 species of Brachyuran crabs including U. pugnax and determined that the female body size principally determines reproductive output in Brachyuran crabs (R = 0.962 for U. pugnax). In other studies of fiddler crabs, Goshima et al. (1996) found a significant correlation ($r^2 = 0.50$) between carapace width and number of eggs for U. tetragonon and Salmon (1984) also shows fecundity positively correlated with female size (r = 0.6021). While all these studies show a relationship between carapace width and fecundity for a number of crustacean species and habitats, most acknowledge that environmental conditions may also influence fecundity. It is important to consider these conditions when comparing fecundity between PC and SHM.

In the reciprocal transplant exposure study, fecundity was slightly greater for PC crabs regardless of sediment treatment and exposure to sediment collected from either PC or SHM did not impact fecundity in crabs collected from either site. Though not statistically significant, fecundity for SHM crabs on PC sediment were greater than SHM crabs on SHM sediment. Given that SHM females were fertilized by SHM males it is unlikely that exposure to PC sediment would increase fecundity. Higher fecundity for SHM crabs on PC sediment likely results from natural variability in fecundity or perhaps error introduced in fecundity calculations. Significant reductions in fecundity would have resulted from ingestion of or direct contact with contaminated sediment.

Fecundity may differ between these sites due to the inherent variability in natural conditions such as food quality, temperature, and salinity. Goshima et al. (1996) reports that body size accounts for only up to 50% of the variance in fecundity in U. tetragonon and partly attributes this variation to differences in nutrition. Bergey and Weis (2008)

cite higher C and N concentrations at PC and hypothesize that higher concentrations of C equate to better food quality and thus larger crabs and higher fecundity at PC. Horng and Taghon (1999), however, cite higher N concentrations at Schooners Creek, a location near SHM, which may mean food quality is higher at SHM as detritivores are known to be N-limited (Marsh and Tenore 1990). Also, higher C content does not necessarily translate into greater available energy as C does not strictly estimate available food energy (Marsh and Tenore 1990). Differences in nutrition may have contributed to differences in fecundity in this study as C, N and C:N clearly differ between sites (Horng and Taghon 1999) but more specific chemical analyses of the sediment are required to understand how food quality may affect U. pugnax fecundity at PC and SHM.

Regarding other site variables, temperatures were similar between sites during the sampling period (D. Haroski, unpublished data) and while fiddler crabs can tolerate high summer temperatures, O'Hara (1973) found that high temperatures accentuate the toxic effects of even small amounts of Cd in U. pugilator. Although contaminants were lower at SHM, O'Hara's study suggests that even low levels of contaminants can be toxic under the right conditions which could affect reproductive effort which may result in decreased fecundity at SHM.

Temperature may also affect fecundity at the sites in other ways besides the synergistic interactions with contaminants. Bergey and Weis (2008) report a longer reproductive season for SHM crabs than PC crabs and hypothesize that increasing water temperatures at SHM (from 1976 to 1990) may result in an increased breeding season. I suggest that a longer breeding season may result in an increased number of broods throughout the season but perhaps a corresponding decrease in batch fecundity per brood.

A decrease in batch fecundity resulting from a longer breeding seasons could explain why batch fecundity is lower for SHM crabs. Future work should investigate egg size per batch as increased egg size may correlate with reduced batch fecundity per brood (Sibly and Calow 1986).

Salinity was lower at PC (21) than SHM (33) and these salinity levels are within those tolerated by U. pugnax (Miller and Maurer 1973, Baldwin and Kirschner 1976). Sudden changes in salinity, however, result in decreased growth, survival, and fecundity in the mole crab Emerita brasiliensis (Lercari and Defeo 1999). The authors hypothesize that fluctuation in local conditions causes stress which inhibits the conversion of food energy into eggs as energy is diverted into maintenance rather than reproduction. Others have shown that the synergistic effects of salinity, temperature, and mercury decrease survival rates in U. pugilator (Vernberg and Vernberg 1972). Given the ability of U. pugnax to tolerate a broad salinity range (Baldwin and Kirschner 1976) it is difficult to predict if salinity contributed to differences in fecundity between sites though this cannot be strictly ruled out. The osmoregulatory capabilities of U. pugnax, however, combined with the fairly constant salinities at both PC and SHM make it unlikely that salinity contributed to fecundity differences between sites. The synergistic effects of food quality, temperature, and other abiotic and biotic factors, however, may have affected fecundity in this study and future studies should examine the role of abiotic parameters.

Variability in environmental conditions may have contributed to differences in fecundity in this study but consideration should also be given to the presence of contaminants at each site. Although contaminant levels were lower at SHM, O'Hara's (1973) study linking high temperatures to increased Cd toxicity implies that synergistic

effects can increases availability and toxicity. It is also possible that even though contaminant levels are lower at SHM, crab bioturbation could be concentrating or making contaminants more bioavailable (Menome et al. 2004) thus increasing the possibility of sublethal effects. Conversely, although contamination is generally higher at PC, crabs can metabolize certain contaminants thus lessening the potential for sublethal reproductive effects (Burns 1976, Mc Enerney and Davis 1979). Also, the presence of contaminants does not necessarily translate into bioavailability as many factors, including physiochemical factors and the chemical form of the contaminant (e.g., dissolved versus particulate phases) (Rand et al. 1995, Blasco et al. 1999), can affect bioavailability especially in short-term laboratory exposures.

Although field studies specifically examining the effects of contaminants on fiddler crab fecundity appear to be limited, contaminants have been shown to affect reproduction. For example, cadmium was shown to inhibit gonadal growth in U. pugilator (Kogan et al. 2000) which could lead to fecundity effects. O'Clair and Freese (1988) found that Dungeness crabs (Cancer magister) near contaminated log transfer facilities had lower fecundity than the control site. Similarly, Zulkosky et al. (2002) found that fecundity was reduced in the benthic crustacean Leptocheirus plumulosus exposed to sewage-impacted sediment. While contaminants could have contributed to decreased fecundity at SHM, more work examining contaminants and the synergistic effects of natural parameters is necessary. The synergistic and [GT1]antagonistic effects discussed here emphasize the dynamic nature of field conditions and the myriad of interactions that can affect contaminant exposure. Understanding the influence of these complex relationships in the field can provide important insights regarding the

interpretation of toxic responses and supports the argument for the collection of field data to support laboratory studies.

While body size, natural variability, and contaminants can affect fecundity, it is important to review fecundity estimation methods and study design. In this study fecundity was estimated gravimetrically where the total number of eggs was extrapolated from the dry weight of three counted subsamples and the dry weight of the total egg mass. Mean fecundity for PC was 1330 and 500 for SHM, but these numbers are lower than fecundity estimates typically given for U. pugnax. For example, Grimes et al. (1989) estimate fecundity ranging from 4,500 to 23,700 eggs although DeCoursay (1979) estimates clutches from 1,500 to 94,000. Bergey and Weis (2008) report estimated egg production of 111,214 eggs/m^2 for PC and 226,030 eggs/m^2 for SHM. Even though fecundity estimates in the literature differ widely, the fecundity estimates in this study still underestimate total clutch size. Error may have been introduced during the handling or weighing of individual eggs or during dehydration. In addition, eggs were not histologically staged for this study so differences in yolk volume or developmental stage may also have introduced variability. Also, a sub-sample of 50 crabs from each location may not adequately represent fecundity for varying size classes. Increasing the number of crabs sampled per size class could improve the calculation of the allometric relationship between carapace width and fecundity.

Larval Morphology

Similar to fecundity research in fiddler crabs, no field studies examining the effects of contaminants on larval morphology could be identified. Most studies examining morphological abnormalities in crustacean larvae are controlled laboratory

exposures. As such, it is challenging to compare the field-collected results of this study to laboratory results given the complex interactions, relationships and fluctuations that occur in nature. In addition, most studies examining exposure effects on larvae expose ovigerous females to water column concentrations of contaminants which may not be the most realistic exposure pathway for U. pugnax.

Ovigerous females of some burrowing Brachyuran crabs, like U. pugnax in this study, plug their burrows during high tide (Botto and Iribarne 2000) thus limiting their exposure to water-borne contaminants. Contaminant exposure most likely occurs through ingestion of sediment or physical contact with sediment (Menome et al. 2004). While water entering open burrows might expose some species, like C. granulata, directly to water-borne contaminants, exposure to interstitial water might be a more realistic exposure pathway for species like U. pugnax. Water column toxicity tests using ovigerous females do not provide a holistic picture of contaminant-mediated reproductive effects for all burrowing Brachyuran crabs especially those that remain in burrows. A suggested method for assessing the effects of estuarine contaminants on some Uca spp. would be to use interstitial water. In this approach, interstitial water is isolated from the sediments and then used for water column toxicity tests (EPA 2007). This method would provide a more accurate exposure pathway for certain crab species like U. pugnax.

Despite potential limitations, comparisons of field data to laboratory results yield some interesting observations. In this study, although the proportion of larval morphological abnormalities was greater at the contaminated site (PC), abnormalities were commonly detected in SHM larvae. It is not clear why abnormalities occur at SHM but as the proportion of naturally occurring abnormalities in all Uca spp. is currently

unknown, future work should attempt to determine the occurrence of abnormalities absent contaminants before comparisons to contaminant-exposed crabs are made. While contaminant levels are lower at SHM, some contaminants are present and crab activity could be trapping these contaminants near crab burrows. Research comparing the effects of two burrowing crabs (C. granulata and U. uruguayensis) on sediment composition and transport find higher water content and organic matter in and around crab burrows (Botto and Iribarne 2000). Further, bioturbation by C. granulata was found to increase concentrations of organochlorine pesticides in crab beds and facilitated the biotransformation of parent compounds (like DDT) into their respective metabolites (Menome et al 2004).

When calculating bioaccumulation factors, Menome et al. (2004) reports higher bioaccumulation from sediments inside the crab burrow. This implies that, for a species like U. pugnax which remains in the burrow while carrying eggs (Henmi and Kaneto 1989a), the potential for bioaccumulation of contaminants could be higher which could translate into reproductive effects. Future studies should measure sediment contaminant levels inside burrows. Additionally, the role of maternal transfer in fiddler crabs has not been researched but should be evaluated as a potential exposure pathway. Recent work examining maternal transfer of metals in cuttlefish found Ag, Se, and Zn were transferred to eggs at least during the last two weeks before spawning (Lacoue-Labarthe et al. 2008). Additionally, egg exposure via direct contact with contaminated sediment and sediment-associated waters should also be addressed

Overall, the percentage of larvae exhibiting morphological abnormalities was 70.5% at PC compared to 32% at SHM, likely resulting from higher contaminant

concentrations at PC. Exposure to contaminated or non-contaminated sediment in the exposure study did not appear to influence abnormalities as exposure to contaminated sediment or clean sediment did not appear to increase or decrease abnormalities, respectively. Interestingly, Bergey and Weis (2008) observed smaller population density and reduced early benthic phase crabs for U. pugnax at PC compared to SHM. The high proportion of abnormal larvae at PC likely reduces survival and recruitment and may be one factor contributing decreased population size at this site. When the abnormalities observed in this study have been observed for the terrestrial land crab C. granulata, stage I zoeae never molted to the next stage and abnormalities proved to be lethal (E. Rodriguez, personal comment) which would likely be the case for larvae in this study. An exception to this occurs for pea crab larvae (Tunicotheres moseri) exposed to Cu where zoeae stage II that hatch with abnormal setation revert back to normal setation when molting to the megalopal stage (Lopez Greco et al. 2001).

The most common morphological abnormalities observed in PC and SHM larvae were unspecific pathologies likely manifested as a general response to pesticides and metals. These abnormalities are similar to those observed in laboratory exposures. Hydropsy, the most common abnormality at SHM and second most common at PC, is typically observed after exposure to diverse pesticides and metals (Zapata et al. 2001). Hypopigmented eyes, the most commonly occurring abnormality in PC larvae, is also an unspecific pathology as it appears after exposure to all metals (Lavolpe et al. 2004). Supernumerary eyes and eye atrophy, both of which occurred in low numbers at PC, have been found to correspond to specific metals like Pb and Cu (Lavolpe et al. 2004) which are present at PC.

Amin et al. (1998) also finds that the teratogenic effects of heavy metals are nonspecific as malformations were similar in larvae exposed to both Cd and Pb. The control larvae in Amin et al. (1998), however, exhibited the same abnormalities as the exposed larvae but simply to a lesser extent. The authors' acknowledgement of abnormal control larvae and the presence of abnormalities from the non-contaminated site in this study raise questions regarding the use of control larvae in laboratory studies. Researchers should take care to identify and quantify all abnormalities in control larvae to ensure accurate comparisons to exposure data. The manifestation of abnormalities in control larvae could result from the unknown presence of contaminants. Future studies should also rule out genetic influences.

Summary

The field data collected in this study found higher fecundity at the contaminated site and abnormal larvae at the non-contaminated site which intuitively would not be expected results given the presence or lack of contaminants, respectively. These results highlight the unpredictability of field data and the deviation of real world conditions from controlled laboratory experiments and predicted outcomes. Ideally, the best understanding of contaminant exposure on reproductive fitness occurs through a combination of baseline field data and multiple lines of toxicity evidence (e.g., single species toxicity tests, ambient tests and *in situ* tests). In addition to this data, it has also been suggested that data on a number of biological variables on several levels of organization, including behavioral, cellular and physiological responses, should also be collected (Hebel et al. 1997).

Although field testing is recommended to address uncertainties that may result from laboratory extrapolations (Graney et al. 1995), few studies could be found which assess reproductive condition in field collected Brachyuran crabs from contaminated sites. Field data can provide insights on possible causality and a more realistic understanding of the contaminant-related impacts on Brachyuran reproduction. This is not to suggest that field validation is required for sediment toxicity tests (Chapman 1995). The complex interactions of biotic and abiotic factors in the field make it difficult and often impossible to validate carefully controlled laboratory studies. Also, while laboratory results may be replicated in the field, a lack of field replication does not imply that laboratory results are untrue. This is not to suggest that laboratory studies are unnecessary as carefully controlled laboratory studies help to quantify the actual effects of contaminant-induced toxicity. A combination of laboratory and field data, however, provides the best viewpoint and perspective on cause-and-effect and, ultimately, assessments of ecological condition. The collection of field data in this study supplements our understanding of laboratory toxicity testing and permits a more holistic picture of contaminant exposure and, ultimately, ecological condition in U. pugnax.

Table 2-1a.

Mann-Whitney rank sum test results (α = 0.05) for the comparison of batch fecundity between Piles Creek (PC) and Sheepshead Meadows (SHM). Fecundity was significantly greater at PC.

Site	N	Median	25%	75%	P
PC	50	1218.32	971.54	1540.3	p<0.001
SHM	50	495.56	390.37	626.7	

Table 2-1b.

Kruskal-Wallis one-factor Analysis of Variance (ANOVA) (α = 0.05) results for the comparison of carapace widths between Piles Creek (PC) and Sheepshead Meadows (SHM). Fiddler crabs were marginally larger at PC.

Site	N	Median	25%	75%	P
PC	50	15.7	14.4	16.4	p=0.003
SHM	50	13.9	12.6	0.23	

Table 2-1c.

Multiple linear regression results for carapace width, site and fecundity for Piles Creek (PC) and Sheepshead Meadows (SHM). With fecundity as the dependent variable, results show that carapace width explains very little of the variability for fecundity while site explains a highly significant amount.

Fecundity = 606.40 + (45.57 * Carapace Width) - (777.42 * Site)

Regression	Coefficient	Std. Error	t	p	R
Constant	606.4	398.52	1.52	0.131	0.615
Carapace Width	45.57	24.46	1.86	0.65	
Site	-777.42	115.78	-6.72	<0.001	
ANOVA	**DF**	**SS**	**MS**	**F**	**P**
Regression	2	18440918.39	9220459.2	29.49	<0.001
Residual	97	30330673.71	312687.36		
Total	99	48771592.1	492642.34		

Table 2-2.

Mean batch fecundity for Piles Creek (PC) and Sheepshead Meadows (SHM) for the reciprocal tranplant exposure study. Sample site was either Piles Creek (PC) or Sheepshead Meadows (SHM) and exposure treatments were: PC crabs on PC sediment; PC crabs on SHM sediment; SHM crabs on SHM sediment; and SHM crabs on PC sediment.

Exposure Treatment	Mean Fecundity (± 95% Confidence Interval)
PC on PC	1011.88 (279.64)
PC on SHM	1012.85 (533.14)
SHM on SHM	462.6 (74.1)
SHM on PC	809.82 (412.87)

Table 2-3.

Two-factor Analysis of Variance (ANOVA) results ($\alpha = 0.05$) for batch fecundity in reciprocal transplant exposure study. Sample site was either Piles Creek (PC) or Sheepshead Meadows (SHM) and exposure treatments were: PC crabs on PC sediment; PC crabs on SHM sediment; SHM crabs on SHM sediment; and SHM crabs on PC sediment. Exposure to sediment collected from either site did not affect fecundity in crabs collected from either site.

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Crab Site	1	1414898.23	1414898.23	6.062	0.019
Sediment Site	1	299734.6	299734.6	1.284	0.265
Crab Site x Sediment Site	1	303095.08	303095.08	1.299	0.262
Residual	36	8402555.12	233404.31		
Total	39	10420283	267186.74		

Table 2-4.
The proportion of eye and body morphological abnormalities in U. pugnax larvae for both the site comparison study and the reciprocal transplant exposure study. Sample site was either Piles Creek (PC) or Sheepshead Meadows (SHM) and exposure treatments were: PC crabs on PC sediment; PC crabs on SHM sediment; SHM crabs on SHM sediment; and SHM crabs on PC sediment.

	Site Comparison Study		Reciprocal Transplant Exposure Study			
Abnormality	**SHM**	**PC**	**SHM on SHM**	**SHM on PC**	**PC on PC**	**PC on SHM**
HYPR-E	0.047	0.041	0.0196	0	0.0019	0.014
HYPO-E	0.06	0.24	0.0039	0.0019	0.016	0.023
AE	0.008	0.01	0	0	0.0019	0.0019
Multiple Eyes	0.0004	0.002	0	0	0	0
Hydropsy	0.13	0.22	0.018	0.018	0.106	0.16
ADS	0.04	0.095	0.0019	0.006	0.11	0.11
AMS	0.03	0.1	0.0019	0.004	0.037	0.043

Hypertrophied eyes (HYPR-E) - eyes have a consistently higher number of ommatidia

Hypopigmented eye (HYPO-E) - the quantity of screening pigments is completely reduced leading to a lighter aspect and/or a heterogeneous distribution of pigments among ommatidia

Atrophied Eyes (AE) - bilateral or unilateral absence of eyes

Hydropsy - this abnormality is a deformity caused by increased tissue volume in the cephalothoracic region

Atrophied Dorsal Spine (ADS) - the dorsal spine is shorter because of incomplete evagination

Atrophied Maxilliped Setae (AMS) - setae are invaginated or scarcely evaginated in the distal segments of the maxillipeds

Table 2-5.

Statistical results for comparisons of larval morphological abnormalities between Piles Creek (PC) and Sheepshead Meadows. For eye abnormalities, HYPR-E and AE were not significantly different between sites. For body abnormalities, hydropsy and AMS were marginally significantly greater at PC .

Abnormality & Site					
HYPR-E	**N**	**Median**	**25%**	**75%**	**P**
SHM	50	0	0	2	p=0.521
PC	50	0	0	2	
HYPO-E	**N**	**Median**	**25%**	**75%**	**P**
SHM	50	1	0	2	p=0.044
PC	50	2	0	24	
AE	**N**	**Median**	**25%**	**75%**	**P**
SHM	0	0	0	0	p=0.844
PC	0	0	0	0	
Hydropsy	**N**	**Mean**	**Std Dev**	**SEM**	**P**
SHM	0	2.5	0	12	p=0.052
PC	0	6	2	17	
ADS	**N**	**Median**	**25%**	**75%**	**P**
SHM	50	1	0	2.00	p=0.174
PC	50	2	0	7	
AMS	**N**	**Median**	**25%**	**75%**	**P**
SHM	50	1	0	2.00	p=0.065
PC	50	2	0	4	

Hypertrophied eyes (HYPR-E) - eyes have a consistently higher number of ommatidia

Hypopigmented eye (HYPO-E) - the quantity of screening pigments is completely reduced leading to a lighter aspect and/or a heterogeneous distribution of pigments among ommatidia

Atrophied Eyes (AE) - bilateral or unilateral absence of eyes

Hydropsy - this abnormality is a deformity caused by increased tissue volume in the cephalothoracic region

Atrophied Dorsal Spine (ADS) - the dorsal spine is shorter because of incomplete evagination

Atrophied Maxilliped Setae (AMS) - setae are invaginated or scarcely evaginated in the distal segments of the maxillipeds

Table 2-6.
Kruskal-Wallis one-factor Analysis of Variance (ANOVA) on ranks ($\alpha = 0.05$) results for differences in eye and body larval morphological abnormalities between exposure treatments. Sample site was either Piles Creek (PC) or Sheepshead Meadows (SHM) and exposure treatments were: PC crabs on PC sediment; PC crabs on SHM sediment; SHM crabs on SHM sediment; and SHM crabs on PC sediment. Hydropsy, atrophied dorsal spine and atrophied maxilliped setae were significantly different between treatments.

Abnormality/Treatment						
HYPR-E	**N**	**Missing**	**Median**	**25%**	**75%**	**P**
SHM on SHM	10	0	0.50	0	2.00	p=0.027
SHM on PC	10	0	0	0	0	
PC on PC	10	0	0	0	0	
PC on SHM	10	0	0	0	1.00	
HYPO-E	**N**	**Missing**	**Median**	**25%**	**75%**	**P**
SHM on SHM	10	0	0	0	1.00	p=0.006
SHM on PC	10	0	0	0	0	
PC on PC	10	0	0	0	1.00	
PC on SHM	10	0	1.00	1.00	2.00	
Hydropsy	**N**	**Missing**	**Median**	**25%**	**75%**	**P**
SHM on SHM	10	0	0	0	2.00	p<0.001
SHM on PC	10	0	0.50	0	2.00	
PC on PC	10	0	3.00	0	9.00	
PC on SHM	10	0	8.50	4.00	12.00	
ADS	**N**	**Missing**	**Median**	**25%**	**75%**	**P**
SHM on SHM	10	0	0	0	0	p<0.001
SHM on PC	10	0	0	0	0	
PC on PC	10	0	3.50	1.00	8.00	
PC on SHM	10	0	4.50	2.00	9.00	
AMS	**N**	**Missing**	**Median**	**25%**	**75%**	**P**
SHM on SHM	10	0	0	0	0	p<0.001
SHM on PC	10	0	0	0	0	
PC on PC	10	0	1.00	1.00	4.00	
PC on SHM	10	0	1.00	1.00	5.00	

Hypertrophied eyes (HYPR-E) - eyes have a consistently higher number of ommatidia

Hypopigmented eye (HYPO-E) - the quantity of screening pigments is completely reduced leading to a lighter aspect and/or a heterogeneous distribution of pigments among ommatidia

Atrophied Eyes (AE) - bilateral or unilateral absence of eyes

Hydropsy - this abnormality is a deformity caused by increased tissue volume in the cephalothoracic region

Atrophied Dorsal Spine (ADS) - the dorsal spine is shorter because of incomplete evagination

Atrophied Maxilliped Setae (AMS) - setae are invaginated or scarcely evaginated in the distal segments of the maxillipeds

Fig. 2-1

Eye and body morphological abnormalities in Chasmagnathus granulata larvae (bar = 10μm) exposed to heavy metals (Lavolpe et al., 2004, used with permission of E. Rodríguez). A = normal eye, B = eye hypertrophy (HYPR-E) and eye hypopigmentation (HYPO-E), C = eye atrophy (AE), D = normal dorsal spine, E = normal maxilliped setae, F = whole control larvae, G= atrophied dorsal spine (ADS), H= atrophied maxilliped setae (AMS), I= Hydropsy.

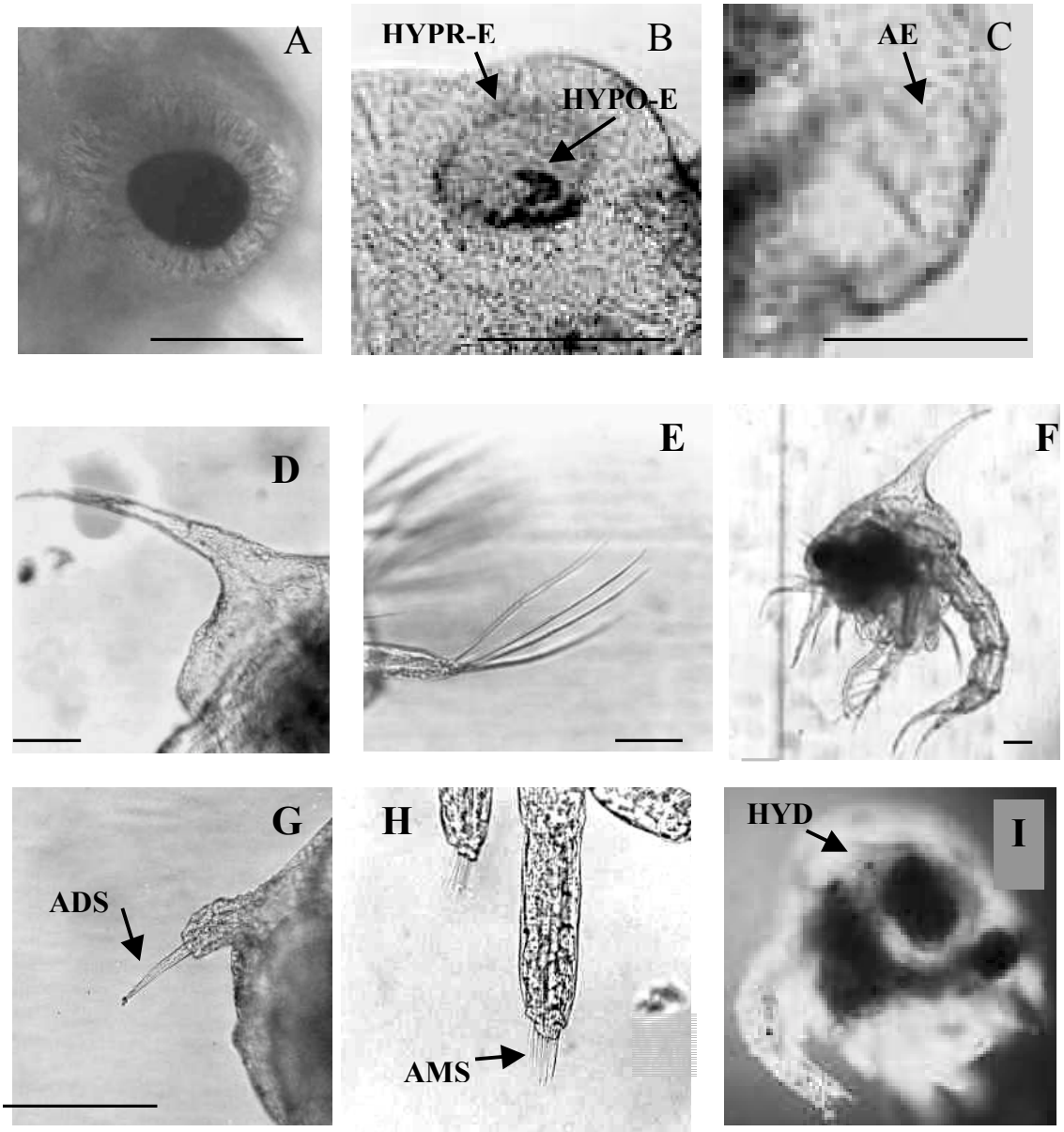
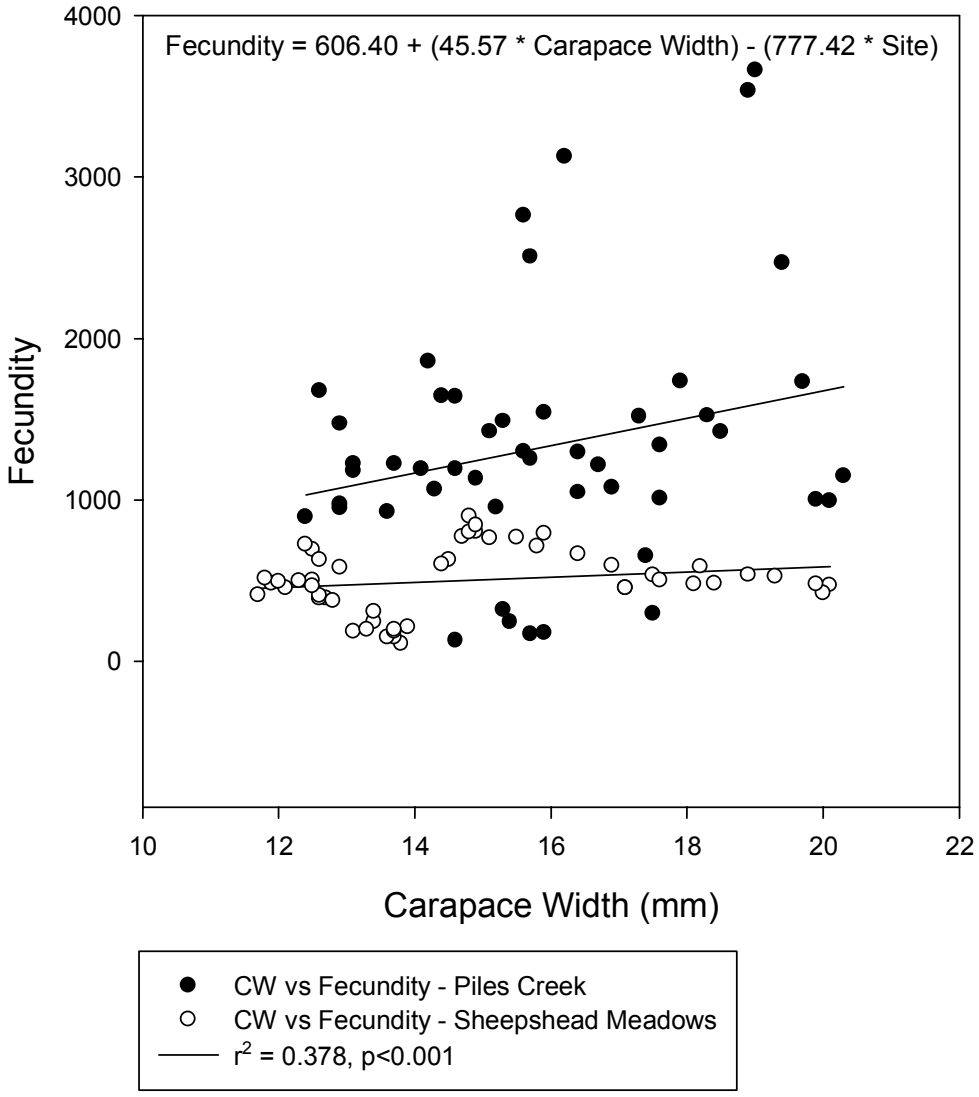


Fig. 2-2.

Multiple regressions for fecundity and carapace width
for Piles Creek (PC) and Sheepshead Meadows (SHM).



CHAPTER THREE

Oophagy in Female Uca pugnax (Smith):

Roles of Food Availability and Contaminants in Filial Cannibalism

ABSTRACT

Oophagy, or egg ingestion, was quantitatively documented for U. pugnax from a contaminated and non-contaminated site. Video observations were used to document crab behavior in both a site comparison and feeding study. The site comparison study simply looked for differences in behavior between the contaminated and non-contaminated site. The feeding study was performed to determine the role of food in oophagy and whether egg ingestion stops or decreases if food pellets are offered. Claw to mouthpart movements were recorded and the duration, activity, and number of eating events was determined. Fecundity was also estimated for all crabs from both studies to determine if oophagy significantly reduced fecundity.

For the site comparison study, the presence of contaminants did not affect oophagy as there were no statistically significant differences in egg ingestion between sites. For the feeding study, crabs from both sites still ingested eggs even if food pellets were present although pellet ingestion was higher than egg ingestion for both sites. Egg and pellet ingestion were similar between the contaminated and non-contaminated site for the feeding study. Egg ingestion in the site comparison study was similar to egg ingestion in the feeding study. This similarity in egg ingestion between the two studies indicates that the presence of food does not decrease or stop egg ingestion.

Egg ingestion almost always occurred in conjunction with abdominal contractions in both the site comparison and feeding study crabs. The only marginally significant

relationships between abdominal contractions and egg ingestion parameters occurred for SHM crabs in the site comparison study. Overall, mean fecundity was higher for crabs from the non-contaminated site in both the site comparison and feeding studies.

INTRODUCTION

Female Atlantic marsh fiddler crabs (Uca pugnax), similarly to other decapod crustaceans, extrude newly fertilized eggs onto the pleopods (Crane 1975) and carry them under the abdomen in a large egg mass or "sponge" (Tankersley et al. 1998). Researchers studying the eggs or released larvae of "berried" crabs often keep individual ovigerous females in the laboratory until the larvae or zoeae are released. Typically, female U. pugnax are not fed during this period as common belief holds that little to no feeding occurs in nature during egg incubation (Christy and Salmon 1984, Naylor et al. 1999). Females actively care for the egg mass, however, and will pump their abdomen while beating the pleopods, presumably to ventilate the eggs (Naylor et al. 1999). This action is similar to the fanning motion initiated by females to facilitate the release of the hatching zoeae (Saigusa 1996).

While observing ovigerous U. pugnax in the laboratory, I noted that egg ingestion (or oophagy) appeared to correlate with abdominal pumping (egg oxygenation). Crabs from both a contaminated and a non-contaminated site were observed yet, at the time, only crabs from the contaminated site ingested eggs. Egg ingestion or filial cannibalism, the eating of one's own offspring (FitzGerald 1992), has not been documented for fiddler crabs, and no accounts of oophagy in crabs could be found in the literature.

Some ecological factors favoring cannibalism include the maximization of lifetime reproductive output via the parental manipulation of brood size, low food availability, or

the parental allocation of resources to future offspring, i.e., some of the offspring produced will actually serve as food to the sibling offspring (Elgar and Crespi 1992). Oophagous behavior in female U. pugnax may occur for any of these reasons, yet hunger, one of the most commonly cited factors instigating cannibalism (Dong and Polis 1992), may be most responsible in non-fed laboratory-confined individuals that may normally be feeding in the field.

As most U. pugnax remain in their burrows during incubation (Crane 1975), the degree to which they may be foraging and the amount of sediment they may be ingesting are unknown. It has been hypothesized that female ocypodids carrying a relatively large number of eggs in a single brood do not feed actively as stress from heat and desiccation require underground incubation and limited foraging (Koga et al. 2000). In contrast, crabs carrying smaller broods experience less stress and feed actively enough on the surface to allow for the internal development of the next brood (Koga et al. 2000). Given that the feeding habits of ovigerous U. pugnax are unknown, a goal of this study was to document the occurrence of oophagy and to determine the role of food availability in egg cannibalism in both fed and non-fed crabs from both the contaminated and non-contaminated site.

There are costs associated with cannibalism, perhaps the greatest being the potential loss of fitness that occurs when a genetically related individual is killed and consumed (Elgar and Crespi 1992). Egg ingestions may be costly in terms of lost fitness but additional consequences, such as effects on population dynamics, may be another negative aspect of cannibalism. It is currently not clear whether cannibalism stabilizes or de-stabilizes populations (Dong and Polis 1992). Before one can assess population level effects, however, an estimate of eggs consumed must be calculated to determine whether

oophagy appreciably decreases clutch size. The second goal of this proposed study, therefore, was to determine whether oophagy affects fecundity.

The goals of this study were to quantitatively document oophagy and some potential stimuli in U. pugnax and determine whether reduced fecundity was a resulting cost of cannibalism. The following three questions were addressed: 1) do contaminants in sediment cause oophagy; 2) does oophagy occur to provide a nutritional benefit to non-fed ovigerous females; and 3) does egg ingestion reduce fecundity in oophagous crabs? The answer to the third question will be relevant to researchers quantifying fecundity in laboratory-held crustaceans. The common practice of withholding food may contribute to or induce oophagy which may reduce fecundity.

RESEARCH LOCATIONS

Sheepshead Meadows (SHM)

The reference or "non-contaminated" site was Sheepshead Meadows (SHM) located within the Barnegat Bay-Little Egg Harbor estuarine system in southern New Jersey (39° 18' N, 74° 12' W). The SHM site is located on the southern tip of a salt marsh peninsula removed from any major industries or point contaminant sources. The system is one of the least disturbed in the northeast United States (Psuty et al. 1993) and, partly as a result, was included in the National Oceanic and Atmospheric Administration (NOAA)-operated National Estuarine Research Reserve System on October 20, 1997 (Able et al. 1996). The estuary is classified as a highly eutrophic estuary based on NOAA's National Estuarine Eutrophication Assessment model (Kennish et al. 2007). The lower estuary is polyhaline with a salinity range of 23.6 to 34.5 at the nearby Rutgers University Marine Field Station (Able et al. 1992). At the nearby Schooners Creek, mean C is 45.7 mg/g, mean N is 4.48

mg/g (Horng and Taghon 1999) and C:N ratios range from 12.1 to 13.6 (G. Taghon, unpublished data). Seasonal water temperatures range from -2º C to 28º C (Able et al. 1992) with a tidal range of 1.1 m near the mouth of Great Bay (Martino and Able 2003). Some low levels of contaminants have been measured at near the site and mean metal levels (µg/g) were: Hg 0.19, Cu 43.8, Pb 73.2, Zn 141, Cd 2.1 (Weis et al. 2001a, Weis et al. 2001b).

Contaminated Site

Piles Creek (PC) (40° 36.5' N, 74° 13.6'W) in Linden, New Jersey is a tributary of the Arthur Kill, the navigational channel between New Jersey and Staten Island, New York. Piles Creek served as the "contaminated" site as the creek is surrounded by industrial sites such as petroleum refineries, oil storage tanks, a sewage treatment plant, and a major highway. The site has likely received contaminants from nearby industries and oil spills in the Arthur Kill (Bergey and Weis 2008). Site contaminants include metals, pesticides, polycyclic aromatic hydrocarbons (PAHs), and some polychlorinated biphenyls (PCBs). Mean metal levels (µg/g) have previously been measured for Hg 6.3, Cu 485, Pb 107, Zn 525, Cd 7.1 (Weis et al. 2001a, Weis et al. 2001b). Salinity measurements at the site consistently ranged from 20 to 23 during the sampling period. Mean C at the site is 44.6 mg/g and N is 3.32 mg/g (Horng and Taghon 1999) while C:N range from 16.3 to 19.6 (G. Taghon, unpublished data).

MATERIALS AND METHODS

Crab Collection and Handling

Female U. pugnax were collected at low tide from the intertidal zone and marsh surface at both sites. Crabs were removed from their burrows by gently inserting a trowel

next to the burrow until the crab emerged for collection. Size and color differences were used to isolate U. pugnax from other Uca species at the site. Immediately upon arrival to the laboratory, crabs were rinsed to remove extraneous sediment and placed into plastic storage containers (approximately 42.5 × 31.5 × 15 cm; 17 L) and arranged on top of laboratory benches. Room temperature (24º C) 0.5 µm filtered sea water obtained from Corson's Inlet, New Jersey was then added to each container until the water covered approximately 75% of the angled bottom. Sea water added to PC containers was diluted with deionized water to a salinity of 21 to approximate site salinity conditions while water for SHM crabs was unadjusted at salinity 33. An air stone was submerged in the deepest part of the water, approximately 3 to 4 cm deep.

Egg-bearing females were placed into individual glass bowls with enough sea water to cover approximately half of their bodies. Females not observed to be carrying eggs were held in large plastic containers and checked for eggs bi-weekly. Once identified as bearing eggs, these females were then removed and placed into individual bowls. Crabs held in the large containers were fed while the isolated ovigerous females were not fed in keeping with the standard practice observed by researchers working with ovigerous fiddler crabs (Nancy O'Connor, pers. comm.).

Site Comparison Study and Feeding Study: Video Observations

Two studies were conducted to compile baseline information about oophagy in U. pugnax. The first study was a site comparison study used to compare differences in oophagy for 20 crabs from SHM and PC. The second study was a feeding study conducted to investigate the role of food in oophagy. In the feeding study, ten crabs from each site were given one pellet of Carolina Biological Supply Fiddler Crab Food to determine if egg

ingestion would stop or decrease if another food source was available. Carapace width was measured for all crabs from both studies to determine if mean body size differed between sites. For the site comparison study, SHM crabs ranged in size from 10.1 to 17.8 mm with a median size of 13.8 mm while PC crabs ranged from 14.6 to 19.2 mm with a median size of 16.1mm. For the feeding study, carapace width for SHM crabs ranged from 11.0 to 17.4 mm with a median size of 13.7 while PC crabs ranged from 13.8 to 18.2 mm with a median size of 15.5mm.

For videotaping in both studies, ovigerous females were placed into individual glass bowls and a piece of plastic was inserted to force the crab to face the camera. Aluminum foil was lightly placed over the top of the bowls to prevent crabs from climbing out. After a 15-minute adjustment period, a video camera was turned on and the room was vacated to ensure the crabs were not disturbed. For crabs in the feeding study, recording was initiated after the 15-minute adjustment period or as soon as the crab began to eat. All crabs were taped for four-hour each as this was the maximum amount of time available on the videotapes used. At the end of the recording period, the crab was removed from the bowl and fecundity was estimated.

When viewing the videotapes, I needed to quantify egg ingestion and food pellet ingestion behavior so I counted the number of times each claw moved an egg or piece of pellet to the crab mouthparts hereafter referred to as Claw to Mouthpart (CM). I then broke CM behavior into following parameters for both egg and pellet ingestion:

CM Events – the total number of feeding events per four-hour observation period. An event was the time that feeding started to when it ended as feeding did not occur continuously. For example, there could be 28 egg feeding events during a four-hour

taping period referred to here as CM Events Egg. Pellet ingestion events are referred to as CM Events Pellet.

CM Activity - the total number of claw to mouthpart movements per four-hour observation period. For example, a crab may move eggs to the mouthparts 228 times during the course of taping period referred to here as CM Activity Egg (or CM Activity Pellet for pellet ingestion).

CM Duration - the total time spent feeding per four-hour observation period. The duration of egg ingestion is referred to as CM Duration Egg and pellet ingestion referred to as CM Duration Pellet.

In addition, I also quantified the total number of abdominal contraction events per four-hour observation period where an event was the time that contractions started to when they ended as contractions did not occur continuously.

Batch Fecundity Estimates

Female Atlantic marsh fiddler crabs extrude eggs several times during the breeding season but the fecundity estimated in this study only examined one batch of eggs. Batch fecundity is referred to simply as fecundity throughout this paper. Eggs were removed with forceps under a dissecting scope (12×) to ensure that all eggs were removed from the pleopods. The eggs were then placed into a small container of sea water and gently agitated to facilitate separation of the eggs.

Given the large number of eggs carried by a female, I used a gravimetric method commonly used in aquaculture (Case 2001) to estimate the number of eggs per female. Aliquots of known numbers of eggs (three aliquots of ten) were used to calculate the average mass of an egg, and a conversion factor was used to estimate the number of eggs in

the total egg mass removed from each crab. All egg samples were oven-dried (60° C) for 24 h and the weight of the dried eggs was calculated for each set of three eggs. The weight of the total egg mass was determined in a similar manner and included the weights from the three sets of 10 eggs. Each of the three weights of 10 eggs were divided by 10 (e.g., Weight #1 /10, Weight #2/10 and Weight #3/10) and the results from these three calculated weights were added together and averaged yielding milligrams per egg (MGPE). Eggs per milligram (EPMG) was then calculated by dividing one by the MGPE (1/MGPE). Finally, the total egg weight was multiplied by the EPMG to yield fecundity. Fecundity estimates were calculated for all 40 crabs from the site comparison study and for all 20 crabs from the feeding study.

Statistical Analyses

Statistical analyses were performed using SigmaStat version 2.03. Descriptive statistics were used to examine basic trends for ingestion parameters for each study. For some tests, the data were natural log or square root transformed to normally distribute the data. In cases where the data could not be transformed, non-parametric tests were utilized. For the site comparison study, egg ingestion parameters between sites were compared using Mann-Whitney Rank Sum tests. Feeding study comparisons also used non-parametric tests except when t-tests on square root transformed data were applicable. Kruskal-Wallis tests were used to compare egg ingestion parameters between the site comparison study and feeding study. The relationship between abdominal contractions and egg ingestion was calculated using Pearson correlations. Fecundity data was analyzed using one-way and two-way ANOVAs as appropriate.

RESULTS

Feeding Behavior

Oophagy was initially only observed in crabs from the contaminated site (PC), but video analysis showed that crabs from both the contaminated and non-contaminated site ingested eggs. Egg removal, versus removal of detritus, was clearly discernable as the entire egg mass moved as individual eggs were tugged away. Evidence for egg removal was further supported when crabs occasionally removed an entire stalk of eggs and proceeded to ingest individual eggs or placed the entire egg stalk into their mouthparts. It appeared, at times, that crabs were choosing which eggs to remove and ingest as there was careful manipulation of the egg mass before an egg was removed and moved to the mouthparts. At other times, crabs ingested eggs very quickly using both claws without any egg mass manipulation. When food pellets were offered, crabs almost immediately moved toward the pellet and pellet ingestion was rapid with both claws quickly moving pieces of pellet into the mouthparts. Typically no pieces of pellet remained in the bowl by the end of a taping period.

Crabs did not always ingest eggs during a four-hour videotape period. For example in the site comparison study, no egg ingestion occurred at SHM for seven out of the 20 videotaped sessions and there were eight cases out of 20 of no egg ingestion for PC crabs. Food pellets were almost always ingested, however, and out of the 10 session recorded where pellets were provided there was only one case per site where the pellet was not ingested. In cases where there was no egg or pellet ingestion the crabs were essentially inactive for almost the entire four-hour taping period.

Egg ingestion almost always occurred in conjunction with abdominal contractions in both the site comparison and feeding study crabs. Pearson correlations were conducted

to determine whether any statistical relationships existed for the number of abdominal contraction events and egg ingestion parameters (i.e., CM Duration Egg, CM Activity Egg and CM Events Egg) for both studies. For the site comparison study, there were marginally significant relationships between contractions in SHM crabs and the three egg ingestion parameters (Table 3-1). For PC crabs in the site comparison study there were no significant relationships between abdominal contractions and any egg ingestion parameters (Table 3-1). Similarly, for both SHM and PC crabs in the feeding study, there were no significant correlations between abdominal contractions and any egg ingestion parameters (Table 3-1).

Site Comparison Study

In the site comparison study, where the only food option was egg ingestion as no food pellets were provided, site comparisons revealed that egg ingestion occurs at both PC and SHM regardless of the presence of contaminants at PC. Although results were not statistically significantly different between sites, egg ingestion parameters (i.e., CM Duration Egg, CM Activity Egg and CM Events Egg) were often higher at SHM (Table 3-2).

Although the duration of egg ingestion was greater for SHM crabs than PC crabs (CM Duration Egg SHM > CM Duration Egg PC, Table 3-2) variability was high at SHM (Table 3-2) and a Mann-Whiney Rank Sum test found no statistically significant difference between sites ($p=0.198$). Similarly, although egg ingestion activity was greater for SHM crabs than PC crabs (CM Activity Egg SHM > CM Activity Egg PC, Table 3-2), a Mann-Whiney Rank Sum test found no there was no statistically significant difference between sites ($p=0.357$). The number of egg ingestion events were similar between sites (CM

Events Egg SHM = CM Events Egg PC, Table 3-2) as confirmed by a Mann-Whitney Rank Sum test (p=0.542).

Feeding Study Results

For the feeding study, the presence of a food pellet did not stop crabs from ingesting eggs but pellet ingestion was greater than egg ingestion at both sites. Typically the entire pellet of food was ingested during the four-hour recording session and crabs began ingesting the pellet as soon as it was placed into the bowl.

Pellet ingestion was compared to egg ingestion for each site individually to determine how the presence of food affected egg ingestion. For SHM crabs, the duration of pellet ingestion was longer than the duration of egg ingestion (CM Duration Pellet > CM Duration Egg, Table 3-3) and a Mann Whitney Rank Sum test revealed this difference in duration to be significant (Table 3-4). Similarly, pellet ingestion activity was greater than egg ingestion activity at SHM (CM Activity Pellet > CM Activity Egg, Table 3-3) and a t-test on square root transformed data revealed this difference in activity to be significant (Table 3-4). The number of pellet ingestion events was also significantly greater than egg ingestion events at SHM (CM Events Pellet > CM Events Egg, Table 3-4).

Results were similar for PC crabs where pellet ingestion was greater than egg ingestion for all parameters (i.e., CM Duration, Activity and Events Pellet > CM Duration, Activity and Events Egg, Table 3-3). T-test results for PC crabs revealed that the duration of pellet ingestion was significantly greater than the duration of egg ingestion (CM Duration Pellet > CM Duration Egg, Table 3-4). Similarly, a t-test revealed that pellet ingestion activity was significantly greater than egg ingestion activity (CM Activity Pellet

> CM Activity Egg, Table 3-4) and that the number of pellet ingestion events were greater than egg ingestion events (CM Events Pellet > CM Events Egg, Table 3-4).

Pellet ingestion and egg ingestion were compared between the two sites to determine if pellet or egg ingestion occurred more or less at a particular site. Overall, egg and pellet ingestion were similar between the two sites. For pellet ingestion, results of a one-factor ANOVA found no significant difference (Table 3-5) in the duration of pellet ingestion at PC compared to the duration of pellet ingestion at SHM (CM Duration Pellet PC = CM Duration Pellet SHM, Table 3-5). Similarly, there was not a statistically significant difference in pellet ingestion activity between SHM and PC (CM Activity Pellet PC = CM Activity Pellet SHM, Table 3-5). Results of a one-factor ANOVA also found no significant difference in the number of pellet ingestion events between PC and SHM (CM Events Pellet PC = CM Events Pellet SHM, Table 3-5).

For egg ingestion, site comparisons revealed that egg ingestion also was similar between SHM and PC. Results of a one-factor ANOVA using square root transformed data found no significant difference in the duration of egg ingestion between sites (CM Duration Egg PC = CM Duration Egg SHM, Table 3-5) and no significant difference in egg ingestion activity between sites (CM Activity Egg PC = CM Activity Egg SHM, Table 3-5). The number of egg ingestion events was also similar between sites (CM Events Egg PC = CM Events SHM, Table 3-5). Mean carapace width was significantly different between sites for the feeding study. T-test results ($p=0.019$) revealed that PC crabs (15.8 mm) were marginally larger than SHM crabs (13.8 mm).

Site Comparison Results versus Feeding Study Results

To determine whether the presence of pellets affected egg ingestion, egg ingestion was compared between the 20 site comparison crabs and the ten feeding study crabs. Kruskal Wallis ANOVA on ranks found no significant differences in egg ingestion between PC or SHM (Table 3-6). These results further indicate that crabs ingest eggs whether or not another food source is available.

Fecundity Results

Overall, mean fecundity (± 95% confidence intervals) was higher for PC crabs in both the site comparison study and feeding study.

Study	SHM Fecundity	PC Fecundity
Site Comparison	500.9 (57.65)	1334.12 (220.14)
Feeding	472.8 (170.5)	875 (200.0)

For the site comparison study, a one-factor ANOVA using natural log transformed data found no significant difference ($p = 0.247$) in fecundity between PC and SHM as variability was high in SHM crabs (Table 3-7).

For the feeding study, a two-factor ANOVA revealed fecundity to be significantly different between sites (Table 3-7) with fecundity at PC significantly higher than fecundity at SHM (Table 3-7). T-test results showed that PC crabs (16.2 mm) were marginally larger ($p=0.037$) than SHM crabs (14.4 mm) in the feeding study which could account for differences in fecundity. Results of a two-factor ANOVA comparing fecundity between site comparison study crabs and feeding study crabs found significant differences between sites but no significant differences between studies and no interactions between studies and site (Table 3-7).

DISCUSSION

General Observations

Although I initially only observed oophagy in crabs from the contaminated site (PC), contaminants do not appear to influence oophagy as crabs from both PC and SHM ingested eggs in this study. Also, even though crabs were slightly larger at PC, crab size does not appear to affect oophagy given the similarities in egg ingestion parameters between sites and sizes. Although the presence of a food pellet decreased egg ingestion in crabs from both sites, crabs still engaged in oophagy indicating that crabs may not be ingesting eggs strictly to meet nutritional requirements.

Filial cannibalism has not been previously documented for U. pugnax in the laboratory or in the field so it cannot be ruled out that egg ingestion may be a laboratory artifact. Initially, filial cannibalism in general was thought to be a laboratory artifact until Trivers (1972) and Rohwer (1978) proposed theories of cannibalism as a reproductive strategy and parental investment. Cannibalistic behavior also occurs across wide taxonomic groups (Elgar and Crespi 1992) in nature offering strong evidence that oophagy in fiddler crabs may have some evolutionary significance yet future experimental studies are clearly required for U. pugnax. Three theories of cannibalism related to selective filial cannibalism, the role of food, and oxygenation of the egg mass are worthy of greater attention and may influence oophagy in U. pugnax.

Selective Filial Cannibalism

Qualitative observations of egg cannibalism by U. pugnax revealed that egg choice often appeared to be selective rather than random (pers. obs.). There were occasional events when egg ingestion was quick with no seeming discretion about the type of egg

chosen. Typically, however, the crabs appeared to select specific eggs for ingestion after careful and lengthy manipulation of the egg mass. These observations allow for the hypothesis that filial cannibalism occurs in U. pugnax because the crabs are selectively removing non-viable eggs. It is possible that crabs, unlike fish which likely identify non-viable eggs by sight, may somehow be using their claws to "feel" for differences in viability.

In only one study of a Brachyuran crab has it been hypothesized that C. granulata removes non-viable eggs with the chelipeds and ambulatory legs (Rodriguez and Pisano 1993) although no data is provided to support this hypothesis. In a study of filial cannibalism in scissortail sergeant (A. sexfasciatus), males that were given food only consume infertile or diseased egg (Manica 2004). In her review of filial cannibalism, Manica (2002) reports several studies where unfertilized, malformed or diseased eggs are preferentially cannibalized by several fish species. Klug et al. (2006) also notes that selective filial cannibalism may be a plausible new hypothesis for oophagy. While it is not known if U. pugnax preferentially ingested nonviable eggs in this study, this possibility should be investigated in future studies.

The Role of Food

Rohwer (1978) suggested that filial cannibalism occurs as parents with limited food options can use offspring as food and thus increase their current and future reproductive success. As ovigerous U. pugnax are believed to primarily remain in their burrows while carrying eggs (Crane 1975), it is possible that oophagy results as a response to limited foraging capability. Egg ingestion may supplement nutrition and provide reproductive benefits for the current or future broods. Unfortunately, there is no data on if or how much

ovigerous U. pugnax feed in the field yet my study clearly shows that laboratory-held ovigerous females ingest eggs and food if offered.

Whether or not ovigerous Ocypodids feed in the field appears to depend on clutch size and the species. Early work by Christy and Salmon (1984) suggests that Uca females who spend most of the incubation period underground feed little if at all yet no data is provided to support these claims. Essentially, there appears to be a trade-off between feeding and clutch size. For example, species that carry relatively large clutches and incubate in burrows, such as U. pugilator, must choose between feeding and strong selection pressures to remain underground thus protecting their clutch from stresses such as temperature and desiccation (Christy and Salmon 1984). In a species that produces many small broods, such as U. vocans, it is more advantageous to spend time actively feeding above ground (Christy and Salmon, 1984). This hypothesis is supported in a study of three ocypodid crabs (Scopimera globosa, Ilyoplax pusillus and Macrophthalmus japonicus) in Japan (Henmi and Kaneto 1989a). S. globosa and I. pusillus produce one to two large broods per year and remain in their burrows without feeding until hatching (Henmi and Kaneto 1989a). M. japonicus, which produces four to five small broods per year, do not remain underground but instead feed actively on the surface during egg incubation (Henmi and Kaneto 1989a).

In an associated study, Henmi and Kaneto (1989b) discovered that egg mortality is high in M. japonicus and lower in S. globosa and I. pusillus which remain in their burrows during egg incubation. The authors hypothesize that increased reproductive fitness results when feeding is traded for underground egg incubation (Henmi and Kaneto 1989b). Another ocypodid crab, U. tetragonon, is a small brood species that actively feeds

aboveground during egg incubation as their small broods are protected by the abdominal flap (Koga et al. 2000). Interestingly, Koga (unpublished data) reports S. globosa (a large brood carrying species) enlarge their burrows after ovulation and that ovigerous U. lactea also have larger burrows presumably to create a suitable microhabitat for incubation (Koga et al. 2000). If burrow sizes are larger in large brood species where aboveground foraging is limited, I hypothesize that limited feeding including oophagy may be occurring underground for a large brood species like U. pugnax thus lessening the tradeoff between underground incubation and active surface feeding.

The studies listed above all presume that no feeding occurs underground for large brood Uca species but none appear to have conclusive data indicating such. If the burrows are large enough, it is possible that limited feeding may be occurring. It is also possible that filial cannibalism of eggs may be providing the energy necessary for reproductive success. The laboratory-held ovigerous U. pugnax in this study ate if food was provided yet no data exists on feeding habits of ovigerous U. pugnax in underground burrows either in the field or laboratory. I personally never observed ovigerous U. pugnax feeding aboveground during the course of this study. Feeding in the laboratory may occur as food is readily available and field stressors (such as predation, heat, and desiccation) do not prevent feeding. However, until conclusive evidence can confirm lack of feeding in burrowing ovigerous Ocypodids, one must question the common practice of not feeding ovigerous crustaceans in the laboratory.

Depending on the types of experiments performed and the data collected, it may be detrimental to not feed ovigerous species such as U. pugnax. Unless a study requires the depuration of entrained food or sediment, providing food to ovigerous U. pugnax may more

closely replicate field conditions and ensure the viability of the embryos and resulting larvae. Future work on the reproductive ecology of Ocypodids should consider the role of filial cannibalism for ovigerous females and attempt to document possible underground feeding activity.

For many species, the high nutritional value of eggs is often attributed as a cause of cannibalism (Via 1999). In the copepod Calanus helgolandicus high egg cannibalism is thought to supplement nutrition as it is associated with increased egg viability (Kang and Poulet 2000). Similarly, high egg cannibalism by neonates in the caterpillar Ascia monuste is attributed to the high nutritional value of eggs (Barros-Bellanda and Zucoloto 2005). The nutritional and energetic gains of oophagy have also been linked to within-clutch cannibalism in the land snail Helix aspersa where oophagy results in increased survivorship and growth (Desbuquois 1997). Additional studies are required examining oophagy in fiddler crabs to determine if filial cannibalism occurs to provide an energetic advantage or increased reproductive success. Simply showing that female fiddler crabs ingest more food than eggs when food is provided does not demonstrate and adaptive tradeoff. More work examining the fitness consequences of oophagy, such as the survivorship of embryos and the reproductive success of larvae, is necessary.

A significant amount of literature has documented filial cannibalism in fish especially in male fish responsible for paternal care. Results are contradictory when examining whether nutritional benefit alone is responsible for partial filial cannibalism in fish. In his review of filial cannibalism in fishes, FitzGerald (1992) reviews the relationship between food availability and filial cannibalism and finds no relationship between the number of eggs eaten and the amount of food supplied in threespine

sticklebacks (Gasterosteus aculeatus) and hypothesizes that food quality may play a role in filial cannibalism. Manica (2002) finds similar contradictions in lab feeding experiments. In only one study (Kvarnemo et al. 1998) was supplementary feeding of gobies found to reduce filial cannibalism. In a study by Payne et al. (2002), filial cannibalism appeared to be unrelated to energetic status in beaugregory damselfish (Stegastes leucostictus). Males fed a supplementary diet of freeze-dried food did grow faster than non-fed males but still engaged in filial cannibalism. There were also no differences in the mortality rate of embryos guarded by fed and non-fed males (Payne et al. 2002). Work by Manica (2004), however, supports the hypothesis that filial cannibalism occurs to provide energy for parental care as the supplementary feeding of scissortail sergeant (Abudefduf sexfasciatus) results in decreased cannibalism of eggs. Clearly, for fish, results are equivocal regarding the role of food in filial cannibalism.

The Role of Oxygen

As U. pugnax in this study engaged in oophagy even if food was available, this suggests there may be other drivers for filial cannibalism for this species. During video observations, it was noted that egg ingestion frequently occurred while the crab pumped its abdomen (pers. obs.) and in one case abdominal contractions were correlated with egg ingestion. Abdominal contractions are believed to occur because the females are ventilating the eggs (Naylor et al. 1999). It is interesting to speculate whether oophagy is mediated by density-dependent egg survivorship in U. pugnax as egg removal may increase available oxygen and thus increase net reproductive success.

Experimentally reducing oxygen levels significantly increases filial cannibalism for male damselfish (S. leucostictus) (Payne et al. 2002). This increase in cannibalism reduces

embryo density and results in increased hatching success for the species. In a follow up study, Payne et al. (2004) conducted a modeling study and found that males are actually able to track oxygen conditions and will reduce clutch sizes in response to low oxygen levels. Conversely, low levels of dissolved oxygen did not increase filial cannibalism in male sand gobies (Pomatoschistus minutus) though it did reduce male body fat resulting from increased fanning for egg ventilation (Lissaker et al. 2003). Oxygen also did not affect egg survivorship in another study on male sand gobies but egg survivorship did increase under simulated partial clutch cannibalism(Klug et al. 2006). Brood care has been found to increase in amphipods when temperature is increased and oxygen decreased although survivorship was not determined (Dick et al. 1998).

It is unlikely that oophagy resulted from low oxygen conditions in the shallow water bowls that housed U. pugnax in the laboratory study. Further studies using varying oxygen levels are required to determine the role of oxygen in oophagy. Field oxygen concentrations within the burrow should also be quantified as this information appears to be limited in the literature. Further, water levels within the burrows should also be quantified as manipulation of the egg mass in water may be limited in the field and may be a laboratory artifact. The role of other abiotic conditions, such as temperature and salinity, should also be investigated in future studies as egg cannibalism may be an adaptive mechanism to resist unfavorable climatic changes such as high temperature (Desbuquois 1998).

Fecundity

Fecundity was estimated to determine whether oophagy causes a statistically significant reduction in egg number. In the site comparison study, there were no

differences in fecundity between sites. In the feeding study, fecundity was significantly lower in SHM crabs but SHM crabs were also significantly smaller than PC crabs. Bergey and Weis (2008) found similar size and fecundity results for PC and SHM crabs and feel size is most likely responsible for higher fecundity at the contaminated site. There were also significant differences in fecundity between sites when comparing results from the site comparison study to the feeding study.

Despite differences in fecundity between sites, to determine whether oophagy truly reduces fecundity, one must compare fecundity between cannibalistic crabs and crabs that do not engage in filial cannibalism or are somehow prevented from doing so. Further studies would then be required to determine whether reduced fecundity results in increased egg survivorship and, therefore, increased reproductive fitness. The fecundity comparisons in this study essentially only indicate that fecundity differs between sites which may or may not be affected by oophagy. Researchers examining fecundity of laboratory-held U. pugnax should be aware that egg ingestion may affect fecundity estimates. It should be noted that decreased fecundity does not necessarily imply reduced reproductive success as cannibalism may actually improve fitness as seen in several studies (Payne et al. 2002, Klug et al. 2006).

Summary

There appear to be a few adaptive explanations for why U. pugnax ingest their eggs: 1) non-viable eggs are selectively being removed to increase egg survivorship; 2) foraging is limited for burrow-bound or laboratory-held crabs and egg ingestion supplements nutrition; and 3) partial cannibalism provides more oxygenation for the egg mass which may result in increased survival. It is likely that filial cannibalism is not mediated by any

one factor and further research is needed to investigate all alternative explanations and the consistency of the behavior in U. pugnax. As this behavior has not been documented in the field it should be noted that oophagy in fiddler crabs may be a laboratory artifact although it has proven otherwise for other species (Manica 2002).

Table 3-1.

Pearson correlation results for abdominal contractions (AC) and egg ingestion parameters for Piles Creek (PC) and Sheepshead Meadows (SHM). Egg ingestion behavior was broken down into three quantitative parameters: CM Events - the total number of feeding events per four-hour observation period; CM Activity - the total number of claw to mouthpart movements per four-hour observation period; and CM Duration - the total time spent feeding per four-hour observation period. The only marginally significant correlation occurred for the site comparison study where there were marginally significant relationships between contractions in SHM crabs and the three egg ingestion parameters.

Study and Site	Comparison	Correlation Coefficient	P
Site Comparison Study - SHM	AC vs. CM Duration	0.469	0.037
	AC vs. CM Activity	0.437	0.0543
	AC vs. CM Events	0.409	0.073
Site Comparison Study - PC	AC vs. CM Duration	-0.01	0.966
	AC vs. CM Activity	0.068	0.777
	AC vs. CM Events	0.378	0.1
Feeding Study - SHM	AC vs. CM Duration	-0.203	0.574
	AC vs. CM Activity	-0.272	0.447

Table 3-2.
Mean egg ingestion activity (± 95% CIs) per four-hour video observation period for the site comparison study for Piles Creek (PC) and Sheepshead Meadows (SHM). Although results were not statistically significantly different between sites, egg ingestion parameters (i.e., CM Duration Egg, CM Activity Egg and CM Events Egg) were often higher at SHM.

	CM Duration	**CM Activity**	**CM Events**
SHM Crabs	1121.6 (1143.2)	126.7 (153.9)	3.4 (2.0)
PC Crabs	245.8 (236.4)	48.7 (52.6)	2.7 (1.9)

Table 3-3.

Feeding study results for egg and pellet ingestion (means ± 95% CIs) per four-hour video observation period for Piles Creek (PC) and Sheepshead Meadows (SHM). For both PC and SHM, pellet ingestion was greater than egg ingestion for all parameters (i.e., CM Duration, Activity and Events Pellet > CM Duration, Activity and Events Egg).

	CM Duration		**CM Activity**		**CM Events**	
	Egg	**Pellet**	**Egg**	**Pellet**	**Egg**	**Pellet**
SHM Crabs	329.5 (399.6)	886.5 (565.6)	31.4 (26.2)	177.7 (151.5)	4.4 (3.8)	9.9 (4.5)
PC Crabs	460.3 (377.5)	1363.6 (723.5)	54.1 (39.0)	167.0 (84.0)	5.5 (3.8)	12.7 (5.5)

Table 3-4.

Statistical results for the feeding study where egg ingestion was compared to pellet ingestion for Piles Creek (PC) and Sheepshead Meadows (SHM). Pellet ingestion was significantly greater than egg ingestion for all parameters (CM Duration, Activity and Events Pellet > CM Duration, Activity and Events Egg) though the number of pellet ingestion events (CM Events) was only marginally significantly greater than egg ingestion at SHM (p = 0.049).

Mann Whitney Rank Sum Results - SHM						
Group	**N**	**Missing**	**Median**	**25%**	**75%**	**P**
CM Duration Pellet	10	0	601.0	447.0	1007.0	0.021
CM Duration Egg	10	0	127.0	0	440.0	
t-test Results - SHM (Square Root transformed)						
Group	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**	**P**
CM Activity Pellet	10	0	11.4	7.3	2.3	0.013
CM Activity Egg	10	0	4.1	4.1	1.3	
t-test Results - SHM						
Group	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**	**P**
CM Events Pellet	10	0	9.9	6.3	2.00	0.049
CM Events Egg	10	0	4.4	5.3	1.68	
t-test Results - PC						
Group	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**	**P**
CM Duration Pellet	10	0	1363.6	1011.3	319.8	0.022
CM Duration Egg	10	0	460.3	527.7	1011.3	
t-test Results - PC						
Group	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**	**P**
CM Activity Pellet	10	0	167.0	117.4	37.1	0.013
CM Activity Egg	10	0	54.1	54.6	17.3	
t-test Results - PC						
Group	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**	**P**
CM Events Pellet	10	0	12.7	7.7	2.4	0.026
CM Events Egg	10	0	5.5	5.4	1.7	

Table 3-5.
One-factor Analysis of Variance (ANOVA) (α = 0.05) results for comparisons of pellet and egg ingestion parameters between Piles Creek (PC) and Sheepshead Meadows (SHM) for the feeding study. Contaminants do not appear to influence oophagy as there were no significant differences between sites the contaminated and non-contaminated site.

CM Duration Pellet					
Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	1	1138122.10	1138122.10	1.38	0.26
Residual	18	14831644.90	823980.30		
Total	19	15969766.95			
CM Activity Pellet					
Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	1	1.18	1.18	0.03	0.87
Residual	18	745.27	41.40		
Total	19	746.44			
CM Events Pellet					
Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	1	39.20	39.20	0.79	0.39
Residual	18	891.00	49.50		
Total	19	930.20			
CM Duration Egg					
Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	1	119.10	119.1	0.631	0.44
Residual	18	3397.70	188.76		
Total	19	3516.80			
CM Activity Egg					
Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	1	17.93	17.93	0.957	0.34
Residual	18	337.08	18.73		
Total	19	355.00			
CM Events Egg					
Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	1	6.05	6.05	0.213	0.65
Residual	18	510.90	28.38		
Total	19	516.95			

Table 3-6.

Kruskal-Wallis one-factor Analysis of Variance (ANOVA) on ranks (α = 0.05) results for comparisons of egg ingestion between the site comparison study and the feeding study for Piles Creek (PC) and Sheepshead Meadows (SHM). There were no significant differences in egg ingestion between the two studies for either site indicating that the presence of food does not stop or decrease egg ingestion for ovigerous U. pugnax.

CM Duration Egg						
Group	**N**	**Missing**	**Median**	**25%**	**75%**	**P**
SHM Site Comparison	20	0	129	0	704.5	0.528
SHM Feeding Study	10	0	127	0	440	
CM Activity Egg						
Group	**N**	**Missing**	**Median**	**25%**	**75%**	**P**
SHM Site Comparison	20	0	10	0	79	0.752
SHM Feeding Study	10	0	14	0	66	
CM Events Egg						
Group	**N**	**Missing**	**Median**	**25%**	**75%**	**P**
SHM Site Comparison	20	0	2	0	5	0.743
SHM Feeding Study	10	0	3	0	8	
CM Duration Egg						
Group	**N**	**Missing**	**Median**	**25%**	**75%**	**P**
PC Baseline	20	0	45	0	253.5	0.093
PC Feeding	10	0	336.5	20	654	
CMEgg Activity						
Group	**N**	**Missing**	**Median**	**25%**	**75%**	**P**
PC Baseline	20	0	2	0	26.5	0.137
PC Feeding	10	0	44	2	87	
Number of CMEgg Events						
Group	**N**	**Missing**	**Median**	**25%**	**75%**	**P**
PC Baseline	20	0	1	0	3.5	0.114
PC Feeding	10	0	4.5	1	10	

Table 3-7.
One- and two-factor Analysis of Variance (ANOVA) (α = 0.05) results for comparisons of fecundity between Piles Creek (PC) and Sheepshead Meadows (SHM). Fecundity between sites was compared using a one-factor ANOVA for the site comparison study (natural log transformed data) and the feeding study. A two-factor ANOVA compared fecundity between both sites and studies. Fecundity was not significantly different between sites for the site comparison study but was significantly different for the feeding study. When both sites and studies were combined, fecundity was also significantly different between sites. Piles Creeks crabs were marginally larger than SHM crabs which likely accounts for differences in fecundity.

Site Comparison Study					
Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Sites	1	0.634	0.634	1.39	0.247
Residual	38	17.39	0.457		
Total	39	18.02			
Feeding Study					
Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Sites	1	808974.71	808974.7	12	0.003
Residual	18	1213490.1	67416.12		
Total	19	2022464.8			
Site Comparison Study vs. Feeding Study					
Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Site	1	1434994.54	1434994.54	9.93	0.003
Study	1	5700.24	5700.24	0.039	0.843
Site x Study	1	73360.16	73360.16	0.51	0.48
Residual	56	8095036.55	144554.22		
Total	59	9554294.14	161937.2		

CHAPTER FOUR

Summary

The research described in this proposal investigated basic and applied aspects of the ecology of contaminant exposure in field-collected Atlantic marsh fiddler crab, U. pugnax (Smith). Specifically, the research examined sublethal effects on crab physiology, reproduction, and behavior by examining: 1) variation in lipid class composition in response to season, molt cycle, and contaminants; 2) contaminant effects on fecundity and larval morphology; and 3) the roles of food and contaminants in filial cannibalism. All data were derived from field-collected organisms in an attempt to provide a more holistic picture of contaminant exposure in U. pugnax as the determination of contaminant effect on estuarine organisms is often confined to laboratory toxicity tests (Hall and Giddings 2000).

The goal to examine lipids seasonally and during the molt cycle originated from a U.S. EPA study where a single snapshot in time of total lipids and contaminants revealed a relationship between the two. Realizing that a single data set from a single sampling point in time did not adequately represent lipid fluctuations over time, this study set out to generate a seasonal lipid profile for U. pugnax while also examining lipids during the molt cycle and in an exposure study. Results revealed that total lipids and lipid classes fluctuate seasonally and during the molt cycle. Total lipids masked much of the variation in lipid classes and fractionation clearly revealed fluctuations in classes, which would be masked by only employing total lipid analysis. Future studies should include lipid class data or, at a minimum, the sum of polar and non-polar lipids (Bergen 1999). Researchers

should also utilize long-term data sets to provide the necessary temporal and/or biological context for the observed lipid concentrations. If long-term data collection is not feasible, lipids should be sampled during periods when lipid concentrations for the species are known.

An additional goal of the lipid study was to clarify the potential utility of using lipid class fluctuations in fiddler crabs as indicators of sublethal effects at contaminated sites. Given the lack of statistical significance for the null hypotheses tested in this study, it cannot be strictly concluded that contaminant exposure resulted in lipid fluctuations. As Nakagawa and Cuthill (2007) point out, however, a null hypothesis in natural systems can rarely be true and can only be exactly true for categorical data. In a broader context, many of the studies cited here have shown contaminant-driven lipid responses for crustaceans and the lipid concentrations in this study often did differ between the contaminated and non-contaminated site. Without more data, however, identifying the influence of abiotic and biotic factors on lipid class fluctuations in U. pugnax, it would be premature to attribute differences in lipids between sites to contaminants. While one can cautiously hypothesize the existence of a biologically relevant, contaminant-driven difference in lipids between sites or this study, longer term studies, in-depth analyses of biotic and abiotic factors, and larger data sets are clearly required.

Results of this study indicate that lipid sampling could be useful when applied to routine (i.e., repetitive) monitoring studies of contaminated areas. Lipids may be less useful when attempting to screen or quickly identify contaminant effects at contaminated sites, as results are best understood within the context of a longer-term seasonal data set.

An exception to this would be at sites with high concentrations of contaminants that are known to impact lipid stores and functioning.

The second study examined batch fecundity and larval morphology in U. pugnax to determine the effects of contaminant exposure on field-collected organisms from a contaminated and non-contaminated site. Again, field data was used to provide a more holistic picture of contaminant exposure in U. pugnax as many reproductive studies typically limit exposure to controlled laboratory experiments. Additionally, no studies for Uca spp. could be identified that compare fecundity or larval morphological abnormalities in field-collected organisms to laboratory exposed individuals. As such, this study attempted to determine whether the sublethal reproductive effects typically observed in laboratory exposures are seen in field-collected U. pugnax.

In this study, fecundity was higher at the contaminated site (PC) for both the site comparison and reciprocal transplant exposure studies. Larger crabs and higher fecundity at PC when compared to SHM was also discovered by Bergey and Weis (2008) although differences in abiotic factors, such as food quality, cannot strictly be ruled out. When examining abnormal larval morphology, 32% of SHM larvae and 70.5% of PC larvae exhibited eye, body, or both eye and body morphological abnormalities. Abnormalities were greater in PC larvae with the exception of hypertrophied eyes which were similar between the two sites and abnormal larvae at the non-contaminated site. It is not clear why abnormalities occur at SHM but as the proportion of naturally occurring abnormalities in all Uca spp. is currently unknown, future work should attempt to determine the occurrence of abnormalities absent contaminants before comparisons to contaminant-exposed crabs are made.

I did not expect to find higher fecundity at the contaminated site and abnormal larvae at the non-contaminated site. These outcomes, however, highlight the unpredictability of field data and the deviation of real world conditions from controlled laboratory experiments and predicted outcomes. Ideally, the best understanding of contaminant exposure on reproductive fitness occurs through a combination of baseline field data and multiple lines of toxicity evidence (e.g., single species toxicity tests, ambient tests and *in situ* tests).

Field data can provide insights on possible causality and a more realistic understanding of the contaminant-related impacts on Brachyuran reproduction. This is not to suggest that field validation is required for sediment toxicity tests (Chapman 1995). The complex interactions of biotic and abiotic factors in the field make it difficult and often impossible to validate carefully controlled laboratory studies. Also, while laboratory results may be replicated in the field, a lack of field replication does not imply that laboratory results are untrue. This is not to suggest that laboratory studies are unnecessary as carefully controlled laboratory studies help to quantify the actual effects of contaminant-induced toxicity. A combination of laboratory and field data, however, provides the best viewpoint and perspective on cause-and-effect and, ultimately, assessments of ecological condition. The collection of field data in this study supplements our understanding of laboratory toxicity testing and permits a more holistic picture of contaminant exposure and, ultimately, ecological condition in U. pugnax.

The goals of the behavioral study were to quantitatively document oophagy and some potential stimuli in U. pugnax and determine whether reduced fecundity was a resulting cost of cannibalism. Results revealed that contaminants do not appear to

influence oophagy as crabs from both RUMFS and PC ingested eggs. For the site comparison study, egg ingestion parameters were typically greater at the non-contaminated site though statistically there were no differences between sites. For the feeding study, crabs from both sites still ingested eggs even if food pellets were present although pellet ingestion parameters were higher than egg ingestion parameters for both sites.

There appear to be a few adaptive explanations for why U. pugnax ingest their eggs: 1) foraging is limited for burrow-bound or laboratory-held crabs and egg ingestion provides energy; 2) partial cannibalism provides more oxygenation for the egg mass which may result in increased survival; and 3) non-viable eggs are selectively being removed to increase egg survivorship. It is likely that filial cannibalism is not mediated by any one factor and further research is needed to investigate all alternative explanations for this previously unstudied behavior. As this behavior has not been documented in the field it should be noted that oophagy in fiddler crabs may be a laboratory artifact although it has proven otherwise for other species (Manica 2002).

This examination into the ecology of contaminant exposure in U. pugnax resulted in several unique findings and provides baseline data for several previously uninvestigated parameters. Prior to this study, no seasonal or molt cycle lipid class profiles existed for U. pugnax collected from both contaminated and non-contaminated sites. The fluctuations observed in total lipids and lipid classes highlight the importance of long-term data sets and lipid fractionation.

While the effects of contaminant exposure on reproductive fitness have been investigated for some crustaceans including U. pugnax, this is the first study to examine

abnormal larval morphology in field-collected U. pugnax. These baseline data will be useful for comparisons to other field sites and for future laboratory studies examining the acute, chronic, and sublethal toxicity of specific contaminants. Further, the presence of abnormalities in control larvae highlights the need to determine the proportion of genetic abnormalities typically occurring in Ocypodid crabs prior to comparing toxicity tests results to control larvae. This will ensure that laboratory exposures of ovigerous females do not overestimate risks from contaminant exposure. Also, porewater exposures should be included in the suite of toxicity tests performed on ovigerous females to better replicate field exposure pathways.

The baseline data on oophagy collected in this study offer several insights into this previously undocumented behavior in U. pugnax. Prior to this work, it was assumed that ovigerous U. pugnax do not feed in the field (Christy and Salmon 1984) and thus do not need to be fed in the laboratory. While my research showed that ovigerous females clearly do eat, field observations of feeding and egg ingestion are necessary. The current and future reproductive benefits of cannibalism also need to be investigated by looking at larval survival and successive broods. While it is not currently known if U. pugnax preferentially ingested nonviable eggs (Rodriguez and Pisano 1993), this possibility should be investigated in future studies.

While contaminant exposure may have affected the physiology, reproduction and behavior of U. pugnax in this study, the synergistic and antagonistic effects of natural parameters should not be overlooked. Many of the studies cited here acknowledge the role of abiotic factors yet recognition often occurs as more of an afterthought than a serious consideration of effect. Our understanding of contaminant exposure in the field

is inevitably defined by abiotic parameters yet many studies choose to focus singularly on the role of contaminants when drawing conclusions while laboratory studies control abiotic factors to minimize their influence. To create a holistic picture of the ecology of contaminant exposure in U. pugnax, future studies must include detailed temporal and spatial investigation of abiotic parameters. Laboratory toxicity tests and the baseline collection of field data are important points of departure, yet long-term multi-parameter investigations are required to truly assess the potential adverse effects of contaminants on U. pugnax.

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Appendix A

Sediment and Tissue Concentrations per Month and Site

Table A1-1a.

Inorganic contaminant concentrations (mg/kg dry weight) in surficial sediment collected from Piles Creek (PC) and Sheepshead Meadows (SHM) from June 2000 to July 2001.

Element	Date	PC	SHM		Element	Date	PC	SHM
Al	Jun-00	15500	6500		Be	Jun-00	1.05	0.315
	Jul-00	20500	8700			Jul-00	0.7	0.425
	Aug-00	18000	7900			Aug-00	0.6	0.35
	Sep-00	12000	7400			Sep-00	0.5	0.41
	Oct-00	13000	7000			Oct-00	0.5	0.37
	Nov-00	16000	6700			Nov-00	0.6	0.4
	Apr-01	17000	11000			Apr-01	1	0.43
	May-01	21000	8600			May-01	0.6	0.7
	Jul-01	13000	11000			Jul-01	0.46	0.6
An	Jun-00	8.5	3.75		Cd	Jun-00	2	0.315
	Jul-00	8.5	10			Jul-00	3.55	0.425
	Aug-00	7.5	4.2			Aug-00	2.5	0.35
	Sep-00	6	4.9			Sep-00	1	0.41
	Oct-00	6	4.45			Oct-00	1	0.37
	Nov-00	7	4.8			Nov-00	1.6	0.4
	Apr-01	5.5	5			Apr-01	1.4	0.43
	May-01	7.5	8.5			May-01	0.6	0.7
	Jul-01	5.5	7.5			Jul-01	1.7	0.6
As	Jun-00	275	16		Ca	Jun-00	4400	2100
	Jul-00	145	6.9			Jul-00	5550	2900
	Aug-00	120	5			Aug-00	3600	2400
	Sep-00	57	4.7			Sep-00	1700	2400
	Oct-00	60	3.2			Oct-00	2800	2400
	Nov-00	120	4.3			Nov-00	2600	2300
	Apr-01	85	15			Apr-01	2600	3100
	May-01	140	10			May-01	3300	3500
	Jul-01	64	14			Jul-01	2300	3600
Ba	Jun-00	3300	20		Cr	Jun-00	585	36
	Jul-00	2350	34			Jul-00	340	41
	Aug-00	2000	23			Aug-00	280	32
	Sep-00	720	22			Sep-00	60	32
	Oct-00	1200	19			Oct-00	81	28
	Nov-00	1500	20			Nov-00	120	29
	Apr-01	1000	32			Apr-01	110	45
	May-01	2000	26			May-01	200	37
	Jul-01	1200	32			Jul-01	99	48

Table A1-1b.
Inorganic contaminant concentrations (mg/kg dry weight) in surficial sediment collected from Piles Creek (PC) and Sheepshead Meadows (SHM) from June 2000 to July 2001.

Element	Date	PC	SHM		Element	Date	PC	SHM
Co	Jun-00	14.5	5		Ms	Jun-00	7700	4000
	Jul-00	19	5.7			Jul-00	8800	5300
	Aug-00	14	4.4			Aug-00	8000	4700
	Sep-00	10	4.7			Sep-00	5200	4700
	Oct-00	13	3.9			Oct-00	5400	4200
	Nov-00	14	4.6			Nov-00	7000	4400
	Apr-01	19	7.5			Apr-01	6400	6300
	May-01	16	7.2			May-01	8500	7100
	Jul-01	13	7.1			Jul-01	6500	7800
Cu	Jun-00	710	16		Mn	Jun-00	255	93
	Jul-00	625	19			Jul-00	305	110
	Aug-00	590	17			Aug-00	280	89
	Sep-00	100	17			Sep-00	190	90
	Oct-00	170	13			Oct-00	180	83
	Nov-00	260	15			Nov-00	250	85
	Apr-01	210	24			Apr-01	300	150
	May-01	460	21			May-01	440	190
	Jul-01	240	25			Jul-01	220	170
Fe	Jun-00	29000	13000		Hg	Jun-00	43	0.13
	Jul-00	44000	13000			Jul-00	34	0.21
	Aug-00	39000	11000			Aug-00	22	0.13
	Sep-00	34000	11000			Sep-00	4.4	0.16
	Oct-00	34000	9700			Oct-00	6.6	0.13
	Nov-00	40000	10000			Nov-00	12	0.17
	Apr-01	47000	22000			Apr-01	6.6	0.3
	May-01	43000	18000			May-01	16	0.17
	Jul-01	33000	22000			Jul-01	7.9	0.22
Pb	Jun-00	635	24		Ni	Jun-00	77	23
	Jul-00	505	26			Jul-00	73.5	14
	Aug-00	430	22			Aug-00	57	12
	Sep-00	88	22			Sep-00	29	13
	Oct-00	160	21			Oct-00	32	11
	Nov-00	260	18			Nov-00	46	10
	Apr-01	210	34			Apr-01	59	18
	May-01	440	23			May-01	47	16
	Jul-01	200	32			Jul-01	38	19

Table A1-1c.
Inorganic contaminant concentrations (mg/kg dry weight) in surficial sediment collected from Piles Creek (PC) and Sheepshead Meadows (SHM) from June 2000 to July 2001.

Element	Date	PC	SHM		Element	Date	PC	SHM
K	Jun-00	3200	1900		Th	Jun-00	0.75	0.275
	Jul-00	4150	2600			Jul-00	0.94	0.31
	Aug-00	3700	2200			Aug-00	1.3	0.36
	Sep-00	2800	2200			Sep-00	0.5	0.415
	Oct-00	2800	2100			Oct-00	0.49	0.365
	Nov-00	3400	2100			Nov-00	0.65	0.42
	Apr-01	3300	3300			Apr-01	0.475	0.55
	May-01	4100	3000			May-01	1.25	1.4
	Jul-01	3000	3400			Jul-01	0.85	1.1
Se	Jun-00	4.8	0.55		V	Jun-00	120	23
	Jul-00	2.4	0.6			Jul-00	97.5	29
	Aug-00	1.3	0.7			Aug-00	80	27
	Sep-00	1.2	0.415			Sep-00	41	25
	Oct-00	0.49	0.75			Oct-00	50	24
	Nov-00	2.6	0.85			Nov-00	52	23
	Apr-01	0.95	1.05			Apr-01	62	39
	May-01	3.05	3.95			May-01	63	31
	Jul-01	2.15	2.75			Jul-01	45	38
Ag	Jun-00	3.9	0.315		Zn	Jun-00	340	300
	Jul-00	5.45	0.425			Jul-00	525	83
	Aug-00	4.1	0.35			Aug-00	420	56
	Sep-00	0.5	0.41			Sep-00	170	53
	Oct-00	0.5	0.37			Oct-00	180	44
	Nov-00	1.8	0.4			Nov-00	380	43
	Apr-01	0.99	0.43			Apr-01	370	86
	May-01	1.7	0.7			May-01	480	86
	Jul-01	2.5	0.6			Jul-01	260	140
Na	Jun-00	16000	9300					
	Jul-00	17000	12000					
	Aug-00	14000	9800					
	Sep-00	9800	12000					
	Oct-00	10000	9500					
	Nov-00	14000	11000					
	Apr-01	7600	14000					
	May-01	13000	25000					
	Jul-01	9000	23000					

Table A1-1d.
Organic contaminant concentrations (µg/kg dry weight) in surficial sediment collected from Piles Creek (PC) and Sheepshead Meadows (SHM) from June 2000 to July 2001.

Compound	Date	PC	SHM
DDD	Jun-00	200	2.95
	Jul-00	1595	3.1
	Aug-00	640	2.85
	Sep-00	160	3.75
	Oct-00	450	2.7
	Nov-00	3900	3.55
	Apr-01	700	4.55
	May-01	7000	5.5
	Jul-01	1300	4.25
DDE	Jun-00	96.5	2.95
	Jul-00	245	3.1
	Aug-00	210	2.85
	Sep-00	75	3.75
	Oct-00	100	2.1
	Nov-00	410	3.55
	Apr-01	150	4.55
	May-01	520	5.5
	Jul-01	290	1.9
DDT	Jun-00	14.25	2.95
	Jul-00	155.5	3.1
	Aug-00	87	2.85
	Sep-00	18	3.75
	Oct-00	28	2.7
	Nov-00	180	3.55
	Apr-01	61	4.55
	May-01	140	5.5
	Jul-01	30	4.25

Table A1-2a.
Inorganic contaminant concentrations (mg/kg dry weight) in whole body fiddler crab tissue collected from Piles Creek (PC) and Sheepshead Meadows (SHM) from June 2000 to July 2001.

Element	Date	PC	SHM		Element	Date	PC	SHM
Al	Jun-00	173.33	170.00		**Be**	Jun-00	0.21	0.20
	Jul-00	205.00	212.50			Jul-00	0.23	0.22
	Aug-00	375.00	210.00			Aug-00	0.24	0.26
	Sep-00	225.00	180.00			Sep-00	0.22	0.22
	Oct-00	230.00	285.00			Oct-00	0.21	0.26
	Nov-00	190.00	165.00			Nov-00	0.18	0.19
	Apr-01	235.00	186.67			Apr-01	0.23	0.17
	Jul-01	370.00	286.67			Jul-01	0.29	0.28
An	Jun-00	0.53	0.50		**Cd**	Jun-00	0.53	0.50
	Jul-00	0.23	0.22			Jul-00	0.55	0.54
	Aug-00	0.55	0.58			Aug-00	0.55	0.58
	Sep-00	0.22	0.22			Sep-00	1.03	0.55
	Oct-00	0.21	0.26			Oct-00	0.80	0.68
	Nov-00	0.18	0.19			Nov-00	0.45	0.47
	Apr-01	0.23	0.17			Apr-01	0.58	0.44
	Jul-01	0.52	0.50			Jul-01	0.70	0.68
As	Jun-00	8.03	19.00		**Ca**	Jun-00	130000	160000
	Jul-00	10.48	22.25			Jul-00	127500	122500
	Aug-00	11.00	18.00			Aug-00	135000	135000
	Sep-00	7.10	24.50			Sep-00	140000	125000
	Oct-00	9.45	17.00			Oct-00	145000	155000
	Nov-00	11.00	13.00			Nov-00	120000	170000
	Apr-01	10.35	15.67			Apr-01	140000	143333.3
	Jul-01	6.00	21.00			Jul-01	160000	210000
Ba	Jun-00	116.67	21.00		**Cr**	Jun-00	3.17	1.40
	Jul-00	94.50	16.00			Jul-00	2.18	1.01
	Aug-00	115.00	17.00			Aug-00	3.70	1.40
	Sep-00	89.50	14.00			Sep-00	2.40	1.60
	Oct-00	90.50	18.50			Oct-00	2.90	1.65
	Nov-00	100.00	19.00			Nov-00	6.80	2.95
	Apr-01	115.00	17.67			Apr-01	4.30	2.23
	Jul-01	120.00	26.33			Jul-01	3.70	2.13

Table A1-2b.
Inorganic contaminant concentrations (mg/kg dry weight) in whole body fiddler crab tissue collected from Piles Creek (PC) and Sheepshead Meadows (SHM) from June 2000 to July 2001.

Element	Date	PC	SHM		Element	Date	PC	SHM
Co	Jun-00	1.23	1.00		**Ms**	Jun-00	8466.67	12000.00
	Jul-00	1.11	1.09			Jul-00	8875.00	10250.00
	Aug-00	1.10	1.20			Aug-00	8900.00	9850.00
	Sep-00	1.10	1.08			Sep-00	9150.00	9350.00
	Oct-00	1.05	1.30			Oct-00	9750.00	11000.00
	Nov-00	0.90	0.98			Nov-00	8300.00	12300.00
	Apr-01	1.18	0.88			Apr-01	9300.00	10666.67
	Jul-01	1.45	1.38			Jul-01	12000.00	16000.00
Cu	Jun-00	210.00	95.00		**Mn**	Jun-00	25.67	23.00
	Jul-00	162.50	112.00			Jul-00	25.25	24.00
	Aug-00	160.00	109.50			Aug-00	23.50	20.00
	Sep-00	180.00	114.50			Sep-00	19.50	14.50
	Oct-00	165.00	99.00			Oct-00	17.50	23.00
	Nov-00	170.00	105.00			Nov-00	26.00	21.50
	Apr-01	170.00	102.33			Apr-01	28.50	21.67
	Jul-01	220.00	126.67			Jul-01	35.00	34.00
Fe	Jun-00	360.00	480.00		**Hg**	Jun-00	0.68	0.19
	Jul-00	592.50	475.00			Jul-00	0.64	0.21
	Aug-00	825.00	380.00			Aug-00	0.72	0.23
	Sep-00	605.00	300.00			Sep-00	0.60	0.20
	Oct-00	585.00	525.00			Oct-00	0.48	0.19
	Nov-00	640.00	455.00			Nov-00	0.53	0.16
	Apr-01	925.00	396.67			Apr-01	0.48	0.18
	Jul-01	830.00	663.33			Jul-01	0.55	0.16
Pb	Jun-00	15.23	4.20		**Ni**	Jun-00	1.07	1.00
	Jul-00	12.75	1.05			Jul-00	1.11	1.09
	Aug-00	14.50	0.97			Aug-00	1.10	1.20
	Sep-00	14.00	1.09			Sep-00	1.10	1.10
	Oct-00	12.00	1.23			Oct-00	1.05	1.30
	Nov-00	16.00	1.06			Nov-00	0.90	65.45
	Apr-01	18.00	1.17			Apr-01	1.18	0.88
	Jul-01	8.10	1.30			Jul-01	1.45	1.38

Table A1-2c.
Inorganic contaminant concentrations (mg/kg dry weight) in whole body fiddler crab tissue collected from Piles Creek (PC) and Sheepshead Meadows (SHM) from June 2000 to July 2001.

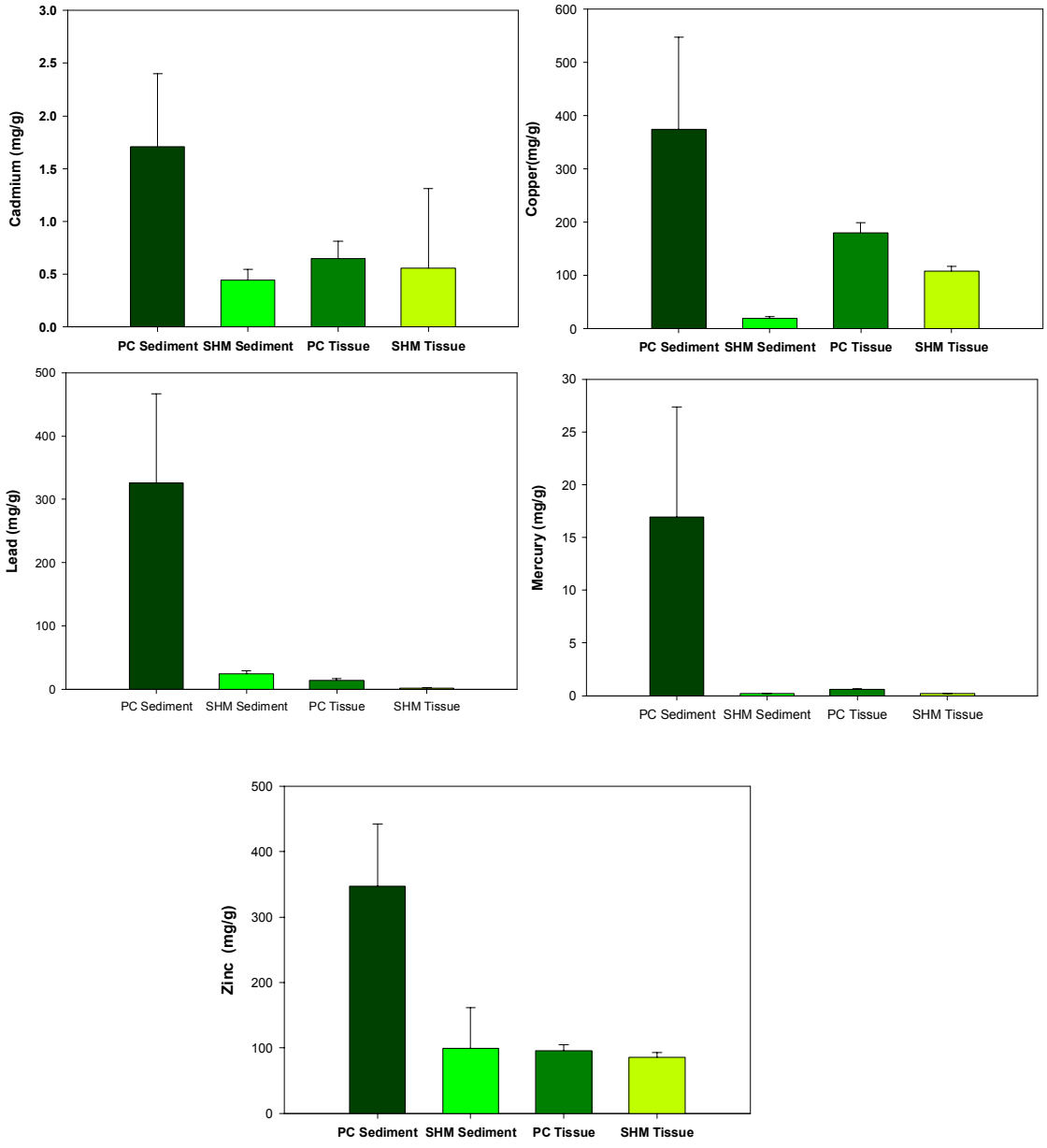
Element	Date	PC	SHM		Element	Date	PC	SHM
K	Jun-00	7566.67	6500.00		**Th**	Jun-00	0.21	0.20
	Jul-00	6600.00	7025.00			Jul-00	0.23	0.22
	Aug-00	7050.00	7700.00			Aug-00	0.24	0.21
	Sep-00	6950.00	7500.00			Sep-00	0.22	0.22
	Oct-00	7050.00	7150.00			Oct-00	0.21	0.26
	Nov-00	6900.00	7550.00			Nov-00	0.18	0.19
	Apr-01	6600.00	6433.33			Apr-01	0.23	0.17
	Jul-01	8000.00	8700.00			Jul-01	0.23	0.36
Se	Jun-00	2.93	1.10		**V**	Jun-00	1.07	1.00
	Jul-00	2.25	1.23			Jul-00	1.11	1.09
	Aug-00	2.25	1.40			Aug-00	1.80	1.20
	Sep-00	2.10	1.06			Sep-00	1.10	1.08
	Oct-00	2.50	1.19			Oct-00	1.05	1.30
	Nov-00	2.00	1.15			Nov-00	2.10	0.98
	Apr-01	2.35	1.10			Apr-01	2.33	0.88
	Jul-01	1.30	0.64			Jul-01	1.45	1.38
Ag	Jun-00	0.53	1.30		**Zn**	Jun-00	97.00	83.00
	Jul-00	0.55	0.88			Jul-00	93.50	78.50
	Aug-00	0.55	0.58			Aug-00	91.00	92.00
	Sep-00	0.55	1.08			Sep-00	93.50	86.00
	Oct-00	0.53	0.68			Oct-00	90.00	78.00
	Nov-00	1.10	1.40			Nov-00	86.00	86.00
	Apr-01	0.58	1.17			Apr-01	96.50	77.00
	Jul-01	0.70	1.02			Jul-01	120.00	103.33
Na	Jun-00	16333.33	14000.00					
	Jul-00	12750.00	21500.00					
	Aug-00	14500.00	17000.00					
	Sep-00	16000.00	19500.00					
	Oct-00	16500.00	19500.00					
	Nov-00	16000.00	22000.00					
	Apr-01	17500.00	15333.33					
	Jul-01	16000.00	20333.33					

Table A1-2d.
Organic contaminant concentrations (µg/kg dry weight) in whole body fiddler crab tissue collected from Piles Creek (PC) and Sheepshead Meadows (SHM) from June 2000 to July 2001.

Compound	Date	PC	SHM
DDD	Jun-00	1280.00	9.75
	Jul-00	1237.50	6.25
	Aug-00	735.00	6.75
	Sep-00	1165.00	7.25
	Oct-00	625.00	7.25
	Nov-00	1600.00	6.50
	Apr-01	120.00	6.67
	Jul-01	2000.00	6.33
DDE	Jun-00	1105.00	8.15
	Jul-00	710.00	5.55
	Aug-00	465.00	6.50
	Sep-00	365.00	7.70
	Oct-00	290.00	7.25
	Nov-00	810.00	6.50
	Apr-01	300.00	2.80
	Jul-01	950.00	6.33
DDT	Jun-00	34.00	9.75
	Jul-00	22.50	6.25
	Aug-00	15.50	6.75
	Sep-00	11.90	7.25
	Oct-00	8.50	7.25
	Nov-00	0.00	0.00
	Apr-01	21.00	13.33
	Jul-01	54.00	6.33

Fig A1.

Mean concentrations (± 95% confidence intervals) of cadmium, copper, lead, mercury and zinc (mg/g) in Piles Creek (PC) and Sheepshead Meadows (SHM) sediment and whole body fiddler crab (U. pugnax) tissue.



Appendix B

Depuration Study Results

INTRODUCTION

Upon collection, fiddler crabs (Uca pugnax) likely have sediment entrained within the digestive tract. As such, it may be necessary to remove this sediment via depuration (where depuration refers to the evacuation of the gut material from an organism retained for a given period in a clean environment) before body burden can be evaluated. The purpose of this study was to determine whether depuration of U. pugnax with and without food affected body burden analyses and if depuration was thereby necessary for accurately determining and presenting contaminant concentrations in fiddler crab tissue.

MATERIALS AND METHODS

Fiddler crabs were collected from Piles Creek (PC), the "contaminated site", a tidal creek and tributary of the Arthur Kill in Linden, New Jersey. The reference or "non-contaminated" site was at Rutgers University Marine Field Station (RUMFS) located within the Great Bay-Little Egg Harbor estuarine system in southern New Jersey. Crabs were collected at low tide from the intertidal zone and marsh surface at both sites. Immediately upon arrival to the laboratory, crabs were rinsed to remove extraneous sediment and placed into plastic storage containers (approximately 42.5 × 31.5 × 15 cm; 17 L) by site and arranged on top of laboratory benches. Room temperature (24º C) 0.5 µm filtered sea water was then added to each container until the water covered approximately 75% of the angled bottom. Sea water added to PC containers was diluted with deionized water to a salinity of 21 to approximate site salinity conditions while water for SHM crabs was unadjusted at salinity 33. An air stone was submerged in the deepest part of the water, approximately 3 to 4 cm deep. Three replicated treatments were used: ND = Non-depurated; DNF = Depurated, non-fed; DF = Depurated, fed.

After a 24 h depuration period, individual jars of crabs (approximately 20-30 crabs per jar) and jars of sediment were analyzed for metals using atomic absorption spectroscopy and for pesticides using gas chromatograph (GC)/electron capture detector (ECD) method. One-way ANOVAs were performed ($\alpha = 0.05$) for the following comparisons: ND vs. DNF and DNF vs. DF.

RESULTS

Sediment concentrations are presented in Table B1. For PC crabs, tissue metal concentrations were generally lower in depurated crabs (Table B2) and were significantly lower in DNF crabs for aluminum, barium, and p, p'-DDT. In some cases food appeared to facilitate increased sediment depuration as concentrations of aluminum, iron, lead and nickel were significantly lower in DF crabs for PC (Table B3). For SHM crabs, depuration decreased metal concentrations for many of the same metals as PC crabs (Table B2) though only sodium and zinc showed significantly lower concentrations (Table B3). There were no significant differences between DNF and DF crabs for SHM (Table B3) although metal concentrations were lower in DF crabs for aluminum, barium, copper, iron and silver (Table B2).

CONCLUSION

Results revealed that depuration resulted in decreased body burden for both SHM and PC crabs. Based on this data, the decision was made to depurate all crabs without food for a 24 h period prior to tissue analyses.

Table B1.

Mean concentrations of metals and pesticides in sediment from Piles Creek (PC) and Sheepshead Meadows (SHM) for the depuration study.

Metals (mg/kg, dry weight)	**PC**	**SHM**
Aluminum	22000.00	8700.00
Antimony	0.00	0.00
Arsenic	140.00	6.90
Barium	2100.00	34.00
Beryllium	0.00	0.00
Cadmium	4.00	0.00
Chromium	290.00	41.00
Cobalt	21.00	5.70
Copper	610.00	19.00
Iron	49000.00	13000.00
Lead	550.00	26.00
Magnesium	9100.00	5300.00
Manganese	320.00	110.00
Mercury	35.00	0.21
Nickel	85.00	14.00
Potassium	4400.00	2600.00
Selenium	2.20	0.00
Silver	4.40	0.00
Sodium	18000.00	12000.00
Thallium	1.00	0.00
Vanadium	85.00	29.00
Zinc	660.00	83.00
Pesticides (µg/kg, dry weight)		
p,p' - DDT	270.00	0.00
p,p' - DDE	380.00	0.00
p,p' - DDD	2600.00	0.00

Table B2.

Mean concentrations of metals and pesticides in U. pugnax tissue for the depuration study for Piles Creek (PC) and Sheepshead Meadows (SHM).

	PC			SHM		
Metals (mg/kg, dry weight)	**ND**	**DNF**	**DF**	**ND**	**DNF**	**DF**
Aluminum	436.67	190.00	126.67	273.33	193.33	123.33
Antimony	0.00	0.00	0.00	0.00	0.00	0.00
Arsenic	7.77	10.63	7.90	16.33	19.33	20.67
Barium	116.67	92.67	96.67	16.67	16.00	15.67
Beryllium	0.00	0.00	0.00	0.00	0.00	0.00
Cadmium	0.00	0.00	0.00	0.00	0.00	0.00
Chromium	4.30	2.20	1.47	0.90	1.00	0.00
Cobalt	0.00	0.00	0.00	0.00	0.00	0.00
Copper	196.67	156.67	163.33	110.00	106.00	97.67
Iron	683.33	590.00	370.00	573.33	470.00	366.67
Lead	9.63	12.67	7.73	0.43	1.00	1.23
Magnesium	9033.33	9400.00	9733.33	10633.33	10666.67	10666.67
Manganese	28.67	26.33	27.67	26.33	26.33	24.67
Mercury	0.76	0.63	0.44	0.19	0.20	0.19
Nickel	2.03	0.00	0.00	0.00	0.00	0.00
Potassium	6900.00	6533.33	6866.67	6900.00	6766.67	7000.00
Selenium	2.43	2.20	2.20	1.23	1.13	1.13
Silver	0.00	0.00	0.00	0.57	0.37	0.33
Sodium	11000.00	130000.00	12666.67	14666.67	19333.33	18333.33
Thallium	0.00	0.00	0.00	0.00	0.00	0.00
Vanadium	0.00	0.00	0.00	0.00	0.00	0.00
Zinc	94.67	91.33	86.00	80.00	75.00	76.00
Pesticides (µg/kg, dry weight)						
p,p' - DDT	0.04	0.02	0.02	0.00	0.00	0.00
p,p' - DDE	1.08	0.67	0.70	0.004	0.00	0.00
p,p' - DDD	1.97	1.08	1.23	0.00	0.00	0.00

Table B3.

One-Factor Analysis of Variance (ANOVA) results (α = 0.05) for metals and pesticides in crab tissue for Piles Creek (PC) and Sheepshead Meadows (SHM).

	Not Depurated vs. Depurated		Depurated, Not Fed vs. Depurated, Fed	
Metals (mg/kg, dry weight)	**SHM**	**PC**	**SHM**	**PC**
Aluminum	0.098	0.008*	0.094	0.009*
Antimony	Not detected	Not detected	Not detected	Not detected
Arsenic	0.371	0.002*	0.778	0.004
Barium	0.561	0.002*	0.643	0.243
Beryllium	Not detected	Not detected	Not detected	Not detected
Cadmium	Not detected	Not detected	Not detected	Not detected
Chromium	0.927	0.027*	0.118	0.418
Cobalt	Not detected	Not detected	Not detected	Not detected
Copper	0.599	0.055	0.370	0.230
Iron	0.147	0.079	0.070	0.002*
Lead	0.272	0.068	0.756	0.017*
Magnesium	0.950	0.547	1.000	0.380
Manganese	1.000	0.391	0.519	0.230
Mercury	0.678	0.202	0.539	0.034*
Nickel	Not detected	Not detected	Not detected	Not detected
Potassium	0.469	0.132	0.346	0.152
Selenium	0.468	0.374	0.978	1.000
Silver	0.782	Not detected	0.950	Not detected
Sodium	0.013*	Not detected	0.468	0.374
Thallium	Not detected	Not detected	Not detected	Not detected
Vanadium	Not detected	Not detected	Not detected	Not detected
Zinc	0.018*	0.493	0.736	0.300
Pesticides (µg/kg, dry weight)				
p,p' - DDT	Not detected	0.009*	Not detected	0.588
p,p' - DDE	0.002*	0.027	Not detected	0.742
p,p' - DDD	Not detected	0.011	Not detected	0.327

Appendix C

Feasibility Study Results
June & July 2000

RESULTS

In June and July of 2000, a feasibility study was conducted to assess fiddler crab sampling techniques and lipid analysis requirements. Initially, both male and female crabs were collected from each site and several crabs were homogenized together by sex for lipid analyses (Table C1). Several male crabs from the depuration study (Appendix B) were combined and homogenized for lipid analyses in July 2000 (Tables C2a and C2b). After reviewing the data and consulting with the contract laboratory, it was decided that future field collections should target individual larger male crabs for lipid analyses. Individual males, and not a homogenized sample of several males, were chosen to provide a more accurate picture of lipids per individual. Whole body crabs were used as this is the simplest method for use in ecological risk assessments. Females were not chosen as visual inspection did not always reveal reproductive state which could influence lipid concentrations.

Table C1.

Lipid class results (µg/g, wet weight) for assorted U. pugnax from Piles Creek (PC) and Sheepshead Meadows (SHM) collected in June 2000.

	Two large ovigerous females	Several small males	Two large males	Several small females	Three large females	Several small males	Three large males	Three large females	Several small ovigerous females
Lipid Class	**Piles**	**Piles**	**Piles**	**Piles**	**Piles**	**Piles**	**Piles**	**Piles**	**SHM**
Hydrocarbons	3.85	48.64	7.56	123.67	30.80	33.63	8.44	25.03	6.34
Steryl Esters/Wax Esters	128.47	8.77	0.00	123.79	8.76	13.90	18.89	30.38	110.49
Methyl Esters	785.74	353.02	192.65	671.68	505.83	475.13	377.97	672.64	200.21
Ethyl Ketones	56.40	0.00	0.00	0.00	50.32	49.51	80.68	0.00	0.00
Triacylglycerol	7066.15	3641.64	1274.23	10997.11	4253.81	2417.50	1394.20	6715.63	13125.14
Free Fatty Acids	328.27	1203.28	630.94	1170.28	621.27	789.71	593.34	595.10	492.06
Alcohols	0.00	0.00	0.00	44.62	0.00	0.00	0.00	0.00	0.00
Sterols	917.07	899.79	570.72	1304.82	587.59	786.05	596.15	733.07	1009.61
Diacylglycerols	268.14	54.61	47.31	306.49	129.15	45.70	48.21	144.96	272.23
Acetone Mobile Polar Lipids	304.82	489.67	308.15	1004.58	347.72	493.87	412.89	447.50	344.41
Phospholipids	4064.72	3047.32	2168.86	5929.39	2956.22	2523.77	1950.04	4137.95	7178.90
Total Lipids	13923.62	9773.74	5200.42	21676.41	9491.46	7628.77	5480.80	13502.27	22739.38

Table C2a.

Lipid class results (µg/g, wet weight) for U. pugnax from Piles Creek (PC) and Sheepshead Meadows (SHM) from the depuration study.

	SHM	PC	SHM	SHM	SHM	PC	PC	PC
Lipid Class	**ND**	**ND**	**DF**	**DNF**	**DF**	**DNF**	**DNF**	**DF**
Hydrocarbons	1.03	178.15	23.53	15.80	14.67	124.00	77.90	112.46
Steryl Esters/Wax Esters	0.00	23.43	3.00	0.75	36.55	24.63	0.00	0.00
Methyl Esters	21.84	426.62	212.70	102.36	147.91	202.15	91.30	436.88
Triacylglycerol	2693.78	5782.33	1254.49	689.28	680.44	5650.83	3553.25	4439.60
Free Fatty Acids	134.21	469.01	509.54	231.70	427.10	353.80	446.46	283.51
Alcohols	3.10	11.53	0.00	0.00	0.00	0.00	0.00	0.00
Sterols	326.13	680.95	543.40	669.60	588.63	558.33	562.64	674.69
Diacylglycerols	80.51	189.09	91.64	57.74	55.98	110.18	71.62	394.33
Acetone Mobile Polar Lipids	119.04	408.69	348.48	249.34	370.01	271.94	334.84	392.83
Phospholipids	1112.51	3883.08	2419.13	2151.97	2922.85	2510.36	3148.03	332.10
Total Lipids	4492.17	12052.88	5405.73	4168.54	5244.14	9806.21	8286.04	10036.41

ND = Not Depurated
DNF = Depurated, Not Fed
DF = Depurated, Fed

Table C2b.
Lipid class results (µg/g, wet weight) for U. pugnax from Piles Creek (PC) and Sheepshead Meadows (SHM) from the depuration study.

	SHM	PC	PC	SHM	SHM	SHM	SHM	PC	PC	PC
Lipid Class	**ND**	**ND**	**DF**	**DNF**	**DNF**	**DF**	**DF**	**DNF**	**DF**	**DF**
Hydrocarbons	55.61	153.18	109.71	36.59	26.72	17.59	12.15	65.05	93.04	132.78
Steryl Esters/Wax Esters	5.05	0.00	0.00	0.00	0.00	0.00	35.73	0.00	2.53	0.00
Methyl Esters	330.07	366.49	447.99	71.64	85.58	70.35	233.83	117.71	13.10	557.08
Ethyl Ketones	22.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Triacylglycerol	2224.73	7213.16	7970.95	373.83	517.55	1154.77	4116.26	4990.05	5353.18	7894.60
Free Fatty Acids	350.12	387.99	303.05	92.21	80.09	337.98	642.66	343.03	1048.07	329.64
Sterols	525.16	936.81	720.49	489.42	390.27	456.30	1387.78	552.14	708.49	837.45
Diacylglycerols	70.98	252.27	152.05	24.70	23.34	63.67	105.63	74.81	263.11	264.64
Acetone Mobile Polar Lipids	210.92	312.22	210.12	184.84	140.85	174.63	635.33	344.27	375.37	301.68
Phospholipids	2313.08	3332.85	7473.63	2677.50	1779.16	2663.68	6193.53	2613.84	2314.11	4072.38
Total Lipids	6098.82	12954.96	17379.00	3950.74	3043.55	4938.98	13362.9	9100.89	10171.62	14390.25

ND = Not Depurated
DNF = Depurated, Not Fed
DF = Depurated, Fed

Curriculum Vitae
Dale Marie Haroski

EDUCATION

- Rutgers University - Ph.D. Graduate Program in Ecology and Evolution, October, 2008
- Rutgers University - M.S. Graduate Program in Ecology and Evolution, 1998
- University of Scranton - B.S. Biology, 1993

WORK EXPERIENCE

U.S. EPA Environmental Protection Agency (U.S. EPA)
January 2002 - Present

Lockheed Martin / Response, Engineering, and Analytical Contract
Aquatic Ecologist, May 1999 - January 2002 (January 1997 to May 1999, Roy F. Weston)

PUBLICATIONS

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