Compensatory partitioning of physiological resource budgets by the grass shrimp (*Palaemonetes pugio*) in association with contaminants encountered in a marsh creek system

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ABSTRACT OF THE DISSERTATION

Compensatory partitioning of physiological resource budgets by the grass shrimp (*Palaemonetes pugio*) in association with contaminants encountered in a marsh creek system

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Resource budgets of the grass shrimp (*Palaemonetes pugio*) were examined for the effects of a highly contaminated field site. Physiological metrics of resource allocation were compared to those of shrimp from a more pristine location. Oxygen, carbon, nitrogen and energy use were compared in shrimp caught from the two sites. The physiological budgets for carbon, nitrogen and energy were examined for evidence of the compensatory partitioning of resources that would allow shrimp from the polluted field site to maintain allocation to growth and reproduction despite contact with a wide variety of contaminants. All components of the budget were measured directly instead of the traditional indirect estimates of production and reproduction. Evidence for compensatory partitioning of resources by grass shrimp at the contaminated field site was found. Ovigerous shrimp from the contaminated site increased reproductive allocation relative to ovigerous shrimp from the clean reference site. Ovigerous shrimp from the two sites had similar rates of resource acquisition through consumption, but ovigerous shrimp from the

contaminated site at Piles Creek had lowed allocation to respiration and ammonia excretion. This allowed them to allocate surplus resources to reproduction. Intermolt shrimp from Piles Creek conversely had reduced consumption and increased respiration in comparison to intermolt shrimp from the clean reference site near Tuckerton. Despite reduced resource acquisition through consumption and increased respiratory costs, these shrimp were able to maintain growth rates similar to those of intermolt shrimp from Tuckerton.

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Chapter 1

Introduction

The grass shrimp (*Palaemonetes pugio*) has a long history as a test subject for the effects of potential toxins and as a major component of models of estuarine function. This study examined the effects of a highly contaminated field site on shrimp using physiological metrics of resource allocation, and compared them to those of shrimp from a more pristine location. Oxygen, carbon, nitrogen, and energy use were compared in shrimp caught from the two sites. The physiological budgets for carbon, nitrogen and energy were examined for evidence of the compensatory partitioning of resources that would allow shrimp from the polluted field site to maintain allocation to growth and reproduction despite contact with a wide variety of contaminants. All components of the budget were measured directly, but not for all treatment groups. Growth and ecdysis were measured for intermolt shrimp, while reproduction was measured for ovigerous shrimp.

Background

Grass shrimp

The grass shrimp (*Palaemonetes pugio*), common to estuarine environments of the East Coast of North America, is an important species in terms of ecological function. *P. pugio* is one of the most widely distributed and abundant shallow water benthic macro-invertebrates found in the Atlantic and Gulf states (Wood 1967, Odum and Heald 1972, Anderson 1985). Grass shrimp are also one of the most important species in estuarine energetics due to their large role in detrital processing (Sikora 1977). In estuaries and salt

marsh habitats they are a primary macroinvertebrate consumer of *Spartina* and *Ulva* detritus, allowing the cycling of detritus into animal biomass (Welsh 1975).

Grass shrimp have not only been found to be important detritivores, but are equally important in their role as epiphyte grazers, encouraging the growth of macrophytes through the effects of grazing and nutrient deposition (McCall and Rakocinski 2007).

P. pugio is also a major prey item for a number of estuarine fishes, and is, indirectly, of great recreational and economic importance (Gunter 1945, Darnell 1958, Diener et al. 1974, Anderson 1985, O'Neil and Weinstein 1987, Buckel and Conover 1997).

Due to its ecological importance, *P. pugio* is widely studied, including a large number of ecological and toxicological studies (Buikema et al. 1980, Wilson 1985, Weber et al. 1996, Wilson 1998, Reinsel et al. 2001).

While the abundance of grass shrimp is one measure of their importance in estuarine systems, their ability to tolerate a range of conditions is equally important. This allows them to dominate in otherwise marginal areas, as well as being common in more diverse habitats (Welsh 1975). They are able to thrive in a wide range of salinity and temperature, and can be found in bays, creeks, and on the marsh surface (Anderson 1985). *P. pugio* is also relatively tolerant of anthropogenic contaminants, including high levels of mercury contamination (Kraus et al. 1988). Grass shrimp are thus a key species throughout their range on the North American eastern seaboard.

Field Collection Sites

The two sites where specimens were collected show two extremes of anthropogenic impact found in New Jersey. The Tuckerton site near Big Sheepshead Creek is relatively pristine (Able et al. 1996). Part of the Mullica River/Great Bay estuary system, it is one of the least disturbed estuaries in the North East. As such it is part of the National Estuarine Research Reserve system. These sites are considered baseline reference sites for establishing the effects of anthropogenic impacts. The Great Bay and Mullica systems are now, and have historically been, pristine and free of excessive nutrient loading from anthropogenic sources. There is little industry or development within the Mullica River drainage basin. The one major source of nutrient loading to Great Bay, a menhaden processing plant, has been closed since 1960. Because the collection site is over 200 meters from the small access road and has no access by boats, it has additional protection from incidental impacts and non-point sources such as motor oil spills and runoff.

Piles Creek lies in a heavily industrialized area with many historical and ongoing sources of contamination. There are several active and abandoned chemical-processing plants near the creek, as well as the Linden Sewerage facility. There is an Exxon Mobil refining center directly adjacent to the site with fuel lines that cross over the site itself. There have historically been both large and point source releases of oil ranging from leaks in the pipes to discharges from small boats. Oil is visible in the sediments and on the surface of the water. High levels of mercury, cadmium, and lead, as well as a wide range

of organic contaminants, have been found at the site for decades (Luther et al. 1980, Khan et al. 1989, Tso and Taghon 1997). Weis et al. (2001) found sediment contaminant levels of 6.3±1 μg/g Hg, 485±46.7 μg/g Cu, 107±20.8 μg/g Pb, 525±67.1 μg/g Zn, 7.1±2.1 μg/g Cd, 0.69 μg/g PCB and 22.8 μg/g PAH. A more recent study found mercury contamination has declined to <5 μg/g (Weis 2002).

Contaminant levels in Tuckerton sediments have been reported as 0.19±0.02 μg/g Hg, 43.8±7.6 μg/g Cu, 73.2± 7.0 μg/g Pb, 141± 13.4 μg/g Zn, 2.1±1.2 μg/g Cd, 0.25 μg/g PCB and 6.0 μg/g PAH (Weis et al. 2001).

Like the marsh creek at Tuckerton, Piles Creek is a tidal creek lined with *Spartina alterniflora* and surrounded by *Spartina patens* high marsh. The tidal creek at Tuckerton receives fresh water inputs from an extensive drainage basin while Piles Creek has more limited fresh water input. Nevertheless, salinity has been reported in the range of 20-25 for both sites (Khan and Weis 1987). During shrimp collection for this study, the upper level of salinity at both sites was similar to the upper level of 25 reported previously, but the Tuckerton site had salinity levels as low as 10 after heavy rains.

Contaminants and stress

Forbes and Calow (1999) recommended that anthropogenic contaminants be viewed as causes of stress leading to loss of fitness within the stressed population. In this view, acute toxicity would be only the most obvious of anthropogenic impacts. Forbes and

Calow argued that population growth models are more appropriate tools for measuring environmental impacts than the traditional "LD-50" models.

Maltby (1999) recommended that models of energy expenditure be used to predict both organism and population level responses to anthropogenic stressors. Calculating an organism's energy budget allows one to determine its "scope for growth" (SfG). Short-term measures of SfG have been shown to correlate well with both growth and reproduction over the long term (Bayne et al. 1985, Maltby and Nayler 1990). It is generally postulated that tolerance to environmental contaminants has metabolic costs (Weis and Weis 1989, Harper et al. 1997, Maltby 1999). Scope for growth is affected by reduced feeding, if it is without a concomitant reduction in respiration (Widdows et al. 1995, Maltby 1992). Klok and de Roos (1996) used a population growth model based on the SfG of individuals to show that a population could be eliminated due to physiological costs of contamination even if there was no direct effect on reproduction. Reproduction in terms of egg production and development may be unchanged in the presence of contaminants, but if SfG is reduced sufficiently, reproduction as a function of body mass will also be reduced. Thus, to determine the impact of anthropogenic changes in a habitat on both individuals and populations, it is necessary to look at an organism's entire energy budget.

Contaminants and physiological budgets

Environmental contaminants can change resource allocation in physiological budgets. The effects of toxicants on resource allocation can potentially be seen in two separate

ways. Weiser (1994) described two major patterns of resource allocation. The first is proportional allocation. In this scenario, the ratio of resources allocated to separate components of the equation remains the same. A reduction in a resource allocated to oxygen metabolism would mean lower resource allocation in all areas. Thus, a single component, like metabolic oxygen demand, could be used as a metric for the entire balanced energy equation for the organism.

Disproportional resource allocation is the more common phenomenon, and occurs in two types. In type one, additive partitioning, budget components such as growth and reproduction remain unchanged despite changes in oxygen use. In a study of this type we could expect to see a rise or drop in metabolic use of oxygen without changes in growth, reproduction or feeding. Alternatively, Weiser (1994) recognized another possibility for resource partitioning. In the second type of disproportional partitioning, compensatory partitioning can also be a factor. Thus, a variable such as oxygen metabolism could remain the same despite changes in activity levels or other influences on oxygen metabolism (Weiser 1989). Changes in other components of the energy equation would compensate for the increased energy demand. In this scenario an observed drop in resources allocated to oxygen metabolism would imply additional resources allocated to some combination of growth and reproduction or an increase in egestion.

In conjunction with the general prediction that there is reduced metabolic demand for oxygen in contaminated environments, there may be a reduced reproductive output. If components of the resource budget are proportionate, a reduced oxidative metabolism in

egg producing females would correspond to a reduced production of eggs. There would be fewer eggs with less stored reserves. Conversely, if shrimp resource budgets respond disproportionately to life in a heavily contaminated environment, there could be an increased reproductive output due to lowered costs of oxygen metabolism, or reproductive output could remain the same.

An example of the possible ways an energy budget can change in response to a contaminant is seen in Stickle et al. (1987). They found that the shrimp *Pandalus borealis* shows disproportionate energy partitioning between consumption and other budget components in response to the water-soluble components of fuel oil. They reported total production, or SfG, rather than separate terms for growth and reproduction, and found that production was determined by feeding rate. The energy budget developed by the investigators measured the costs of respiration and ammonia excretion and found that energy budget costs did not change significantly in response to differing doses of fuel oil, while feeding rate was dose dependent. In fact, at low doses, feeding rates increased, showing evidence of hormesis (the hypothesized tendency for low doses of contaminants to have beneficial results), while at high doses, feeding rates decreased. Given that the metabolic costs measured by Stickle et al. (1987) remained relatively constant at all dosages, only consumption determined production in their experiment. Metabolic costs did not change proportionately with energy intake. Nitrogen excretion showed some dose response, but was considered relatively unimportant because it accounted for only 10-20% of metabolic costs, while oxygen metabolism accounted for 80-90% of costs. Energy intake determined production rather than metabolic costs.

Thus the differences in both the individual components of an energy budget, as well as the partitioning between components, may have effects on an organism's function in a given habitat. Single metrics of metabolic function are not adequate to determine either long-term population effects of anthropogenic contamination or effects on individual organisms.

Studies of the bioenergetics of other crustaceans have shown large differences in the impact of contaminants on resource allocation and production. Stickle et al. (1987) found increased feeding in the shrimp *Pandalus borealis* in response to aromatic hydrocarbons without a concomitant increase in metabolic costs, such as respiration. Thus, total production actually increased in response to aromatic contaminants. Conversely, blue crabs (*Callinectes sapidus*), when exposed to the same weathered fuel oil contaminants, showed a decrease in feeding rate (Wang and Stickle 1987). In both studies, metabolic costs such as respiration remained relatively constant while feeding rate changed in response to contamination. Clearly, these examples show disproportionate partitioning of resources. Feeding rates responded differently in the two species without changes in oxygen use. Thus, total production of a species may either increase or decrease depending on the organism in question or the contaminant. Whether this is due to compensatory or additive disproportionate partitioning is not clear. The components of production were not analyzed in either study.

Elements of an organism's energy budget may move together, or may respond disproportionately to various impacts. Rowe (1998) found a closely related grass shrimp (*Palaemonetes paludosus*) had an increased standard metabolic rate (SMR), as measured by oxygen consumption, when exposed to coal combustion products under field conditions. He speculated that compensatory partitioning suppresses other portions of the balanced energy equation, such as growth and reproduction, as a response to the lower SMR. The general understanding that contaminants will have a metabolic cost, and that portions of an energy budget are likely to respond similarly to such impacts, warrants such speculation. While often this may be true, there is no fixed pattern. Only through measuring every component of a resource budget is it possible to ascertain if reduced allocation in one component compensates for reduced consumption or increased allocation to another component.

Contaminants and *P. pugio*

The impact of anthropogenic contamination on *P. pugio* has not been fully determined. The studies by Dillon (1981, 1983) and Hutcheson et al. (1985) found that shrimp exposed to certain contaminants in the laboratory can show changes in the physiological budgetary component of oxygen metabolism, but field-collected shrimp show the ability to adapt to or at least mitigate the effects of contaminants. Kraus et al. (1988) found that grass shrimp captured from an area of high contamination (Piles Creek) had increased resistance to lethal effects of mercury in the laboratory. Klerks (1999) concurred that grass shrimp exposed to single contaminants, such as metals and polyaromatic hydrocarbons (PAHs), developed resistance to those compounds, while shrimp exposed

to a variety of contaminants simultaneously did not develop resistance. This may be typical of organisms in field conditions. Weis and Weis (1989) found that mummichog from Piles Creek had developed tolerance to organic mercury but not to the more rarely occurring inorganic mercury. There also was no cross tolerance with organic contaminants such as PCBs or even with other metals such as lead. Single contaminant dosing does not give a clear picture of physiological responses to the wide range of contaminants found simultaneously in the field.

Generally, when an organism is exposed to contaminants, function is reduced. Wall et al. (2001) found that grass shrimp from a highly contaminated estuary had lower reproductive output, as measured by brood size and mass, than shrimp from a cleaner reference site. This is in contradiction of the study by Santiago Bass et al. (2001), which found increased reproductive output in shrimp from Piles Creek. In some cases, grass shrimp are able to obscure the effects of pollutants through various methods of tolerance. One method for tolerating a reduction in one component of a resource budget is to move resources from another component, as investigated in this study. Compensatory partitioning may explain many of the conflicting results found in previous studies.

Vernberg and Piyatiratitivorakul (1998) determined the energy budget for *P. pugio* when various environmental factors, such as salinity or temperature, are altered. There has been no study, however, of the effect environmental contamination in the field has on shrimp energetics. Particularly lacking are studies examining changes in oxygen

metabolism due to exposure to contamination in the field, combined with the possible impacts on reproductive output.

Earlier studies have primarily relied on a single proxy metric, such as oxygen consumption, to determine changes in metabolic rates by *P. pugio* in response to environmental contamination. Stickle et al. (1987) looked at bioenergetics in other crustaceans, but focused on the effects of single contaminants on production, rather than effects of a suite of contaminants present in a heavily impacted habitat. The respiration portion of the equation has been measured in relation to several contaminants, but no researcher has looked at it in conjunction with differences in allocation between somatic growth and reproductive output.

Oberdorster et al. (1999) conducted one of the few studies of shrimp (*Palaeomonetes* sp.) metabolism designed to show effects of a contaminated habitat rather than laboratory dosing, but due to the high variance in the measured variables, were unable to find any significant effects. The investigators measured heat protein 63 (a stress indicator) and cytochrome P450 1A (an indicator of metabolic function) and found no differences despite heavy lead contamination along a gradient marked by reduced shrimp abundance. While shrimp tested after exposure in the field showed no differences, when shrimp were exposed to the same sediments in the laboratory they showed raised metabolism as measured by cytochrome P450 1A.

Previous studies have demonstrated that organic and metal contaminants can have a significant effect on shrimp metabolism as measured by respiration rate (Anderson et al. 1974, Dillon 1981, Dillon 1983, Hutcheson et al. 1985, and St. Amand et al. 1999). None of these studies, however, has specifically investigated ovigerous shrimp. While ovigerous shrimp offer some complicating factors, such as difficulty in measuring growth and ecdysis, these sources of uncertainty are outweighed by the advantages. Ovigerous shrimp allow complete certainty as to the sex of a shrimp and its life history stage. Focusing on a single, easily identifiable life history stage as well as the more usual intermolt shrimp, the present study demonstrates the advantages of factoring life history information into metabolic studies. By studying shrimp whose life history stage is uniform, it is possible to increase the ability to determine treatment effects. Also, because previous studies have not focused on females with eggs, this study provides resource allocation budgets for a critically important stage in the life of an otherwise well studied organism.

While previous studies have shown inconsistent results, some general trends are recognizable. There is a tendency for laboratory dosing studies to show a reduction in physiological functions in response to contaminants. This may be particularly true for embryonic and reproductive shrimp. Adult *P. pugio* have a different metabolic budget than either larval or juvenile shrimp (Vernberg and Piyatiratitivorakul 1998). It has not previously been tested whether ovigerous shrimp, like juveniles, also show significantly different energy budgets than the budgets of adult intermolt shrimp.

These studies highlight some of the difficulties in relating laboratory studies to field studies. Exposure in the field can be very different from exposure in the laboratory. The act of collecting sediments may uncover toxicants previously bound to sediments and sequestered, and thus expose shrimp to contaminant levels beyond what they are exposed to in the field. Alternatively, shrimp may develop behavioral tolerance in the field by avoiding areas of contamination, but may be unable to do so in the laboratory. The variance in physiological responses due to a variety of tolerance strategies makes it more difficult to draw conclusions from studies based on field-caught specimens. The study by Oberdorster et al. (1999) highlights the need to use methods that could potentially reduce the variance typically found in metabolic studies.

Hypothesis

This study determines whether compensatory allocation of resources allows shrimp from a contaminated environment to have similar rates of assimilation and production to shrimp from uncontaminated habitats, or if these compensatory mechanisms are overwhelmed, as was found by Klerks (1999). In addition, the lack of prior work on the most influential life history stage in shrimp populations has been addressed.

Measuring resource acquisition and allocation provides a more sensitive test of anthropogenic impacts on a population than the more usual toxicological approach of determining lethality (LD-50) for separate contaminants. Stickle et al. (1984, 1985) found that due to factors such as tolerance over time, the change in bioavailability of varying aromatic hydrocarbons due to weathering and adsorption, and behavioral factors

such as filter feeding or burrowing, an index of bioenergetics is a more sensitive metric than lethality, particularly for benthic organisms such as shrimp.

The contaminated site chosen here, Piles Creek, near Elizabeth, New Jersey, has been the subject of many studies involving anthropogenic contaminants and grass shrimp. (Luther et al. 1980, Weis and Khan, 1990, Kraus and Kraus, 1986, Khan and Weis 1987, Khan et al. 1989, Weis and Weis 1989, Khan and Weis 1993, Smith et al. 1995, Tso and Taghon 1997, Zhou and Weis 1999, and Schmalz et al. 2002). A study by Santiago Bass et al. (2001) found that Piles Creek shrimp are larger and more numerous than Tuckerton shrimp with a higher proportion of ovigerous females. Several explanations have been advanced for this difference.

A series of papers has described the effects of contaminants on interactions between the grass shrimp and its primary piscine predator, *Fundulus heteroclitus* (Kraus and Kraus, 1986, Khan and Weis 1987, Weis and Weis 1989, Weis and Khan, 1990, Zhou and Weis 1999, Weis et al. 2001, Weis 2002). These papers have drawn the connection between the physiological and behavioral effects of contaminants, particularly compounds of organic mercury, and reduced predation on grass shrimp. Similarly, a study by Santiago Bass et al. (2001) shows a trophic cascade, with reduced predation attributed to anthropogenic contaminants. The authors did not attribute the differences in the shrimp populations to the direct effects of contaminants on the shrimp themselves, because they found no significant differences in time to reproduction, or shrimp growth rates when the shrimp were tested in the laboratory. Because Piles Creek does have a lower fresh water

input than Big Sheepshead Creek in Tuckerton, salinity was also examined and found not to be a factor in growth rates. The authors concluded that the increased shrimp biomass at Piles Creek was due primarily to lowered predation from *Fundulus heteroclitus*, and was not the direct effect of contaminants on the shrimp themselves. The positive effects of lowered predation overwhelmed any negative effects on the shrimp due to contaminants. Nevertheless, the differences in the two creek systems are so extreme in terms of anthropogenic disturbance that it is likely that contaminants at Piles Creek have a direct effect on shrimp physiology as well as the observed indirect effect through the suppression of predation.

An alternative hypothesis to the one proposed by Santiago Bass et al. (2001) is possible. The shrimp are indeed affected by living in a contaminated habitat, but they are able to compensate for the effects of contaminants and allocate more to growth and reproduction by lowering resource allocation to other components of their resource budgets. Lowered predation helps lead to a positive effect, but it is compensatory partitioning of resources by the shrimp that allows growth and reproduction to be maintained in a contaminated habitat.

A previous study on grass shrimp from two highly polluted marsh creeks near Piles Creek, in the Arthur Kill system, found reduced feeding in field-caught specimens. This reduction in feeding was also induced in shrimp from a clean reference site (Tuckerton) by exposing them to contaminated sediments (Perez and Wallace 2004). If Piles Creek shrimp show reduced feeding, as predicted by previous studies, then increased allocation

to growth must be compensated for by a decrease in resources allocated to another portion of the physiological budget. An increase in the proportion of resources obtained by feeding to growth could then be possible and allow Piles Creek shrimp to have growth rates similar to those from Tuckerton as shown by Santiago Bass et al. (2001).

The present study measures how compensatory allocation of resources allows the shrimp exposed to contaminants in the field to maintain production despite reduction in some components of shrimp physiological budgets. It also provides complete physiological budgets for ovigerous and intermolt *Palaemonetes pugio* utilization of C, N, and energy as well as oxygen uptake by early and late embryos. This provides information on rates of assimilation for ovigerous and intermolt shrimp, allows comparisons of usual estimates of reproductive output with measured reproductive output, and highlights differences in budget allocation associated with field exposure to contaminants.

Chapter 2

Lowered oxygen uptake in association with anthropogenic contaminants encountered in the field by ovigerous grass shrimp (*Palaemonetes pugio*)

Introduction

This study measures the resting metabolic rate of the grass shrimp *Palaemonetes pugio.* *P. pugio* is common in estuarine environments of the East Coast of North America, and is an important species in terms of ecological function. *P. pugio* is one of the most widely distributed and abundant shallow water benthic macro-invertebrates found in the Atlantic and Gulf states (Wood 1967, Odum and Heald 1972, Anderson 1985), and plays an important role in estuarine energetics due to its large role in detrital processing (Sikora 1977). In some estuaries it is the primary consumer of *Spartina* and *Ulva* detritus, allowing the cycling of detritus into animal biomass (Welsh 1975). *P. pugio* is a major prey item for a number of estuarine fishes and is thus, indirectly, of great recreational and economic importance (Gunter 1945, Darnell 1958, Diener et al. 1974, Anderson 1985, O'Neil and Weinstein 1987, Buckel and Conover 1997). Due to its ecological importance *P. pugio* is widely studied, including a large number of both ecological and toxicological studies (Buikema et al. 1980, Wilson 1985, Weber et al. 1996, Wilson 1998, Reinsel et al. 2001). Here, I compare the metabolic rates of grass shrimp from a highly contaminated site at Piles Creek, near Linden, NJ, and from a relatively pristine site in Little Egg Harbor near Tuckerton, NJ.

Previous studies have shown that larval and adult grass shrimp found in Piles Creek have some tolerance to mercury contamination (Kraus et al. 1988). Furthermore, shrimp from Piles Creek are both larger and more abundant than shrimp from Tuckerton, with similar times to reproduction (Santiago Bass et al. 2001). Santiago Bass et al. (2001) also found that shrimp from these two sites have equivalent growth rates, and concluded that the increased size and abundance of Piles Creek shrimp are due to differences in predation by *Fundulus heteroclitus*. The *F. heteroclitus* found at Piles Creek have reduced size, abundance, and predatory ability compared to Tuckerton fish, which is attributed to heavy metals and other contaminants (Khan and Weis 1987, Weis and Weis 1989, Weis et al. 2001).

Nevertheless, tolerance is not without costs. Kraus et al. (1988) attributed the tolerance of Piles Creek shrimp to mercury to metallothionein production and increased depuration. Protein production and increased excretion are energetic costs that would slow growth if there were not a compensatory adjustment in some other portion of the shrimps' metabolic budget. If Piles Creek shrimp are able to maintain growth and reproduction expenditures in the presence of toxicants at Piles Creek, there must be some compensatory saving in another metabolic function. Respiration in *P. pugio* and other decapod shrimp can be influenced by both heavy metal and organic contaminants. In most cases respiration is depressed by the presence of anthropogenic contaminants (Anderson et al. 1974, Dillon 1981, Dillon 1983, Hutcheson et al. 1985, and St. Amand et al. 1999).

Previous studies on respiration have focused on the effects of contaminants given in laboratory dosing studies. One of the few studies that measured respiration in a *Palaemonetes* sp. in response to field exposure found no significant correlation between respiration and exposure, despite the same shrimp showing a significant reduction in respiration when exposed to the same contaminant (lead) when the shrimp were dosed in the laboratory (Oberdorster et al. 1999). Field exposure showed too much variance for significant results, yet is a much better measure of the actual impact of a contaminant as encountered by shrimp under field conditions. In the current study, shrimp were also exposed to contaminants in the field and respiration was measured in the laboratory. This preserved the signal from exposure in the field while controlling for possible environmental variables such as temperature and salinity. Measuring respiration in the well-defined life history stage of ovigerous shrimp also reduced variance.

Materials and methods

Grass shrimp were sampled from two New Jersey coastal marshes. Shrimp were collected during their breeding cycle from late June through early September. Shrimp were collected from each site on alternating weeks so that seasonal differences would not obscure site differences. One group was taken from a highly polluted, heavily industrialized salt marsh along the Arthur Kill in the New York/New Jersey harbor. These shrimp came from Piles Creek, a small marsh creek that runs past active refineries and chemical plants. An abandoned plant that produced photographic material is also located nearby. High levels of mercury, cadmium, and lead, as well as a wide range of organic contaminants, are present in the environment (Luther et al. 1980, Khan et al.

1989). Mercury levels in Piles Creek sediment are in the range of 10-20 μg/g and lead levels are up to 3000 μg/g (Weis and Weis 1989). More recent studies have found mercury contamination has declined to <5 μg/g (Weis 2002). Other metals include 6 μg/g Cd, 620 μg/g Cu, and 630 μg/g Zn (Khan and Weis 1987). The second group of shrimp was taken from the estuary of the Mullica River near the Rutgers Marine Field Station at Tuckerton, NJ. This estuary is considered one of the least disturbed estuaries in the northeastern United States (Able et al. 1996) and is located within the Jacques Cousteau National Estuarine Research Reserve. Metal levels in Tuckerton sediments have been reported as (in μg/g) 0.054 Hg, 0.13 Cd, 12.9 Cu, and 7.7 Zn (Khan and Weis 1987). The tidal creek at Tuckerton receives fresh water inputs from an extensive drainage basin while Piles Creek has more limited fresh water input. Nevertheless, salinity has been reported in the range of 20-25 for both sites (Khan and Weis 1987). During shrimp collection for this study, the upper level of salinity at both sites was similar to the reported upper level of 25 reported previously, but the Tuckerton site had salinity levels as low as 10 after heavy rains.

Shrimp were captured using one meter square umbrella nets baited with ribbed mussel (*Geukensia demissa*) and placed in the marsh creeks at the two sites. Mussels used for bait were collected fresh at the collection sites and used immediately after collection. The mussels were cracked and placed in the middle of the nets. The nets were placed in the marsh creeks along the bed, as close as possible to the bank without tipping. Nets were set between the tides. No shrimp were collected at either high or low tides due to their absence at these times. The first shrimp collected coincided with the first ovigerous

females found in the nets, and collection continued until ovigerous females were no longer found. The appearance of ovigerous young-of-the-year females in mid to late July was also noted.

Adult ovigerous and intermolt shrimp of similar sizes were selected, placed in five-gallon buckets and taken to the laboratory. Shrimp less than 250 mg wet weight were assumed to be juveniles and excluded from the experiment. Shrimp acclimatized for 24 hours in the laboratory. Salinity of 20 and temperature of 20°C were kept constant throughout the experiment. Any shrimp that had softened exoskeletons due to recent shedding were not included in the analysis.

The sex of individuals was noted when possible. Identifying ovigerous females is quite easy, requiring only a quick look to inspect for the dark egg mass. Females that had previously carried eggs are also relatively easy to determine. The tagma of the abdomen extend down forming a vessel for holding eggs between the spread pleopods. Often it is possible to see the abdomen itself between the pleopods due to the spreading effect of egg bearing. Males and juveniles do not show the spreading of the pleopods or enlargement of the tagma. Thus individuals without the characteristics of egg bearing, found after the onset of the breeding season, and before young-of-the-year reach comparable size (~250mg), can be assumed to be males. It is not possible to determine the sex of individuals under endocrine control by parasites such as *Bopyrid* sp. Obviously parasitized shrimp were excluded from these experiments.

Resting oxygen consumption, as opposed to active oxygen consumption, was measured for each shrimp. Shrimp were placed individually in 250 ml Erlenmeyer flasks filled completely to eliminate air space. Before the shrimp were added, the dissolved oxygen content of each flask was measured using a YSI oxygen meter (model # 57). After a shrimp was added, each flask was sealed with a rubber stopper and left to stand with minimal disturbance for one hour. One flask with no shrimp was measured for every ten flasks containing shrimp. At the end of an hour each flask's dissolved oxygen content was measured again. The oxygen level in the control flask was never significantly lower at the end of the hour. Oxygen levels both before and after were verified to be above the lower limit determined by Cochran and Burnett (1996) below which oxygen consumption is reduced and anaerobic pathways begin to be utilized. CO_2 concentration was not measured, as Cochran and Burnette (1996) found no effect on oxygen uptake in grass shrimp across a wide range of concentrations. No attempt was made to assess the effects of salinity or temperature at the collection sites on subsequent laboratory measurements. As mentioned above, McFarland and Pickens (1965) found that little if any thermal acclimation of oxygen consumption occurs in *Palaemonetes sp*., and that the rate is predictable and a function of temperature, not past thermal or salinity regimes, or seasonality. After oxygen measurements were taken, each shrimp's length and wet weight were measured. Total egg mass was also measured for ovigerous shrimp.

Eggs were measured separately for oxygen consumption, and the rate of oxygen consumption by each egg mass was subtracted from the total rate of oxygen consumption of each ovigerous shrimp. The eggs were left relatively undisturbed, attached to the

pleopods of the abdomen, which was separated from the respiratory structures of the thorax. The eggs and tail sections were measured for oxygen uptake as above immediately after their removal from the thoracic sections. As a control, tail sections without eggs were also measured for oxygen uptake, but no measurable uptake was found. After the measurements, each egg mass was examined with a dissecting microscope to determine the developmental stage of the embryos. Embryos with visible eyespots were termed "late development", and embryos without visible eyespots were termed "early development".

A total of 23 ovigerous TK, 23 ovigerous PC, 24 intermolt PC and 27 intermolt TK shrimp were measured.

All statistical analysis was performed using GraphPad Prism version 5.02 for Windows (GraphPad Software 2008). For the purposes of analysis, shrimp were evaluated in groups with similar characteristics. Thus, ovigerous shrimp were compared with ovigerous shrimp, intermolt with intermolt, TK with TK and PC with PC. Physiological parameters were not converted to mass corrected ratios due to the distorted results such techniques can produce (Packard and Boardman 1987, 1999). Rather, multiple regressions compared the scaling effects of oxygen consumption to shrimp mass for each population. A log/log ANCOVA compared the slopes of the regressions between groups. However, when the slopes of two groups being compared were different, the ANCOVA could not be performed, and a mass corrected T test of oxygen uptake was used.

Results

When the four treatment groups were compared simultaneously using ANCOVA, the slopes were significantly different (Figure 2.1 and Table 2.1). This showed that there were differences between treatment groups, which were investigated through pairwise ANCOVAs.

When comparing ovigerous shrimp, PC shrimp used significantly less oxygen than TK shrimp (Figure 2.1 and Table 2.1). Respiration rates were more variable for PC shrimp than TK shrimp. The combined slope for both groups was 1.07 ± 0.34. This slope of resting metabolic rate was close to the predicted value of 0.75 for allometric scaling of the standard metabolic rate (SMR) (Savage et al. 2004).

Intermolt shrimp presented very different results from the ovigerous subjects. No significant differences were found in a comparison of the resting respiration of intermolt shrimp from the two sites (Figure 2.1 and Table 2.1). Body mass was not highly predictive of oxygen use in either population of intermolt shrimp tested (Table 2.2). As seen in PC ovigerous shrimp, PC intermolt shrimp showed a much greater range of respiration than TK intermolt shrimp.

Ovigerous PC and TK shrimp used significantly less oxygen than intermolt PC and TK shrimp (Figure 2.1 and Table 2.1).

No significant differences were found between the respiration rates of PC and TK eggs (Figure 2.2 and Table 2.3). The slopes were not statistically different, nor did the rates of respiration differ. PC and TK eggs showed very little relationship between mass and respiration rate (Table 2.2). There were significant differences in egg respiration, however, when the eggs were divided into early and late development groups. Dividing PC and TK eggs into different developmental stages increased the correlation coefficients for all groups and made it possible to compare developmental stages using pairwise ANCOVAs.

When the ANCOVA was preformed an all treatment groups together, no significant differences were seen, but as seen with shrimp respiration, when pairwise ANCOVAs were used, significant differences were clear. When egg respiration is divided into oxygen use by early and late embryos, only early PC eggs without a visible eyespot show a significant difference in respiration rate. PC eggs with embryos in an earlier stage of development without a visible eyespot use less oxygen than PC eggs in a later stage of development (Figure 2.3 and Table 2.4). Early PC eggs also showed the greatest range in respiration rates.

Early TK eggs showed a negative slope that was significantly different from the slopes of other groups (Tables 2.3 and 2.4). This made a rate comparison using ANCOVA impossible for this group, and because the slope crossed that of the other groups, t tests were not significant.

PC and TK late development eggs showed no significant differences in slope or overall respiration rates (Table 2.4). They also showed similar ranges in their respiration rates (Figure 2.3).

Discussion

Ovigerous shrimp used less oxygen than intermolt shrimp from the two sites, and ovigerous shrimp from the contaminated site used less oxygen than ovigerous shrimp from the pristine site. It is not clear why ovigerous shrimp had a lower resting respiration rate than intermolt shrimp, but the difference in respiration between ovigerous shrimp can reasonably be attributed to high levels of contamination at Piles Creek. There may have been abiotic differences in the two sites not attributable to anthropogenic sources, such as salinity or temperature, but these should have had little effect on the shrimp after the 24 hour acclimation period in the laboratory (McFarland and Pickens 1965). The differences found in respiration must be considered a resting metabolic rate because the shrimp were not active during the measurements, and they had not been given food that would require energetic costs for digestion. The 24 hour period where shrimp were held without food may have lowered respiration slightly as different energy pathways were utilized to replace the usual oxidation of carbohydrates (Wolvekamp and Waterman 1960).

It is likely that there would be treatment effects on active respiration rates and that active rates might give an even less ambiguous signal, however, using resting oxygen uptake as a proxy of metabolic rates in this study has shown significant differences between

treatment groups. These differences are consistent with the majority of dosing studies that show a lowering of respiration in response to contamination (Hutcheson et al. 1985, Kobayashi et al., 1991, St. Amand et al. 1999). Unfortunately, it is not possible to state which contaminants might produce the effect found. The effects of single contaminants on oxygen uptake are by no means uniform. Nevertheless, a reduction in resting metabolic rate is what is predicted for shrimp living with multiple contaminants.

It is not clear why the respiration rates of the egg treatment groups did not show differences more strongly. Developing embryos would be expected to be even more sensitive to contaminants than adults. The lowered respiration in the early PC eggs may be a sign of this, but the negative slope of the early TK eggs made comparisons difficult.

Nevertheless, previous studies have failed to find a significant reduction in the respiration of field-caught specimens while this study found several significant differences. A study by Oberdorster et al. (1999) highlights the need to use methods that could potentially reduce the variance typically found in metabolic studies. That study measured respiration in intermolt shrimp exposed to a concentration gradient of lead under field conditions. Due to the high variability in response, no significant reduction in respiration was found despite Oberdoster et al. demonstrating that lead reduces the respiration of shrimp in the laboratory.

Other studies of the effects of contaminants encountered by grass shrimp in the field have used physiological tolerance as a metric such as that found in PC grass shrimp by Kraus

et al. (1988), or behavioral effects such as those found in PC grass shrimp by Kraus and Kraus (1986). These studies looked at intermolt shrimp and in the first study, larval survival, but made no determination of sex or reproductive status.

This study addressed the issue of high variability of response in several ways. Rather than attempting to find differences along a gradient as did Oberdorster et al. (1999), this study compared sites with maximal differences in anthropogenic disturbance. Variance was also reduced in this study by controlling for sex and life history stage with the easily recognizable ovigerous shrimp. Out of over 1000 studies in an EPA review that listed toxicant effects in *P. pugio*, none measured the response of ovigerous shrimp, and the vast majority tested only the ambiguous "intermolt" life history stage (Orme and Kegley 2006).

Ovigerous shrimp are often excluded from dosing studies in an attempt to eliminate variance. For the most part, studies focus on intermolt shrimp, often of indeterminate sex. While ovigerous shrimp offer some complicating factors, such as the need to account for possible metabolic differences in the developing embryos, these complications are outweighed by the advantages. This study demonstrates that investigating ovigerous shrimp can actually reduce variance by providing complete certainty of both sex and life history state, and thus a greater measure of comparability. This study found no differences in the comparison of respiration in PC and TK intermolt shrimp, but did find statistical differences in the more specific life history stages of embryos and ovigerous shrimp.

By focusing on a single, easily identifiable life history stage, as well as the more usual intermolt shrimp, this study demonstrates the advantages of factoring life history information into metabolic studies. The uniformity of life history stage increased the ability to measure effects of collection sites. Furthermore, as previous studies have not focused on ovigerous females, this study has provided information on oxygen uptake for a critically important stage in the life of an otherwise well-studied organism. The usefulness of examining ovigerous shrimp rather than just intermolt adults is clearly demonstrated by the higher correlation between shrimp mass and oxygen uptake in ovigerous shrimp than in intermolt shrimp (Table 2.1). This may be due to life history effects. All ovigerous shrimp are at the same life history stage, while intermolt shrimp may be composed of males, large juveniles, and adult females without eggs. Furthermore, female shrimp without eggs may be undergoing vitellogenesis, with a corresponding increase in metabolic expenditure. Given the considerable differences in oxygen uptake in varying life history stages, as shown by the cited dosing studies, it is not surprising that shrimp oxygen use shows a higher correlation with shrimp mass when confined to a single life history stage. Given the difficulties with determining sex in grass shrimp at many of their life history stages, and the extreme difficulty in determining whether a shrimp is undergoing vitellogenesis, ovigerous shrimp present the simplest life history stage to determine with ease. The high correlation between respiration and mass in ovigerous shrimp makes site-specific effects more readily apparent.

What is not clear is why ovigerous PC shrimp show the lowest per gram use of oxygen of any group. On a gram for gram basis, one would expect ovigerous shrimp to use more oxygen than intermolt shrimp. Ovigerous shrimp fan their eggs with their pleopods, which should increase respiration. Nonetheless, ovigerous shrimp from the contaminated site had the lowest respiration rate of any of the groups studied. The lower variance found in ovigerous shrimp allows site differences to be seen that are obscured when examining intermolt shrimp. Nevertheless, respiration by ovigerous PC shrimp is lowered far enough that there is also a within site difference between ovigerous and intermolt PC shrimp. Why a contaminated habitat should have such a strong effect on the ovigerous shrimp is uncertain due to the lack of dosing studies conducted on egg-bearing shrimp. It is impossible to tell if this conforms to a general trend. Several studies have shown differences in toxicant effects on different life history stages, but these effects were observed in juvenile or larval shrimp rather than egg-bearing females (Kobayashi et al. 1991, St. Amand et al. 1999). The lowered oxygen demand in ovigerous PC shrimp may indicate compensatory partitioning. The lowered oxygen use may enable egg-bearing shrimp to allocate proportionally more energy to growth or reproduction than if oxygen use were similar to shrimp without eggs. After oxygen uptake by developing embryos is subtracted from oxygen uptake by ovigerous shrimp, ovigerous shrimp from both sites are shown to use less oxygen than intermolt shrimp from both sites, but those from a contaminated environment use the least (Table 2.1). If other portions of the PC ovigerous shrimp metabolic budget have higher costs, or there is less energetic input in the form of food, the reduced oxygen demand may represent compensatory partitioning of resources. Alternatively, there may be a comprehensive lowering of measurable

physiological functions in contaminated shrimp, with oxygen demand as simply one lowered component.

One would assume a general lowering of metabolic function in step with the lowered respiration would imply a lowered growth rate. The study by Santiago Bass et al. (2001) found no such lowering of growth, and found that PC shrimp are larger. Perhaps the low respiratory expenditure of PC shrimp is due to increased investment in growth, explaining the large size of PC shrimp. Nevertheless, Santiago Bass et al. found no differences in growth rate between PC and TK shrimp. Further studies that are the subject of later chapters are necessary to determine where in the total metabolic budget the extra energy is going.

While the lowered resting metabolic rate of PC ovigerous shrimp implies metabolic resources that can be allocated elsewhere, it also implies a significant cost to the shrimp. Oxygen metabolism is the basis for physical activity in the shrimp. Thus a lowered respiration would imply less energy available for feeding and predator avoidance.

While previous studies have shown inconsistent results, some general trends are recognizable. There is a general tendency for laboratory dosing studies to show a reduction in metabolic oxygen demand in response to a specific contaminant. By decreasing the variance in metabolic rate due to uncertain life history stage, focusing on ovigerous shrimp has shown clearly that anthropogenic contaminants encountered by shrimp under field conditions do indeed depress the respiration. Further studies will

show if the depressed respiration of PC ovigerous shrimp is one aspect of generally depressed metabolic functioning, or if the depressed respiration acts in a compensatory fashion. Compensatory partitioning of metabolic resources and life history dependent effects are two possible reasons that previous studies of the effects of contaminants encountered in the field have failed to show significant effects on respiration. However, without more field based measures of organism responses to contaminants it is difficult to formulate accurate risk assessments or impact statements.

Shrimp respiration (PC intermolt =TK intermolt) > TK ovigerous >PC ovigerous						
ANCOVA	Are the slopes of the regression lines statistically different?			Is there a statistical difference in total respiration by treatment group?		
	Df	F stat.	P value	Df	F stat.	P value
	3,88	4.199	0.008	See pairwise ANCOVA		
Pairwise ANCOVA	Are the slopes of the regression lines statistically different?			Is there a statistical difference in respiration rates by treatment group?		
	Df	F stat.	P value	Df	F stat.	P value
TK ovigerous > PC ovigerous	1,42	3.878	0.056	1,43	4.134	0.048
TK intermolt = PC intermolt	1,46	0.293	0.591	1,47	2.449	0.124
PC intermolt > PC ovigerous	1,43	2.272	0.139	1,44	21.628	<0.001
TK intermolt > TKovigerous	1,46	0.817	0.371	1,47	9.455	0.004

Table 2.1

	N	r^2	Slope ± 95% CL
Respiration			
PC ovigerous	23	0.68	1.31 ± 0.20
TK ovigerous	26	0.20	0.64 ± 0.28
PC intermolt	23	0.15	0.52 ± 0.27
TK intermolt	27	0.14	0.35 ± 0.17
Egg respiration			
PC shrimp	23	0.13	0.18±0.20
TK shrimp	26	0.003	0.05±0.36
Early and late egg respiration			
Early PC eggs	14	0.15	0.18 ± 0.27
Early TK eggs	11	0.29	-0.76 ± 0.88
Late PC eggs	9	0.45	0.17 ± 0.17
Late TK eggs	15	0.04	0.14 ± 0.40

Table 2.2

Egg respiration PC eggs = TK eggs						
ANCOVA	Are the slopes of the regression lines statistically different?			Is there a statistical difference in total respiration by treatment group?		
	Df	F stat.	P value	Df	F stat.	P value
	1, 45	0.413	0.524	1,46	0.073	0.788

Table 2.3

Egg respiration PC early eggs > PC late eggs Other pairwise comparisons not significant						
ANCOVA	Are the slopes of the regression lines statistically different?			Is there a statistical difference in total respiration by treatment group?		
	Df	F stat.	P value	Df	F stat.	P value
	3,41	2.345	0.087	3,44	2.428	0.078
Pairwise ANCOVA	Are the slopes of the regression lines statistically different?			Is there a statistical difference in respiration rates by treatment group?		
	Df	F stat.	P value	Df	F stat.	P value
PC early eggs = TK early eggs	1,21	5.137	0.034	(t test) 23	(t test) t=1.923	0.067
PC late eggs = TK late eggs	1,20	0.020	0.89	1,21	2.343	0.14
PC early eggs > PC late eggs	1,19	0.003	0.956	1,20	7.036	0.015
TK late eggs = TK early eggs	1,22	4.971	0.036	(t test) 24	(t test) t=0.742	0.465

Table 2.4

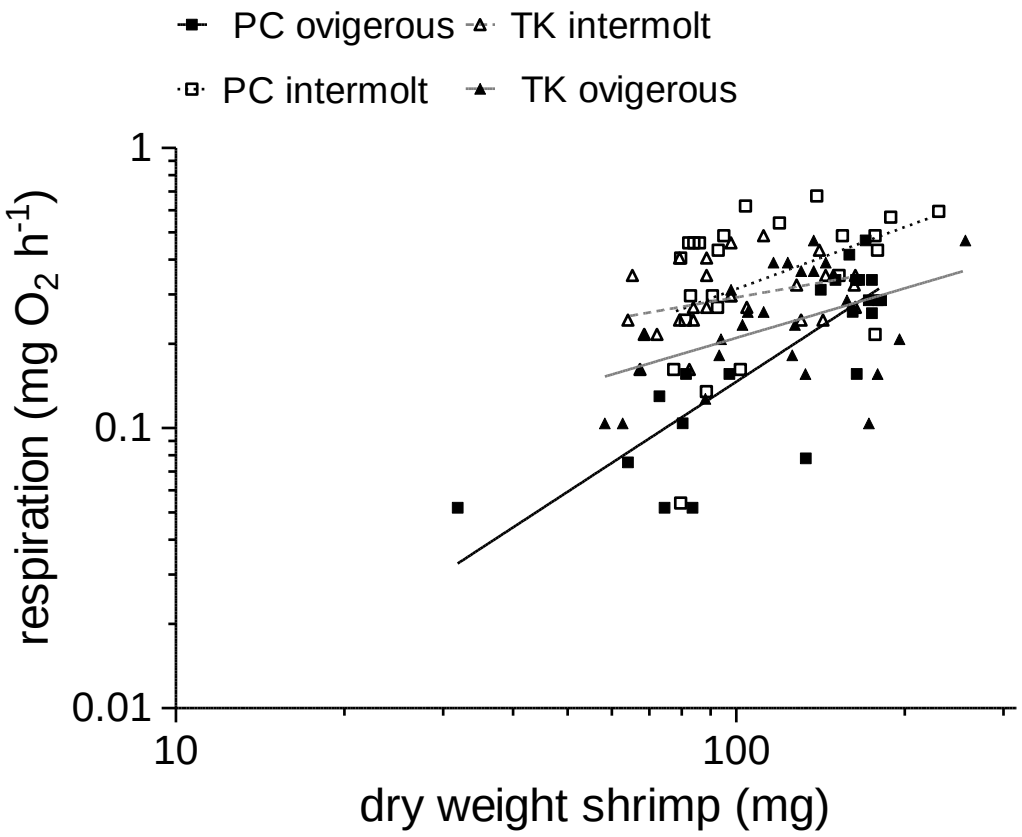


Figure 2.1. Respiration by ovigerous and intermolt PC and TK shrimp.

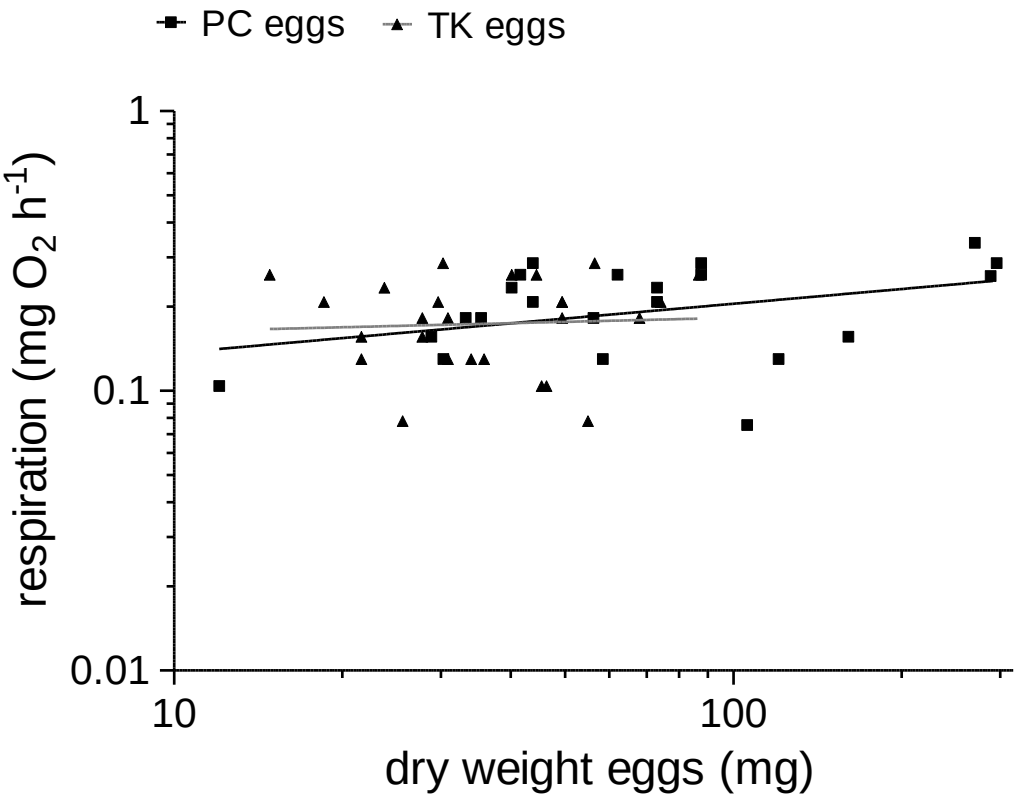


Figure 2.2 Respiration by PC and TK shrimp eggs.

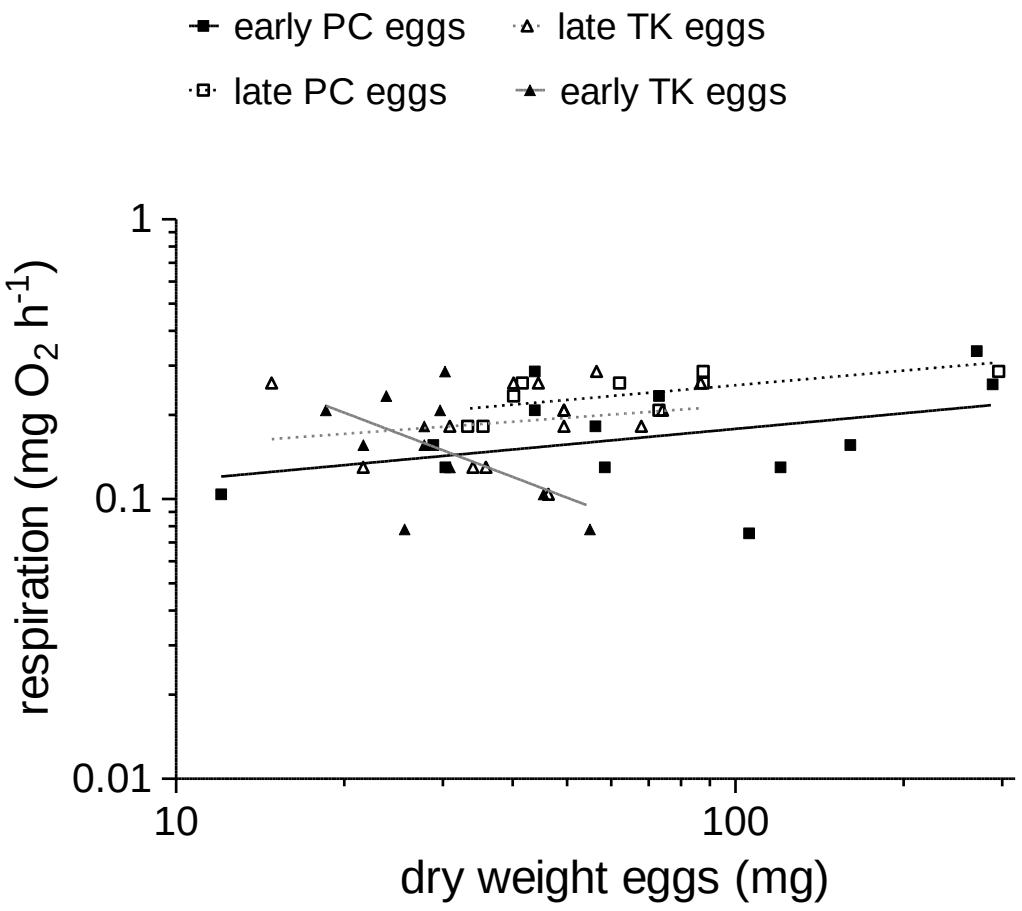


Figure 2.3. Respiration by PC and TK shrimp eggs separated by developmental stage.

Chapter 3

Compensatory partitioning of carbon budgets in association with anthropogenic contaminants encountered in the field by the grass shrimp (*Palaemonetes pugio)*

Introduction

The genus *Palaemonetes* is abundant in many fresh water and estuarine systems worldwide. Members of the genus are commonly used for toxicological dosing studies and the results used to build models of ecological risk due to anthropogenic contaminants. Carbon assimilation has been studied in *P. pugio,* but no study has looked at how assimilation might be influenced by anthropogenic contaminants (Adams and Angelovic 1970). If there are anthropogenic effects on carbon assimilation in grass shrimp, this would represent a major unmeasured impact on the carbon budget of multi-cellular organisms in estuaries and near shore environments. The influence of anthropogenic contamination on carbon assimilation has implications for predicting the environmental impact of contaminants, for models of estuarine function, and trophic transfer from the dominant macroscopic detrital processor to species of direct economic importance.

Several studies have shown changes in respiratory rates in crustaceans in response to a variety of anthropogenic contaminants. Respiration has been measured as a dose response to fuel oil, coal ash and PAH derivatives (Anderson et al. 1974, Wang and Stickle1987, and Stickle et al. 1987), organochlorides (McKenny et al. Dillon 1981, Dillon 1983), and heavy metals (Hutcheson et al. 1985, and St-Amand et al. 1999). Few

studies, however, have examined the total carbon budget of a crustacean, nor has any study examined the impact of anthropogenic contaminants on such a budget. Furthermore, existing dosing studies, the majority of which focus on respiration rates, are far from conclusive. The results vary by species, contaminant, and dosage level.

Laboratory dosing studies are designed to control the number of variables, but usually do not examine synergistic or antagonistic effects of multiple contaminants. Few studies have related effects on respiration to total carbon assimilation through measures of feeding and excretion. Most studies simply determine dosages leading to lethality. The EPA database of all toxicant dosing studies done since 1972 on *Palaemonetes pugio* shows that out of 1016 studies, 65% measured lethality as the end point, while the combined total of studies measuring respiration, growth, feeding, or reproduction is only 5% (U.S. Environmental Protection Agency 2006). No studies surveyed combined effects such as respiration and growth, no studies considered excretion rates, and no studies examined the carbon budget, except as a function of respiration.

Carbon assimilation is an excellent metric for estimating the place of *P. pugio* in estuarine function, because it measures the element most associated with biological function. Furthermore, an analysis of the carbon budgets of field-caught shrimp supplies data lacking in laboratory dosing studies. Although laboratory dosing studies provide important information on the effects of specific contaminants, a comparison of total carbon budgets in field-caught shrimp is more relevant in terms of anthropogenic disturbance in the field and their effects on ecosystem function.

This study provides the first complete carbon budget for *P. pugio* that includes direct measurement of carbon allocated to the eggs of ovigerous females. In addition, the relative allocation of carbon for each component is compared for shrimp obtained from salt marshes that display the full range of anthropogenic impacts found in New Jersey. The implications of observed differences in allocation can then be examined in the context of over two decades of studies by previous researchers on the effects of contaminants on predator-prey relations at these sites.

Materials and methods

Shrimp were collected at two sites. The Tuckerton site at Big Sheepshead Creek is relatively pristine (Able and DeLuca 1996). As part of the Mullica River/Great Bay estuary system, it is one of the least disturbed estuaries in the northeast and is part of the National Estuarine Research Reserve System. The Great Bay and Mullica systems are now, and have historically been, pristine and free of excessive nutrient loading from anthropogenic sources. There is very little industry or development within the Mullica River drainage basin. The one major source of nutrient loading to Great Bay, a menhaden processing plant, has been closed since 1960. The collection site is over 200 meters from a small access road and has no access by boats, and so has additional protection from incidental impacts and non-point sources such as motor oil spills and runoff.

The Piles Creek site represents a heavily impacted marsh creek. There are several active and abandoned chemical-processing plants near the creek as well as the Linden Sewerage facility. There is an Exxon Mobil refining center directly adjacent to the site, with fuel lines that cross over the site itself. There have historically been both large and point source releases of oil, ranging from leaks in the pipes to discharges from small boats. There is oil visible in the sediments and on the surface of the water. Mercury concentrations in the sediment ranging from 10-20 μg/g at Piles Creek have been reported, whereas Tuckerton sediments are less than a hundredth of that at 0.05 μg/g (Koepp et al. 1980, Callahan and Weis 1983). More recently mercury concentration at PC have been reported to have declined to <5 μg/g (Weis 2002). Other contaminants found in Piles Creek sediment include cadmium (5.8 μg/g) and copper (623 μg/g), with Tuckerton showing much lower levels (Cd 0.13 μg /g and Cu 12.9 μg/g) (Kahn et al. 1989, Eckenfelder 1991).

Shrimp collection

Shrimp were collected from each site during the breeding cycle from late June through early September of 2000-2005 on alternating weeks so that seasonal differences would not obscure site differences. Shrimp were captured using 1meter square umbrella nets baited with ribbed mussel (*Geukensia demissa*). Mussels used for bait were collected fresh at the collection sites, cracked, and placed in the middle of the nets. The nets were placed in the marsh creeks along the bed, as close as possible to the bank without tipping. Shrimp were collected during the middle two hours of the incoming or outgoing tide, when they were most active. The first shrimp collected during a collection season

coincided with the first ovigerous females found in the nets, and collection continued until ovigerous females were no longer found. The appearance of ovigerous young-of-the-year females in mid to late July was also noted.

Adult ovigerous and intermolt shrimp of similar sizes were selected, placed in five-gallon buckets and taken to the laboratory. Shrimp less than 250mg wet weight were assumed to be juveniles and excluded from the experiment. Shrimp acclimatized for twenty-four hours in the laboratory at 20° C in water with a salinity of 20. Salinity and temperature were kept constant throughout the experiment. Any shrimp that had softened exoskeletons due to recent shedding or that shed during the holding period and analysis were not included.

Sex of individuals was noted when possible. Obviously parasitized shrimp were excluded from these experiments.

Carbon budgets

Carbon allocation was measured according to the equation developed for grass shrimp energy budgets by Vernberg and Piyatiratitivorakul (1998), modified to exclude the term for ammonia excretion as irrelevant to carbon budgets. Carbon allocation is described as $C = Pg + Pr + R + Ex + F$ where C is consumption, Pg is the portion of total production allocated to somatic growth, Pr is the production allocated to reproduction, R is respiration, Ex is exuvia, and F is egestion. More properly the equation can be

understood as carbon assimilation (C-R-F) equaling total carbon allocated to production (Pg+Pr+Ex).

Consumption and egestion

Consumption and egestion was measured during the shrimp breeding season of 2002 and 2003. Each week between the beginning of June and the first week in September ten shrimp were caught from each site in alternation. A total of 60 shrimp were caught from each site. Each batch of ten shrimp was preselected to include five with eggs and five without eggs for consumption and egestion analysis. After the 24 hour acclimation period in the laboratory, shrimp were dried with paper towels and then weighed to the nearest milligram. Shrimp were placed in individual glass fingerbowls in 650 ml of 1μm filtered seawater with a salinity of 20. They were provided with food in excess of what they could consume in a twenty-four hour period. The food consisted of tissue taken from live ribbed mussels that was frozen, and then freeze dried for twenty-four hours in a Labconco freeze dryer at -50°C in a 5 micron Hg vacuum. A solid piece of freeze-dried tissue (approximately 50mg, weighed to the nearest hundredth of a milligram) was placed in each fingerbowl with the shrimp. Solid pieces of mussel tissue allowed minimal loss of tissue through handling by the shrimp. The fingerbowls were covered with nylon screening to prevent the shrimp from jumping out. A method blank was included so that in addition to the ten fingerbowls containing shrimp and mussel tissue, there was one fingerbowl that contained only mussel tissue. The method blank was treated identically to the other samples containing shrimp.

After 24 hours, the shrimp were removed from the fingerbowls, dried with paper towels, and then weighed to the nearest milligram. Fingerbowls that contained dead shrimp, shrimp that had released their eggs, or shrimp that had undergone ecdysis were emptied and re-washed. Fingerbowls with live, healthy shrimp were carefully examined for any remaining ribbed mussel tissue. The mussel tissue was removed, frozen, freeze-dried, and then weighed to the nearest hundredth of a milligram. The remaining 650 ml of seawater containing both fecal pellets and liquid excretion products was filtered through 47 mm Whatman GF/F filters pre-baked at 500º for five hours.

The carbon consumption rate was determined from the difference in mussel tissue mass before and after each twenty-four hour experimental period. To convert mussel mass to carbon content, ten samples of mussel tissue were analyzed for carbon content as described below. The samples consisted of ribbed mussel tissue collected from Tuckerton ribbed mussels. The mussel tissue was homogenized in a blender, combining mantle tissue from six individual mussels, and then freeze-dried for twenty-four hours. The resulting dried cake was ground using a mortar and pestle that had been baked in a muffle furnace at 500° for five hours to remove carbon residue, and individual samples were taken from the resulting powder. The mean percent carbon by weight of the samples was multiplied by the total food consumed by each shrimp to derive the carbon consumption rate.

The filtrate was extracted using a vacuum pump kept to a low pressure. Before use, each filter was weighed to the nearest hundredth of a milligram using a Sartorius

microbalance. Fecal matter was analyzed using the methods of Verardo et al. (1989), modified for use with glass fiber filters and an absence of inorganic carbon in the samples. Each filter was thoroughly rinsed with de-ionized water and then placed in a drying oven. After a trial run, no acidification step was used. The filters were then weighed to the nearest hundredth of a milligram using a Sartorius microbalance. The dried filters were then cut into four equal pieces and wrapped in tin. The tin sample boats containing the quartered glass fiber filters were kept in the drying oven at 60°C for 24r hours or until analyzed.

Carbon lost to egestion was measured through analysis of the carbon content of fecal pellets. Data from each site for ovigerous and intermolt shrimp were combined for analysis of excretion. Fecal pellets were defined as all material retained by a Whatman GF/F filter (nominal pore size 0.7 μm), thus including all particulate organic carbon. This necessarily included the carbon content of food particles lost during maceration by the shrimp. All glassware used was first washed with detergent, and then rinsed with de-ionized water. The glassware was then thoroughly rinsed with a solution of 12.5% sulfuric acid. The glassware was then re-rinsed with de-ionized water, and baked in a muffle furnace at 500° for five hours.

Respiration

Resting respiration, as opposed to active oxygen consumption, was measured for shrimp captured during the summer of 2000. Shrimp were held without food for 24 hours, and then placed individually in 200 ml flasks. The flasks were filled with 1 μm filtered

seawater (salinity 20) and kept at 20° C throughout the experiment. Before adding the shrimp, the dissolved oxygen concentration in each flask was measured using a YSI oxygen meter (model # 57). Each flask was then sealed and left to stand with minimal disturbance for one hour, after which the dissolved oxygen concentration was measured again. One blank flask with no shrimp was measured for every ten flasks containing shrimp. Oxygen concentrations both before and after were verified to be above the lower limit determined by Burnett and Cochran (1996), below which oxygen consumption is reduced and anaerobic pathways begin to be utilized. CO_2 concentration was not measured, as it was found by Burnett and Cochran (1996) to have no effect on oxygen uptake in grass shrimp across a wide range of concentrations. No attempt was made to assess the effects of salinity or temperature at the capture sites on subsequent laboratory measurements. McFarland and Pickens (1965) found that little if any thermal acclimation of standard oxygen consumption occurs in *Palaemonetes sp*., and that the standard rate is predictable and a function of temperature, not past thermal or salinity regimes or seasonality. After oxygen measurements were taken, each shrimp's length and weight were then measured. Total egg mass was also measured for the ovigerous shrimp.

Oxygen uptake was converted to carbon loss using an RQ of 0.74 for shrimp held without food (Wolvekamp and Waterman 1960).

The abdomen of each ovigerous shrimp was removed from the cephalothorax leaving the eggs attached with as little disturbance to the eggs as possible. The egg masses were then

measured for oxygen uptake in the same manner as described above. After the oxygen measurement the eggs were removed, patted dry with paper towels and weighed in the same manner as the shrimp. Oxygen uptake was then calculated for ovigerous shrimp independently of the mass and oxygen uptake of the eggs.

Ecdysis

Shed exoskeletons were collected during the experiment designed to measure carbon allocated to somatic growth as described previously. The exoskeletons were collected on a daily basis during the four weeks of the experiment. Each of the shrimp being measured for growth shed at least once, and the exoskeletons were collected, washed with de-ionized water, and freeze-dried. Ten exoskeletons from each study site were measured for total carbon content. The calcium carbonate was removed through acidification. Shed exoskeletons were not collected from ovigerous shrimp because egg-bearing females do not shed. They do, however, shed at the end of each 14-day reproductive cycle, so the carbon lost to ecdysis for ovigerous shrimp was assumed to be similar to the carbon lost by intermolt grass shrimp, with a similar periodicity to their exoskeleton formation. The total carbon lost in each exoskeleton was divided by the 14 day period to determine the daily carbon lost to ecdysis per 24 hours.

Somatic Growth

Twenty shrimp from each site were collected in early September. Only intermolt shrimp were selected. Shrimp were dried with paper towels and then weighed to the nearest milligram. Shrimp were placed individually in 400 ml beakers containing 400 ml of 1μm

filtered salinity 20 seawater. All beakers were first washed, rinsed with de-ionized water, and then baked at 500° for five hours. Each beaker was covered with an aluminum foil cap, individually aerated and placed in a walk-in incubator maintained at 20°. Once per day the shrimp were fed ~50mg freeze-dried ribbed mussels ground into a coarse powder. The food was the same as that used to measure consumption and egestion except for homogenization in a blender. This allowed the mussel tissue to be prepared and freeze dried in a single batch prior to use. The amount of food provided was calibrated to insure maximum feeding rates at all times. At no time were shrimp allowed to be without food. Any food remaining from the previous day's feeding was removed. The water in the beakers was changed every other day for the duration of the experiment. Each day the shrimp were checked for ecdysis and the shed exoskeletons removed, rinsed with de-ionized water, and saved for later analysis. Any shrimp that could not right itself was removed. After four weeks all shrimp were removed from the beakers and weighed to the nearest milligram.

To convert the change in shrimp mass into a measure of carbon assimilation, whole shrimp were measured for total somatic carbon content. Ten ovigerous and ten non-ovigerous shrimp were captured at each site and freeze-dried for 24 hours. The shrimp were weighed both wet and dry to determine the relationship between wet and dry shrimp masses. Shrimp tissue was analyzed for carbon content as below. Ten ovigerous and ten intermolt samples were analyzed from each site. Whole shrimp were ground using mortars and pestles that had been baked in a muffle furnace at 500° for five hours to remove carbon residue. A 30mg sub-sample was taken from individual homogenized

powdered shrimp. The sub samples were acidified in a HCl saturated atmosphere for 24 hours to remove inorganic carbon.

Reproduction

The daily carbon allocated by ovigerous shrimp to egg production was calculated as the total carbon content of individual egg masses divided by 28 days given the 14-day reproductive period where shrimp are ovigerous and assuming prior egg production for an additional 14-day intermolt period. A total of 33 ovigerous TK shrimp and 23 ovigerous PC shrimp were captured as above in 2000 and the eggs were removed after measuring their respiratory rate. The individual egg masses were separated from each shrimp with a scalpel. The egg masses were freeze-dried, then weighed using a Sartorius microbalance. The egg masses were powdered, and a 20mg sub-sample was taken and acidified prior to organic carbon analysis.

Carbon analysis

Samples were analyzed for carbon content using a Carlo Erba NA-1500 elemental analyzer. A standard curve was developed using six acetanilide standards ranging from 1mg to 0.01 mg. Any standard curve showing more than a 0.0001 deviation from the regression was rejected. Each analysis run was preceded by two empty sample tin boats run as instrument blanks. For every six samples an acetanilide check standard was used to monitor accuracy. If the check standard exceeded a 1% deviation from expected results a second check standard was run to verify the deviation. If the percentages of C and N continued to deviate by more than 1% the run was discontinued

Oxygen consumption was converted to carbon lost to respiration using a respiratory quotient of 0.74 for shrimp held without food (Brody 1945 and Wolvekamp and Waterman 1960).

Statistical analysis

All statistical analysis was performed using GraphPad Prism version 5.02 for Windows (GraphPad Software 2008). All error estimates represent 95% confidence levels for the data. ANCOVA was used to compare slopes between all groups and the combined slope (weighted average) from each budget component was found. The average slope was then used to calculate the mean values for each treatment group for each component using an average shrimp size of 116.1 mg (Packard and Boardman 1987, 1999).

The total carbon budget for each treatment group was derived from the mean rate of consumption minus the mean allocation for each component. The error bars are cumulative showing the 95% confidence intervals.

Results

Consumption

There was no difference in carbon consumption between ovigerous shrimp or between intermolt shrimp (Figure 3.1 and Table 3.2). Nor were there any within site differences. Carbon intake by TK shrimp was more closely correlated with shrimp weight than carbon intake by PC shrimp (Table 3.1). PC shrimp showed much greater variation in their rate

of consumption than TK shrimp, with one individual consuming more during 24hours than any TK shrimp. Five ovigerous PC shrimp and seven intermolt PC shrimp consumed no measurable carbon (< 0.04 mg C), while all TK shrimp consumed measurable carbon.

Intermolt PC and TK shrimp showed a closer correlation between shrimp weight and carbon consumption than ovigerous shrimp (Table 3.1).

Respiration

Ovigerous shrimp lost significantly less carbon to respiration than intermolt shrimp, with ovigerous PC shrimp losing the least amount of carbon to respiration of any group (Figure 3.2 and Table 3.2). Conversely, intermolt PC shrimp had higher carbon losses to respiration than intermolt TK shrimp. PC shrimp had a greater range in carbon respired than TK shrimp with intermolt PC shrimp having the greatest range of any group.

Reproduction

Ovigerous PC shrimp allocated significantly more carbon to reproduction than ovigerous TK shrimp (Figure 3.3 and Table 3.2). There were also significant differences in the carbon content of the eggs but they did not significantly change total allocation and are not likely to be biologically relevant. Dry TK eggs contained slightly more carbon by weight than PC eggs, with PC shrimp eggs containing 47.15% C ±1.676 while TK eggs contained 49.41% C ± 0.4411 ($p=0.002$ $n=17$). The heavier egg masses of ovigerous PC shrimp overwhelmed the small differences in the proportion of carbon, and thus the total

carbon allocation to reproduction was higher for ovigerous PC shrimp even though TK eggs had slightly more carbon as a percentage of weight.

There was no significant correlation between shrimp weight and carbon allocated to reproduction in PC shrimp, while the relationship was significant for TK shrimp (Table 3.1). PC shrimp also have a slightly greater range of reproductive allocation by individuals than TK shrimp.

Egestion

PC shrimp lost significantly more carbon to egestion than TK shrimp (Figure 3.4 and Table 3.2). TK shrimp did not show a significant correlation between somatic carbon content and egestion rate, while there was a significant correlation in PC shrimp (Table 3.1). TK shrimp showed a much greater range in carbon lost to egestion than PC shrimp and had the highest and lowest egestion rates by individual shrimp.

Somatic growth

There was no significant difference in carbon allocated to growth between PC and TK shrimp (Figure 3.5 and Table 3.2). Both groups showed similar correlation coefficients (Table 3.1). PC shrimp contained slightly more carbon as a percentage of dry body weight (39.60%±0.40%) than TK shrimp (38.33%±0.56%). The difference was statistically significant ($P=0.001$ $n=38$), but not enough to cause a difference in the total carbon allocated to growth, nor is the difference likely to be biologically relevant.

Ecdysis

There was no significant difference in carbon allocated to the production of exoskeletons between PC and TK shrimp (Figure 3.6 and Table 3.2). Neither PC shrimp nor TK shrimp showed a significant slope between somatic carbon and carbon allocated to ecdysis (Table 3.1).

Carbon allocation model

Ovigerous PC and TK shrimp had similar rates of carbon consumption but ovigerous PC shrimp were able to allocate more carbon to reproduction through a reduction in carbon lost to respiration. Thus the proportional reproductive allocation was higher for ovigerous PC shrimp than it was for ovigerous TK shrimp. Intermolt PC shrimp, on the other hand, had a lower consumption rate and a higher respiration rate than intermolt TK shrimp. They showed no evidence of compensatory partitioning of resources yet were able to allocate proportionately more resources to growth than intermolt TK shrimp. The growth rates were low for both groups, but represented a larger proportion of available resources for intermolt PC shrimp.

Ovigerous shrimp allocated approximately 1/3 of carbon consumed to egestion, 1/3 to respiration and 1/3 to production. Due to their lower consumption, PC shrimp allocated proportionately more carbon to egg production than TK shrimp.

Intermolt TK shrimp allocated approximately 1/3 of carbon consumed to egestion, over half to respiration and approximately 3% to growth. Intermolt PC shrimp allocated

slightly less than half of consumption to egestion, over half to respiration and 3% to growth.

Discussion

Ovigerous PC shrimp transferred carbon allocation away from respiration and into increased reproductive output. They were able to compensate for increased losses to egestion without increasing consumption by this reduction in respiration, leaving surplus resources for allocation to reproduction. Intermolt PC shrimp showed a very different pattern with reduced consumption and increased respiration. Nevertheless, they were still able to maintain carbon allocation to growth at a rate similar to intermolt TK shrimp. Also relative to TK shrimp, PC shrimp showed an increased range of response. The increased variability and the lowering of some physiological rates are expected results of contaminant exposure. Through compensatory partitioning, PC shrimp were able to ameliorate the effects of the exposure and maintain carbon allocation to somatic growth and reproduction.

The partitioning of resources away from respiration allowed ovigerous PC shrimp to allocate a higher proportion of carbon consumed to assimilation than ovigerous TK shrimp. It is not as clear how intermolt PC shrimp maintained somatic growth because of the higher respiration rates, lower consumption and slightly higher excretion rates. Intermolt PC shrimp may have reduced reproductive allocation to increase somatic allocation.

When measuring the response of physiological parameters such as respiration to a variety of toxicants, generally the treatment group exposed to the toxicants can be expected to have a wider range of response than the control group (Forbes and Depledge 1996). Piles Creek shrimp show a broader range of response than those captured at Tuckerton for consumption, respiration and reproduction (Figures 3.1, 3.2 and 3.3). The increased variance found in shrimp exposed to contaminants implies that these shrimp are potentially at greater risk to further impacts. With the range of most physiological responses being increased, some individuals will be much closer to the lower end of resource allocation that would allow for survival and reproduction.

Reduced feeding is a common response to contaminants (Weis et al. 2001), and this was found for intermolt PC shrimp. The lowered rate of consumption by intermolt PC shrimp is still statistically significant even when individuals with no consumption are eliminated from the calculations. Nevertheless, the complete cessation of feeding by a few individuals in the two PC treatment groups appears to be the result of the combination of field exposure to contaminants and the stress of testing in the laboratory. The cessation in feeding by some PC shrimp may be the result of a lowered ability in PC shrimp to tolerate stress. A lowered ability to tolerate stress has been correlated with exposure of shrimp to contaminants (Petrović et al. 2004). If lowered stress tolerance due to contaminant exposure is combined with the increased variability and lowered consumption, then it can be expected that some individuals may halt consumption entirely.

Those individuals with measurable but lowered carbon consumption would also be at risk for all physiological processes requiring carbon. Somatic growth and reproduction are the most important end points of carbon allocation, and in order to maintain these at optimal levels, individual shrimp would be forced to lower allocation to other portions of the carbon budget.

Respiration is generally depressed after exposure to contaminants, as has been found in other studies of shrimp toxicant dosing (Anderson et al. 1974, Hutcheson et al. 1985, and St-Amand et al. 1999), but this appears to work in combination with lowered respiration found in ovigerous shrimp from both sites. Thus, ovigerous shrimp exposed to contaminants have greatly reduced losses of carbon when compared to intermolt shrimp not exposed to contaminants. This allows ovigerous PC shrimp to have a similar reproductive allocation to that of ovigerous TK shrimp despite a lowered rate of consumption.

Nevertheless, it should be remembered that respiration is measured at a very different time scale than the components of production. Growth and reproduction represent changes over 28 days while respiration represents carbon allocation over a single hour. There may be large differences between carbon lost to respiration in an hour in the laboratory after 24 hours of starvation and the abrupt transition to an Erlenmeyer flask than carbon lost to respiration over longer time scales.

There were several other areas of uncertainty due to the methods used. It was not possible to measure growth directly in ovigerous shrimp, or to make a direct measurement of the reproductive allocation of intermolt shrimp, so it is not clear if there is further partitioning of resources between the life history stages. The decreased consumption and increased respiration by intermolt PC shrimp may indicate that these shrimp are allocating more resources toward growth and away from reproduction when compared to intermolt TK shrimp. Conversely, ovigerous PC shrimp may partition resources away from growth and toward reproduction. It is clear that intermolt PC shrimp are able to maintain growth similar to that of intermolt TK shrimp, and ovigerous PC shrimp are able to maintain a reproductive effort greater than that of ovigerous TK shrimp. As an ecological and evolutionary strategy it is most important that intermolt shrimp grow as much as possible and ovigerous shrimp carry as many eggs as possible. PC shrimp are able to do this despite similar or lowered consumption through compensatory partitioning of resources.

Ecdysis was only measured for intermolt shrimp, and the carbon losses to sheds by ovigerous shrimp may have differed. If so, there still would have been little difference in the overall carbon budget due to the relatively small proportion of carbon intake allocated to ecdysis.

The slightly higher rate of egestion found in PC shrimp relative to TK shrimp is not supported by the equal or lower feeding rate found in PC shrimp, nor is it predicted by the general assumption that contaminant exposure reduces physiological rates. Unlike

the increased variability seen in consumption and respiration, PC shrimp show reduced variability in egestion, suggesting that egestion is constrained by some factor.

While all portions of the carbon budget were measured for these shrimp, it was not possible to measure every component simultaneously in a single experiment nor was it possible to measure every component of every treatment group. Thus the carbon budget includes several assumptions and estimates upon which the final results are based. For example in production allocation, somatic growth and ecdysis could only be measured for intermolt shrimp. Ovigerous shrimp have the same period between molting as intermolt shrimp (Anderson 1985), and the carbon content of the exoskeleton is not likely to differ between the two groups, so the estimate of carbon allocated to ecdysis for ovigerous shrimp should be a good approximation of actual allocation. On the other hand, the estimate of carbon allocated to somatic growth should be considered an upper limit for ovigerous shrimp because intermolt shrimp are likely to choose a strategy maximizing potential for growth while ovigerous shrimp will choose a strategy maximizing reproductive output.

Shrimp respiration was measured under resting conditions without the increased energetic costs of food handling and capture or predator avoidance. All shrimp were held without food for 24 hours so the respiration rate did not include any component representing the respiratory requirements of food digestion. Thus the respiration rate measured over a single hour may be the lower measure of what shrimp engaging in necessary survival activities over a longer time scale would require.

Finally, carbon intake represents a short-term measure of intake after food was withheld for 24 hours, rather than what may be a slightly lower rate for many shrimp when presented with constant food over a longer time period. Thus, the shrimp growth rate during the 28 days of the growth experiment may be related to a lower rate of consumption than that found in the 24 hour feeding experiment. Conversely, the longer period of the growth experiment may have allowed intermolt PC shrimp a longer acclimation time so that they did not show the cessation of feeding seen in the consumption experiment.

Taken together these estimates indicate that the results of the carbon budget are likely to be contingent upon the experimental design. Nevertheless, the relative differences in allocation between the two treatment groups arose under conditions constant within each experiment and thus represent a good estimate of the relative impact of anthropogenic contaminants on the components of the carbon budgets of grass shrimp.

High reproductive allocation in ovigerous shrimp is an expected result given that reproduction is the focus of this life history stage. The much lower assimilation by intermolt shrimp is a function of the higher respiration and lower consumption among these shrimp. The low assimilation rate for intermolt PC shrimp is the result of these shrimp having the lowest carbon intake while at the same time having the highest egestion and respiration. The lower assimilation in all treatment groups in this study, as opposed to that of Vernberg and Piyatiratitivorakul (1998), is partly a function of the

much higher egestion rate. Vernberg and Piyatiratitivorakul found only 2.16% of ingested energy was lost to egestion, while in this study, over 1/3 of carbon ingested was lost to egestion by TK and PC shrimp. The high egestion rates in this study were likely caused by the lower handling efficiency of mussel tissue as opposed to the *Artemia* naupli used in the Vernberg and Piyatiratitivorakul study. There may also have been some residual gut loading that was not expelled during the twenty-four hour acclimation period but which was then expelled during the feeding experiment, thus increasing the measured egestion.

The models developed by this study for ovigerous shrimp differed in major ways from previously published budgetary models of *P. pugio*. Although for PC shrimp the reduced feeding by intermolt shrimp and reduced respiration by ovigerous shrimp show the negative impact of anthropogenic contaminants, they are able to maintain production and thrive, as evidenced by field observations and laboratory results. The higher reproductive allocation found in this study replicates that found by Santiago Bass et al. (2001) as does the similarity of intermolt growth rates. Through compensatory partitioning of physiological resources, the shrimp are able to compensate for the metabolic consequences of their environment.

Consumption	N	r^2	Slope ± 95% CL
PC ovigerous	34	0.23	0.04 ± 0.02
TK ovigerous	28	0.44	0.04 ± 0.02
PC intermolt	33	0.30	0.07± 0.04
TK intermolt	25	0.52	0.05± 0.02
Respiration			
PC ovigerous	23	0.64	0.02 ± 0.006
TK ovigerous	26	0.18	0.008 ± 0.008
PC intermolt	23	0.19	0.01 ± 0.01
TK intermolt	27	0.09	0.006 ± 0.008
Egestion			
PC shrimp	15	0.39	0.004 ± 0.003
TK shrimp	28	0.02	0.002 ± 0.006
Ecdysis			
PC	9	0.06	0.00002 ± 0.00004
TK	10	0.10	0.00002 ± 0.00004
Growth			
PC	17	0.06	-0.001± 0.001
TK	16	0.10	-0.003 ± 0.004
Reproduction			
PC ovigerous	12	0.01	0.001 ± 0.003
TK ovigerous	12	0.37	0.003 ± 0.002

Table 3.1. Slopes for regression lines of the carbon budget components showing 95% confidence intervals and correlation coefficients.

	PC ovigerous	TK ovigerous	PC intermolt	TK intermolt
Consumption	3.97±1.31	4.22±0.75	4.02±1.28	5.19±0.88
Respiration	1.67±0.20	1.94±0.25	3.14±0.49	2.53±0.26
Reproduction	0.82±0.16	0.64±0.09	n/a	n/a
Egestion*	1.85±0.22	1.55±0.25	1.85±0.22	1.55±0.25
Growth**	n/a	n/a	0.12±0.03	0.14±0.05
Ecdysis***	n/a	n/a	0.002±.0006	0.002±0.0002

Table 3.2. Components of the carbon budgets for ovigerous and intermolt shrimp from Piles Creek and Tuckerton. Resource allocation (mg carbon $(24h)^{-1}$) for a standard-sized shrimp of 116.1 mg dry weight. Data are means±95% confidence intervals. Egestion* represents pooled values from combined intermolt and ovigerous shrimp data. Growth** was based on intermolt shrimp only. Ecdysis*** was based on intermolt shrimp only.

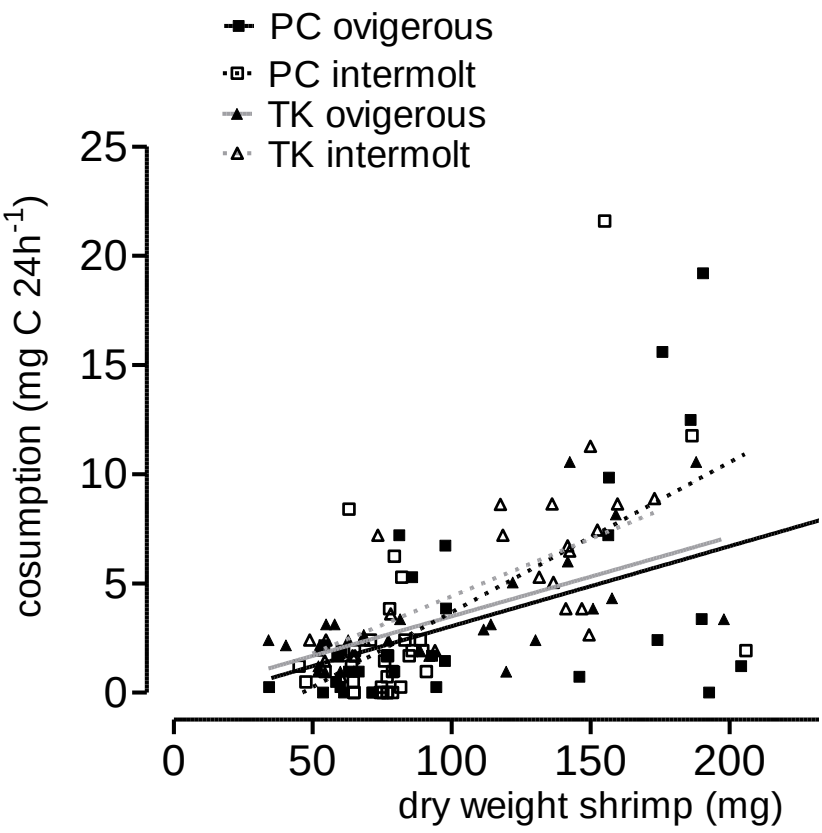


Figure 3.1. Consumption by ovigerous and intermolt Piles Creek and Tuckerton shrimp. All regression lines are statistically parallel with a pooled slope of 0.04 (P=0.34).

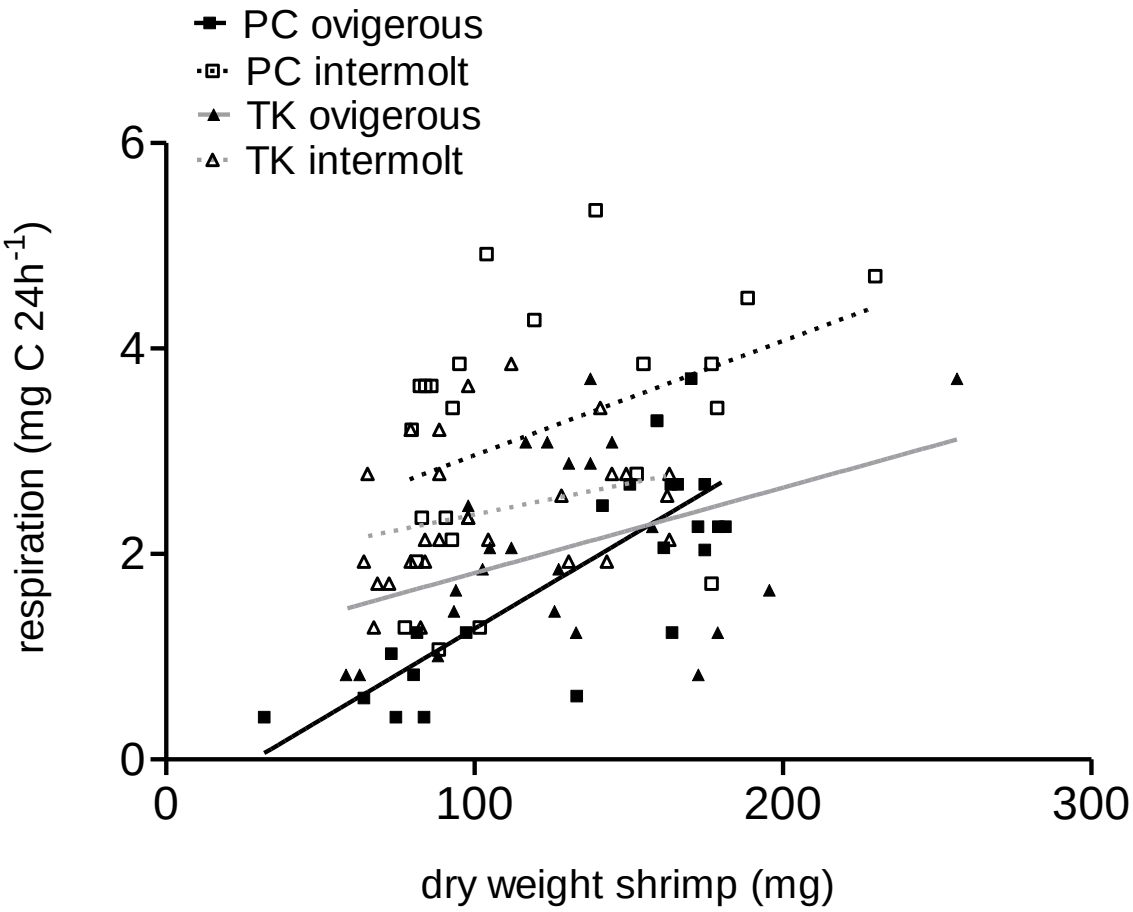


Figure 3.2. Respiration by ovigerous and intermolt Piles Creek and Tuckerton shrimp. All regression lines are statistically parallel with a pooled slope of 0.011 (P=0.22).

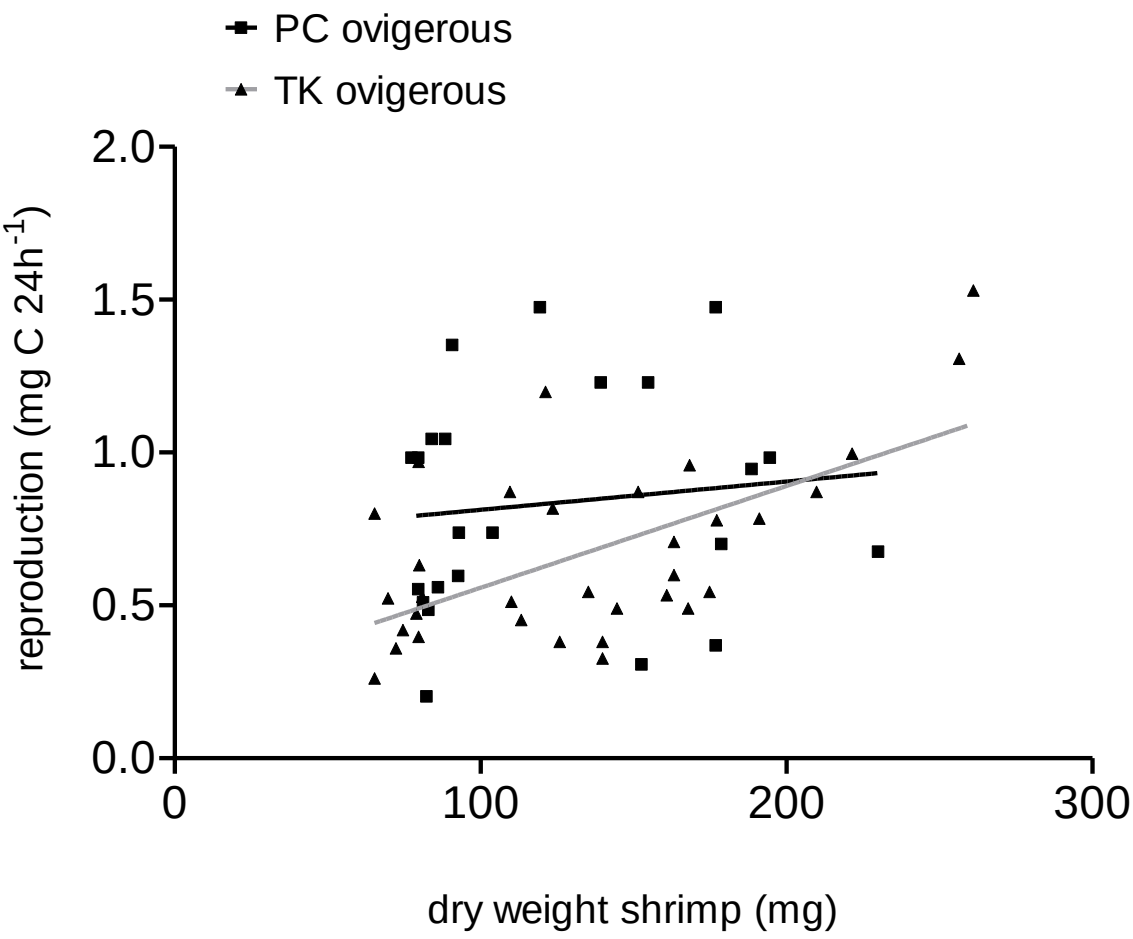


Figure 3.3. Reproduction by ovigerous Piles Creek and Tuckerton shrimp. All regression lines are statistically parallel with a pooled slope of 0.002 (P=0.16).

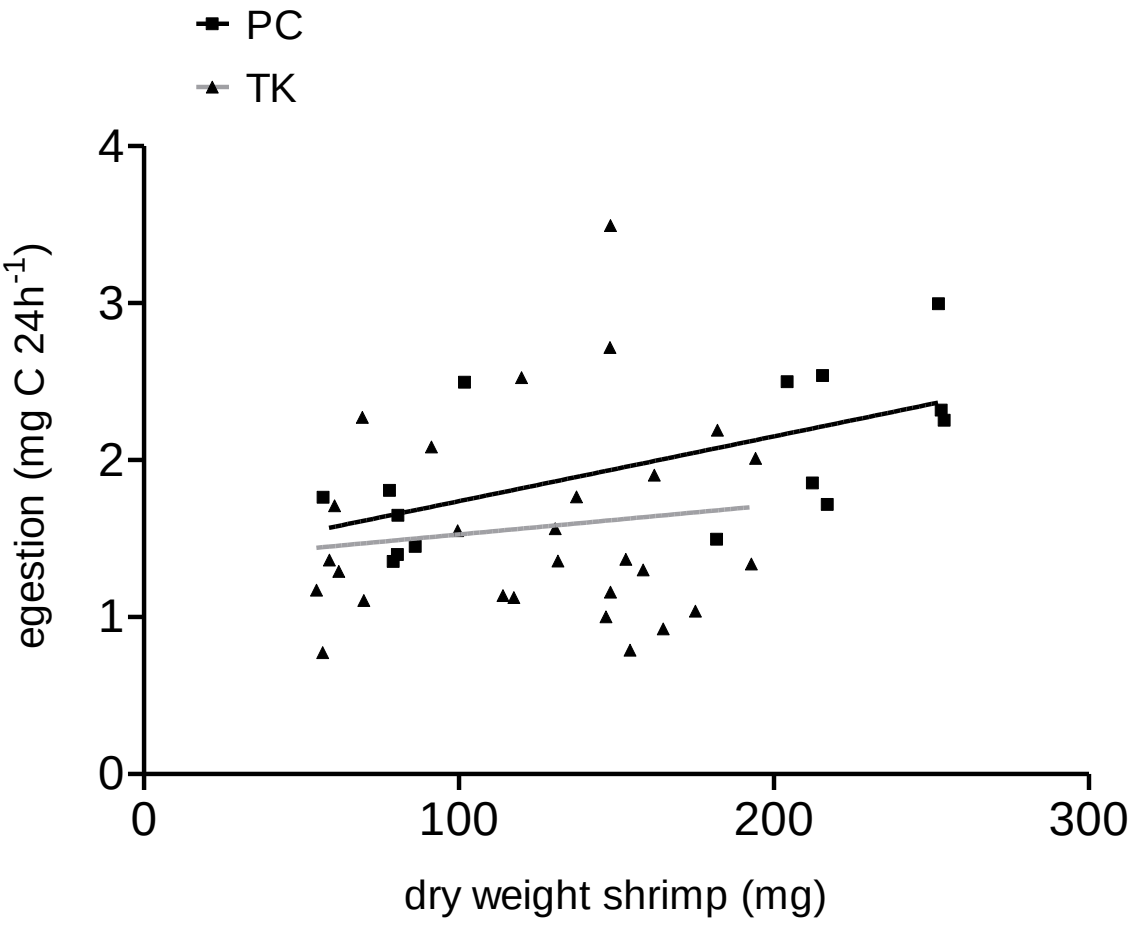


Figure 3.4. Egestion by Piles Creek and Tuckerton shrimp. Intermolt and ovigerous shrimp are grouped together for statistical purposes. The slopes do not differ significantly and have a pooled slope of 0.003 (P=0.49).

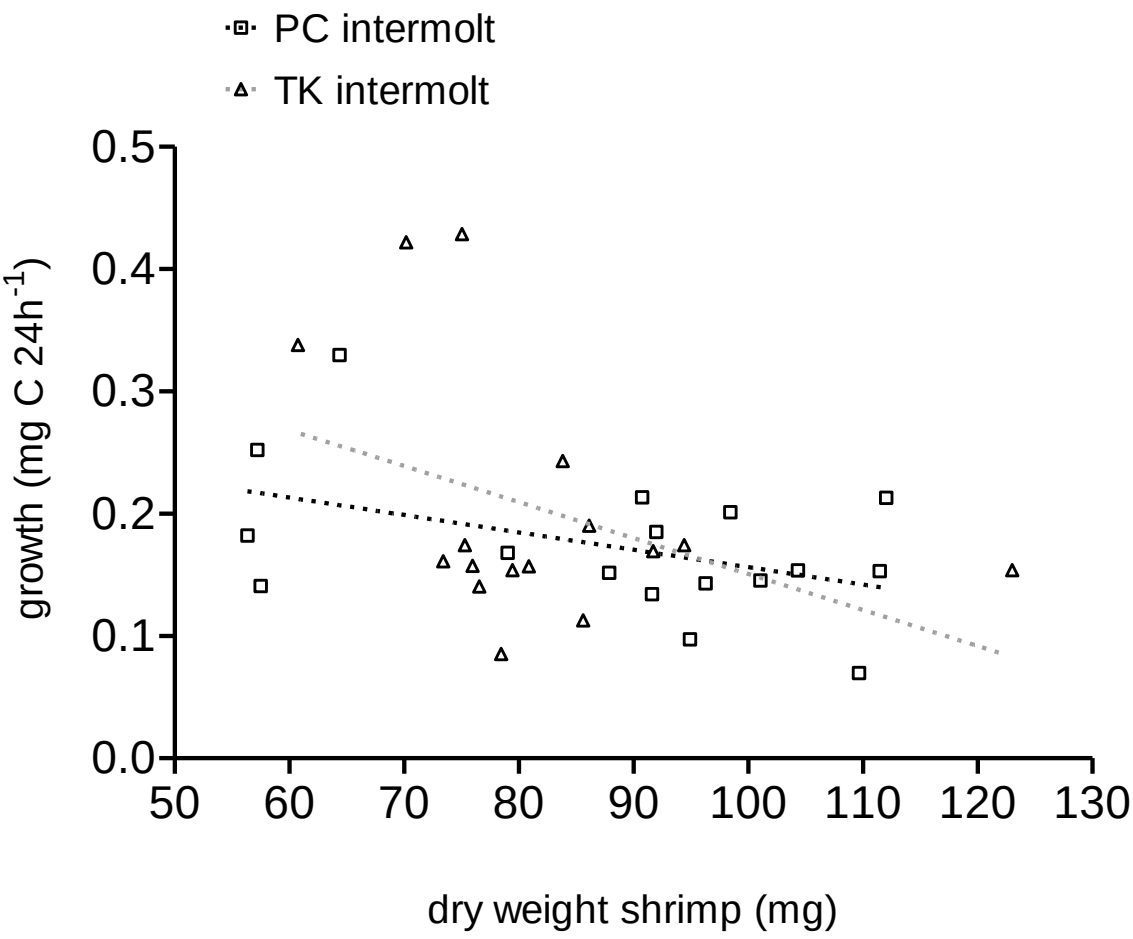


Figure 3.5. Growth by intermolt Piles Creek and Tuckerton shrimp. All regression lines are statistically parallel with a pooled slope of -0.002 (P=0.41).

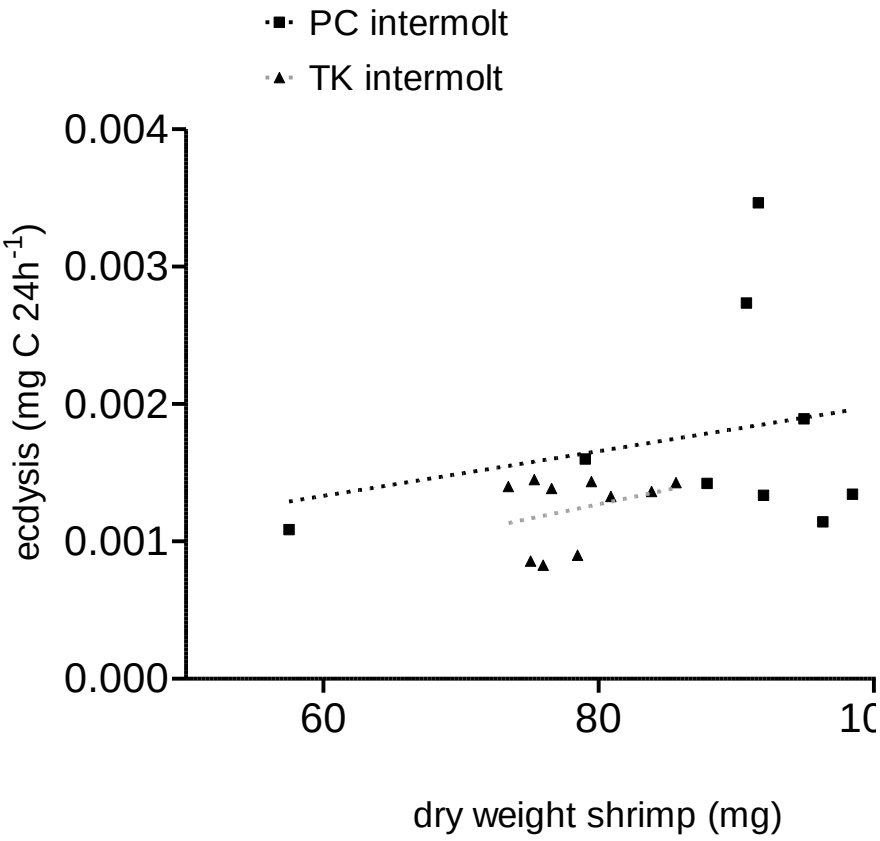


Figure 3.6. Ecdysis by intermolt Piles Creek and Tuckerton shrimp. All regression lines are statistically parallel with a pooled slope of 0.00002 (P=0.93).

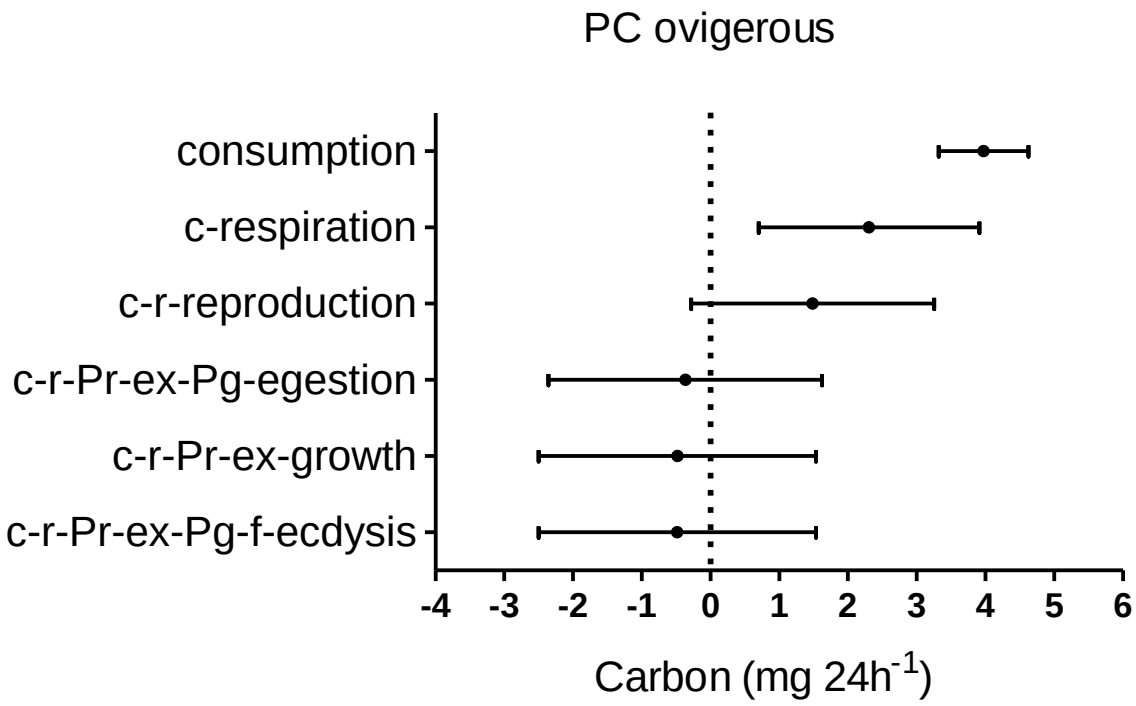


Figure 3.7. Carbon budget for ovigerous Piles Creek shrimp. Means were calculated using an average shrimp size of 116 mg. The error bars show 95% confidence intervals around the mean values. Physiological costs were subtracted line by line in a cumulative fashion from consumption. The ranges of the error bars are also cumulative. Error bars were calculated with the high end of the range representing maximum consumption with minimum costs, and the low end representing minimum consumption with maximum costs. (c=consumption, r=respiration, Pr=reproduction, ex=excretion, Pg=growth, and f=egestion)

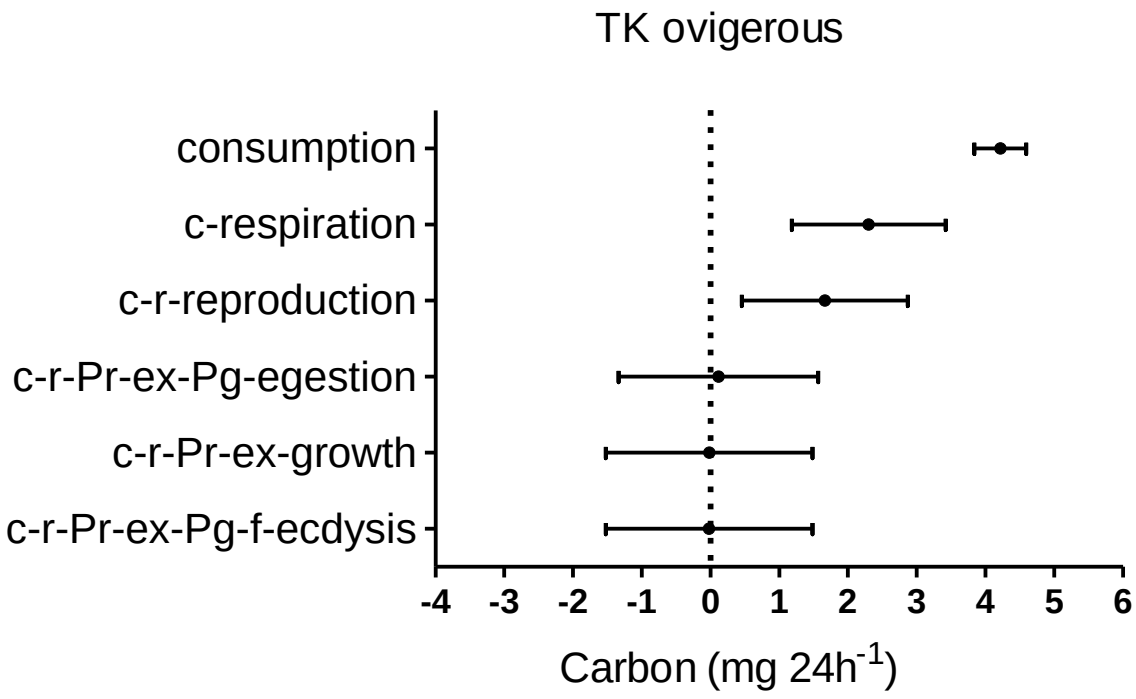


Figure 3.8. Carbon budget for ovigerous Tuckerton shrimp. Means were calculated using an average shrimp size of 116 mg. The error bars show 95% confidence intervals around the mean values. Physiological costs were subtracted line by line in a cumulative fashion from consumption. The ranges of the error bars are also cumulative. Error bars were calculated with the high end of the range representing maximum consumption with minimum costs, and the low end representing minimum consumption and maximum costs. (c=consumption, r=respiration, Pr=reproduction, ex=excretion, Pg=growth, and f=egestion)

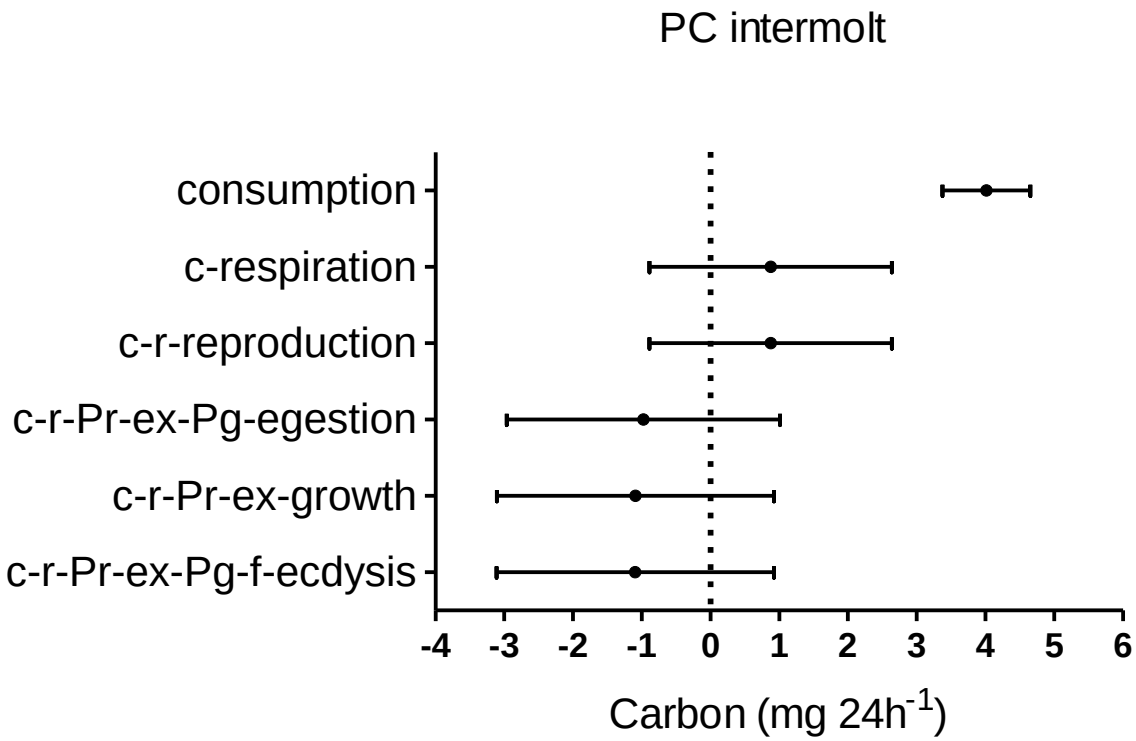


Figure 3.9. Carbon budget for intermolt Piles Creek shrimp. Means were calculated using an average shrimp size of 116 mg. The error bars show 95% confidence intervals around the mean values. Physiological costs were subtracted line by line in a cumulative fashion from consumption. The ranges of the error bars are also cumulative. Error bars were calculated with the high end of the range representing maximum consumption with minimum costs, and the low end representing minimum consumption with maximum costs. (c=consumption, r=respiration, Pr=reproduction, ex=excretion, Pg=growth, and f=egestion)

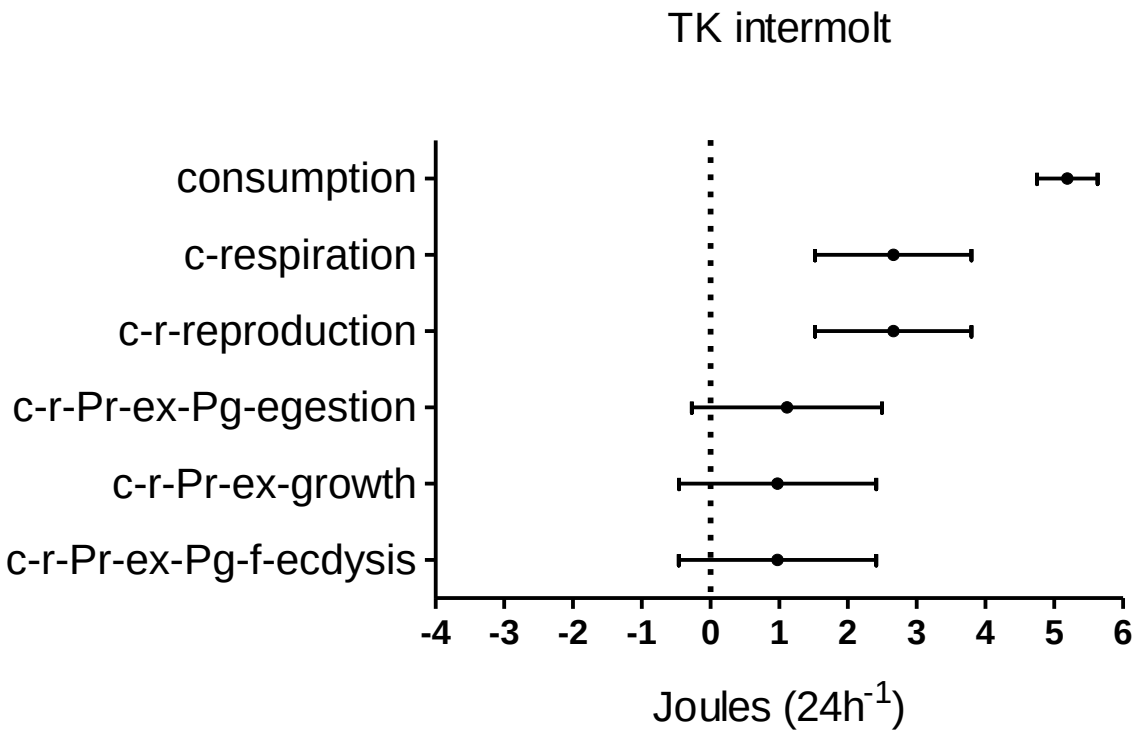


Figure 3.10. Carbon budget for intermolt Tuckerton shrimp. Means were calculated using an average shrimp size of 116 mg. The error bars show 95% confidence intervals of the mean values. Physiological costs were subtracted line by line in a cumulative fashion from consumption. The ranges of the error bars are also cumulative. Error bars were calculated with the high end of the range representing maximum consumption with minimum costs, and the low end representing minimum consumption with maximum costs. (c=consumption, r=respiration, Pr=reproduction, ex=excretion, Pg=growth, and f=egestion)

Chapter 4

Compensatory partitioning of nitrogen budgets in association with anthropogenic contamination in the daggerblade grass shrimp (*Palaemonetes pugio*)

Introduction

The nitrogen cycle is an inherent property of ecosystems. Nitrogen is necessary for protein production in all organisms, but an overabundance leads to eutrophication and habitat degradation. Most previous research on the nitrogen cycle in estuaries has focused on nitrogen throughput rates and budgets as a measure of anthropogenic disturbance (Wang et al. 2004). This study examines the nitrogen budget and the effects of anthropogenic contaminants on nitrogen utilization of a single species, the daggerblade grass shrimp *Palaemonetes pugio*. *Palaemonetes pugio* is abundant and ubiquitous in estuarine systems of the eastern United States, and so is an ideal subject for determining the role of anthropogenic contaminants such as heavy metals and organic compounds have on the nitrogen budget at the organism level.

P. pugio has been the subject of many toxicology and physiology studies (Roesljadi et al. 1976, Tatem et al. 1976, Kraus et al. 1988, Vernberg and Piyatiratitivorakul 1998, U.S. EPA, 2007). Nevertheless, in over 1000 toxicological studies collected by the EPA, no studies examined the nitrogen budget or any component of the nitrogen budget of this shrimp (U.S. EPA, 2007). Overall, studies of physiological responses to contaminants are rare in *P. pugio* (U.S. EPA, 2007), despite findings that short-term studies of

physiological responses to contaminants are good predictors of long-term population effects and are more sensitive than lethality studies (Maltby 1999).

It is necessary to understand intake, output, and assimilation of nitrogen in order to model the role of an organism on the nitrogen budget of an ecosystem. There are also organism-level responses in the nitrogen budget that reflect the role of tolerance and acclimatization to toxicants. Nitrogen-containing proteins are important in the sequestration of toxicants, other proteins are involved in stress reactions caused by toxicants, and ammonia excretion can be linked to toxicant depuration (Kraus et al. 1988). Other consequences of living in a habitat with significant toxicant levels include the development of heat shock proteins, increased protein turnover, and increased rates of depuration (Kraus et al. 1988, Maltby 1999). These all could have implications for a nitrogen budget. In addition, feeding—and thus nitrogen uptake—is generally found to be depressed in response to toxicants, and is a more sensitive measure of toxicant effects than respiration (Maltby 1999, Walace et al. 2000).

As Anger (1990) found, nitrogen uptake and assimilation can also be influenced by life history stage, with protein assimilation increasing with age in the decapod *Hyas araneus*. This study compares nitrogen budgets of ovigerous and intermolt shrimp to measure differences in allocation due to life history stage, as well to examine the relationship between life history stage and physiological responses to anthropogenic contaminants. By measuring nitrogen allocation in field caught shrimp from clean and contaminant laden study sites, this study shows the relative differences between the sites in shrimp

nitrogen allocated to each budgetary component. This reveals differences in allocation as a result of field conditions and thus the potential impact of a contaminated environment on the role shrimp play in nitrogen cycling within the habitat. A total nitrogen budget provides a sensitive metric of the effects of living in a contaminated environment, as well as providing information for models of nitrogen utilization and revealing some possible costs of tolerance to potential toxins.

Materials and methods

Field Collection Sites

The two collection sites demonstrate the full range of anthropogenic impacts found in New Jersey. The Tuckerton site at the Rutgers Marine Field Station at Big Sheep's Head Creek (TK) is relatively pristine (Able et al. 1996). As part of the Mullica River-Great Bay estuary system, it is one of the least disturbed estuaries in the North East. Located in the Jacques Cousteau National Estuarine Research Reserve, it is part of the National Estuarine Research Reserve system. These sites are considered baseline reference sites for establishing the effects of anthropogenic impacts. The Great Bay and Mullica systems are now, and have historically been, pristine and free of excessive nutrient loading from anthropogenic sources. There is very little industry or development within the Mullica River drainage basin. The one major source of nutrient loading to Great Bay, a menhaden processing plant, has been closed since 1960. The collection site is over 200 meters from a small access road and has no access by boats, and so has additional protection from incidental impacts and non-point sources such as motor oil spills and

runoff. Metal levels in TK sediments have been reported as 0.054 μg Hg/g, 0.13 μg Cd/g, 12.9 μg Cu/g, and 7.7 μg Zn/g (Khan and Weis 1987).

The Piles Creek site (PC) represents a heavily impacted marsh creek. There are several active and abandoned chemical-processing plants near the creek as well as the Linden Sewerage facility. An Exxon Mobil refining center lies directly adjacent to the site, with fuel lines crossing over the site itself. There have historically been releases of oil ranging from leaks in the pipes to discharges from small boats. There is visible oil both in the sediments and on the surface of the water. Mercury levels in PC sediment have been found in the range of 10-20 μg/g and lead levels have been found up to 3000 μg/g (Weis and Weis 1989). More recent studies have found mercury contamination has declined to <5ppm (Weis 2002). Other metals include 5.9 μg Cd/g, 623.5 μg Cu/g, and 627.9 μg Zn/g (Khan and Weis 1987).

Collection methods

Shrimp in marsh creeks were captured using one-meter square umbrella nets baited with ribbed mussel (*Geukensia demissa*). Mussels used for bait were collected fresh at the collection sites and used immediately after collection. The mussels were cracked and placed in the middle of the nets. The nets were placed in the marsh creeks along the bed, as close as possible to the bank without tipping. Nets were set between the tides. No shrimp were collected at either high or low tides due to their absence at these times. The first shrimp collected coincided with the first ovigerous females found in the nets, and

collection continued until ovigerous females were no longer found. The appearance of ovigerous young-of-the-year females in mid to late July was also noted.

Adult ovigerous and non-ovigerous shrimp of similar sizes were selected, placed in five-gallon buckets and taken to the laboratory. Shrimp less than 0.25 g were assumed to be juveniles and excluded from the experiment. After collection, the shrimp acclimatized for 24 hours in the laboratory at 20° in water with a salinity of 20. Salinity and temperature were kept constant throughout the experiment.

The sex of individuals was noted when possible. Obviously parasitized shrimp were excluded from these experiments.

Consumption, Egestion, and Excretion

Each week between the beginning of June and the first week in September in 2002 and 2003 ten shrimp were caught from each site in alternation. A total of 60 shrimp were caught from each site. Each batch of ten shrimp was sorted and five with eggs and five without eggs were chosen for excretion analysis. The shrimp acclimatized for 24 hours in the laboratory. Acclimatization was done under the same conditions as the rest of the experiment. Shrimp were kept at 20° in 1μm filtered seawater with a salinity of 20 in five gallon buckets. After 24 hours, the shrimp were dried with paper towels and then weighed to the nearest milligram. Shrimp were placed in individual glass fingerbowls in 650 ml of 1μm filtered seawater with a salinity of 20. They were provided with food in excess of what they could consume in a 24 hour period.

The food consisted of tissue taken from live ribbed mussels that was frozen and then freeze dried for 24r hours. A solid piece of freeze-dried tissue approximately 50mg, weighed to the nearest hundredth of a milligram, was placed in each fingerbowl with the shrimp. Nitrogen content of the mussel tissue was measured as below. The fingerbowls were covered with nylon screening to prevent the shrimp from jumping out. A method blank was included so that in addition to the ten fingerbowls containing shrimp and mussel tissue, there was one fingerbowl that contained only mussel tissue. The method blank was treated identically to the other samples containing shrimp.

After 24 hours, the shrimp were removed from the fingerbowls, dried with paper-towels, and then weighed to the nearest milligram. Fingerbowls that contained dead shrimp, shrimp that had released their eggs, or shrimp that had undergone ecdysis were emptied and re-washed. Fingerbowls with live healthy shrimp were carefully examined for any remaining ribbed mussel tissue. The mussel tissue was removed, frozen and freeze dried for 24 hours in a Labonco freeze dryer at -50°C in a 5 micron Hg vacuum. The tissue was then weighed to the nearest tenth of a milligram. The remaining 650 ml of seawater containing both fecal pellets and liquid excretion products was filtered through 47 mm Whatman GF/F glass microfiber filters using a low pressure vacuum pump. Each filter was baked at 500° for five hours and then kept sealed until use. Before use, each filter was weighed to the nearest hundredth of a milligram using a Sartorius microbalance. The filtrate was retained, and a 10 ml sub-sample was frozen for analysis of nitrogen content. All glassware used in sample preparation for nitrogen analysis was first washed with

detergent, and then rinsed with de-ionized water. The glassware was then thoroughly rinsed with a solution of 12.5% sulfuric acid. The glassware was then re-rinsed with de-ionized water, and then baked in a muffle furnace at 500° for five hours.

Nitrogen losses to gaseous diffusion were not measured due to the insignificant loss rate under the experimental conditions. Ammonia in aqueous solution exists as ammonium ions (NH_4^+) and for significant gaseous diffusion they must be converted to ammonia at a pH of 10.5-11.5 and the temperature increased to at least 75ºC (Chidgopkar 1996).

Somatic Growth

Twenty shrimp from each site were collected in early September 2003 using baited umbrella nets as above. Only intermolt shrimp were selected. Shrimp were dried with paper towels and then weighed to the nearest milligram. Shrimp were placed individually in beakers containing 400 ml of 1μm filtered salinity 20 seawater. All beakers were first washed, rinsed with de-ionized water, and then baked at 500° for five hours. Each beaker was covered with an aluminum foil cap, and individually aerated. The beakers were placed in a walk-in incubator maintained at 20°. Once per day the shrimp were fed 50mg freeze-dried ribbed mussels ground into a coarse powder. The food was the same as used to measure consumption and egestion except for homogenization in a blender. This allowed the mussel tissue to be prepared and freeze dried in a single batch prior to use. The amount of food provided was calibrated to insure maximum feeding rates at all times. At no time were shrimp allowed to be without food. Any food remaining from the previous day was removed. The water in the beakers was changed every other day for

the duration of the experiment. Each day the shrimp were checked for ecdysis and the shed removed, rinsed with de-ionized water, and saved for later analysis. Any shrimp that could not right itself was removed. After 28 days all shrimp were removed from the beakers and weighed to the nearest milligram.

To convert the change in shrimp mass into a measure of nitrogen assimilation, whole shrimp were measured for total somatic nitrogen content. Ten ovigerous and ten non-ovigerous shrimp were captured at each site and freeze dried. The shrimp were weighed both wet and dry to determine the relationship between wet and dry shrimp masses. Whole dried shrimp were ground using mortars and pestles that had been baked in a muffle furnace at 500° for five hours. A 30mg sub-sample was taken from individual homogenized powdered shrimp and analyzed for nitrogen content.

Ecdysis

Shed skins were collected during the experiment designed to measure nitrogen allocated to somatic growth as described previously. The exuvia were collected daily. Each of the shrimp being measured for growth shed at least once, and the exuvia were collected, washed with de-ionized water, and freeze-dried. Ten exoskeletons from each study site were measured for total nitrogen content as above. Shed exoskeletons were not collected from ovigerous shrimp because egg-bearing females do not shed. They do, however, shed at the end of each 14-day reproductive cycle, so the nitrogen lost to ecdysis for ovigerous shrimp was assumed to be similar to the nitrogen lost by intermolt grass shrimp, with a similar periodicity to their exoskeleton formation. The total nitrogen lost

in each exoskeleton was divided by the 14-day period to determine the daily nitrogen lost to ecdysis.

Reproduction

The daily nitrogen allocation by ovigerous shrimp to egg production was calculated as the total nitrogen content of individual egg masses divided by 28 days given the 14-day reproductive period where shrimp are ovigerous and assuming prior egg production for an additional 14-day intermolt period. A total of 33 ovigerous TK shrimp and 23 ovigerous PC shrimp were captured as above in 2000 and the eggs were removed. The individual egg masses were separated from each shrimp with a scalpel. The egg masses were freeze-dried then weighed using a Sartorius microbalance. As above, the egg masses were powdered, and a 2 mg sub-sample was taken for nitrogen analysis.

Nitrogen analysis

Fecal matter was analyzed using the methods of Verardo et al. (1989). Fecal pellets were defined as all substances that did not pass through the filter. Each filter was thoroughly rinsed with de-ionized water and then placed in a drying oven. The filters were then weighed to the nearest hundredth of a milligram using a Sartorius microbalance. The dried filters were then cut into four equal pieces and wrapped in tin. The tin sample boats containing the quartered glass fiber filters were kept in the drying oven at 60° for 24 hours or until analyzed.

Each filter was analyzed for nitrogen content using the Carlo/Erba NA-1500 Analyzer gas chromatography instrument. A standard curve was developed using six acetanilide standards ranging from 0.01 to 1mg. No more than 20 filter quarters were run at a single time. Each analysis run was preceded by two empty sample tin boats run as instrument blanks. Every six samples an acetanilide check standard was used to monitor accuracy. If the check standard exceeded a 1% deviation from expected results a second check standard was run to verify the deviation. If the nitrogen content continued to deviate by more than 1% from expected, the run was discontinued.

After particulate nitrogen was removed through filtration, a 50 ml sub-sample was collected from the resulting solution. Following the methods of Seitzinger et al. (1997, 2005), total dissolved nitrogen (TDN) was measured using high temperature combustion of the sample to produce nitric oxide. Total nitric oxide was measured using chemoluminescent detection with an Antek Modell 7000 Total N Analyzer. All aqueous samples were kept frozen until analysis.

Nitrogen budgets

Nitrogen allocation was measured according to the equation developed for grass shrimp energy budgets by Vernberg and Piyatiratitivorakul 1998) modified to exclude the term for respiration as irrelevant to nitrogen budgets. Nitrogen allocation is described as $C = Pg + Pr + E + Ex + F$ where C is consumption, Pg is the portion of total production allocated to somatic growth, Pr is the production allocated to reproduction, E is excretion,

Ex is exuvia, and F is egestion. More properly the equation can be understood as nitrogen assimilation (C-E-F) equaling total nitrogen allocated to production (Pg+Pr+Ex).

Statistical analysis

All statistical analysis was performed using GraphPad Prism version 5.02 for Windows (GraphPad Software 2008). All error estimates represent 95% confidence levels for the data. ANCOVA was used to compare slopes between all groups and the combined slope (weighted average) from each budget component was found. The average slope was then used to calculate the mean values for each treatment group for each component using an average shrimp size of 116.1 mg (Packard and Boardman 1987, 1999).

The total nitrogen budget for each treatment group was derived from the mean rate of consumption minus the mean allocation for each component. The error bars are cumulative showing the 95% confidence intervals.

Results

Consumption

Ovigerous PC and TK shrimp consumed similar amounts of nitrogen, while intermolt PC shrimp showed reduced consumption in comparison to intermolt TK shrimp (Figure 4.1 and Table 4.2) (dry mussel tissue=9.47±0.12% nitrogen). TK shrimp also showed less variance in response than PC shrimp (Figure 4.1 and Table 4.2).

Ovigerous PC shrimp showed a much greater range of response than ovigerous TK shrimp. Four ovigerous PC shrimp consumed no food during the course of the experiment, while all ovigerous TK shrimp consumed measurable amounts. Some individual ovigerous PC shrimp also consumed more food than any ovigerous TK shrimp. There was no correlation between shrimp mass and consumption for ovigerous shrimp (Table 4.1)

Intermolt PC shrimp consumed less nitrogen per24 hours than intermolt TK shrimp (Figure 4.1 and Table 4.2). There was a significant relationship between nitrogen consumption and shrimp mass for intermolt PC and TK shrimp (Table 4.1). In a pattern similar to that seen in ovigerous shrimp, intermolt PC shrimp showed a greater range of consumption than intermolt TK shrimp. Six out of the 32 intermolt PC shrimp consumed no food during the experiment, while all intermolt TK shrimp did. Some individual PC intermolt shrimp also consumed more food than any TK intermolt shrimp.

Excretion

Ovigerous shrimp from the Tuckerton site excreted more soluble nitrogen (NH_4^+) as a function of body mass than ovigerous shrimp from Piles Creek (Figure 4.2 and Table 4.2). There was no significant relationship between shrimp mass and excretion for either ovigerous PC shrimp or ovigerous TK shrimp (Table 4.2). Ovigerous PC shrimp had a greater range of excretion than ovigerous TK shrimp.

Intermolt TK shrimp had a statistically similar excretion to that of intermolt PC shrimp (Figure 4.3 and Table 4.4). Intermolt PC shrimp did not show a significant relationship between mass and excretion, but intermolt TK shrimp did (Table 4.2). Intermolt PC shrimp showed a greater range of excretion than intermolt TK shrimp.

There were no within-site differences for TK shrimp, but ovigerous PC shrimp excreted significantly less nitrogen than intermolt PC shrimp (Figure 4.2 and Table 4.2). Only intermolt TK shrimp showed a significant relationship between body mass and the rate of excretion (Table 4.1).

Egestion

Piles Creek shrimp egested more nitrogen as solid waste than Tuckerton shrimp (Figure 4.3 and Table 4.2). Nitrogen egestion was not significantly correlated with body mass in TK shrimp, but it was in PC shrimp (Table 4.2). TK shrimp showed a greater range of egestion than PC shrimp.

Reproduction

There was no significant difference in the percentage of nitrogen in shrimp eggs from the two sites. PC shrimp eggs were 10.97% N ± 0.54 (n=8) while TK shrimp eggs were 11.09%N ± 0.27 (n=9). PC shrimp egg masses were proportionately larger than TK egg masses and thus the total nitrogen allocated to eggs was significantly higher in PC shrimp (Figure 4.3 and Table 4.2).

Ecdysis and somatic growth

The somatic nitrogen content was similar between shrimp from the two sites. PC shrimp were 11.25±0.37 % nitrogen by dry weight (n=8) and TK shrimp were 10.84±0.45 % nitrogen (n=9).

Piles Creek and Tuckerton shrimp had similar rates of nitrogen allocation to somatic growth. The slope of shrimp mass to nitrogen allocation rates was statistically similar for each study site (Figure 4.5 and Table 4.1), and there were no differences in growth rates (Table 4.2). The slopes for both sites were negative, indicating that smaller shrimp allocate more nitrogen to somatic growth than larger shrimp. Nevertheless, there was no statistically significant relationship between shrimp mass and somatic growth. PC and TK shrimp had similar ranges of somatic nitrogen allocation and were similar in their correlation between dry shrimp weight and somatic nitrogen allocation over time.

Shed exoskeletons from PC and TK shrimp differed significantly in nitrogen content. PC dried exoskeletons were 3.29±0.23% nitrogen (n=9), while TK exoskeletons were 2.74±0.25% nitrogen (n=10). Despite the higher nitrogen content of PC shrimp exuvia, there was no significant difference in the rate of nitrogen loss through ecdysis between the two sites (Figure 4.6 and Table 4.2). Neither group showed a significant correlation between shrimp dry weight and nitrogen allocated to shed exoskeletons (Table 4.1). PC shrimp showed a much greater range of nitrogen allocation to ecdysis than TK shrimp.

Nitrogen Budget

The nitrogen budgets for almost all treatment groups were statistically balanced with the error bars crossing the zero line after all costs had been subtracted (Figures 4.7-4.10). Intermolt TK shrimp were the exception with a small positive remainder of nitrogen (Figure 4.10)

Ovigerous PC shrimp had similar rates of consumption to those of ovigerous TK shrimp and higher egestion and reproduction. The higher reproductive allocation was balanced by reduced excretion. Allocation to growth was similar between TK and PC intermolt shrimp despite a lowered consumption rate in PC intermolt shrimp. Intermolt PC shrimp did not compensate for lowered consumption with lowered excretion.

Discussion

Ovigerous PC shrimp utilized compensatory partitioning to allocate more nitrogen to reproduction than Ovigerous TK shrimp. The reduction in excretion by PC ovigerous shrimp allowed them to allocate correspondingly more resources to reproduction even though there consumption rates were similar to those of ovigerous TK shrimp, and their egestion rates were higher.

Previous studies predict that feeding levels are likely to be lower due to toxins (Maltby 1999) while others (Kraus et al. 1988) predict increased depuration. If increased depuration is linked to ammonia excretion, then it appears there would be a deficit in the nitrogen budget, especially if the growth rates of shrimp are similar, as found in this

study and that of Santiago Bass et al. (2001). Reduced feeding was indeed found among intermolt shrimp exposed to a contaminated habitat, as has been found by previous studies, but was not found in ovigerous shrimp. This has not been found in previous studies because previous studies have not looked at ovigerous shrimp. Also contrary to expectations, there was no evidence of increased depuration via excretion by PC shrimp, but rather a reduction in excretion by ovigerous PC shrimp. By maintaining consumption, and reducing excretion, ovigerous PC shrimp had more resources to allocate to reproduction than ovigerous TK shrimp.

The increased egestion found in PC shrimp was balanced by decreased ammonia excretion in ovigerous PC shrimp (Table 4.2). The decrease in excretion exceeded the increase in egestion sufficiently to allow surplus nitrogen to be allocated to reproduction. This was evidence of compensatory partitioning of resources by ovigerous PC shrimp.

Most studies of toxicant effects on *P. pugio* are dosing studies performed in the laboratory on uniform populations of grass shrimp. Few studies attempt to measure effects of contaminants encountered by shrimp in the field. One of the few such studies on *Palaemonetes* sp. by Oberdorster et al. (1999) did not find significant differences in the physiological budgets of field-caught shrimp despite finding differences in metabolic functions of shrimp exposed to the same contaminants in the laboratory. Nevertheless, there is evidence of population differences between shrimp found in the heavily polluted Piles Creek as opposed to shrimp found in the relatively clean marsh creek near Tuckerton. PC shrimp were found to be larger and more numerous than TK shrimp

despite the high toxicant levels at Piles Creek (Santiago Bass et al. 2001). Santiago Bass et al. (2001) attributed this to lowered predation at Piles Creek as evidence by growth rates that were not significantly different between the two populations. Nevertheless, growth is only one aspect of the budget of an organism.

In the present study, intermolt PC shrimp showed statistically similar growth rates when compared to intermolt TK shrimp, however, the nitrogen available for growth was lower for PC shrimp due to their lower rate of consumption (Table 4.2). Thus a larger proportion of nitrogen consumed is allocated to growth.

A similar situation is seen in nitrogen allocation to reproduction. Ovigerous PC shrimp showed a higher allocation of nitrogen to reproduction than that of ovigerous TK shrimp as measured by total allocation to eggs (Tables 4.2). Nevertheless, consumption was the same for ovigerous shrimp from the two sites; thus significantly more of the nitrogen consumed by PC ovigerous shrimp was allocated to reproduction. The increased proportion of nitrogen consumed allocated to reproduction in ovigerous PC shrimp must come at the expense of another portion of the nitrogen budget. Another component of the budget must be reduced to compensate for the higher reproduction in ovigerous PC shrimp. This can be seen in the reduced excretion in these shrimp.

Similarly to the increase in the proportion of nitrogen consumed allocated to reproduction seen in ovigerous PC shrimp, the increase in allocation to growth among intermolt shrimp must come at the expense of another component of the budget. Excretion in PC intermolt

shrimp, however, was not significantly different than excretion in TK intermolt shrimp (Table 4.2). To balance the nitrogen budget, there must be a reduction in nitrogen allocated to another component. The only component not measured in these shrimp is the reproductive allocation. Thus it appears as though intermolt PC shrimp increase allocation to growth through a reduction in allocation to reproduction, while ovigerous PC shrimp increase allocation to reproduction through decreased allocation to excretion.

The budgets for intermolt shrimp assumed zero reproductive allocation. The small positive remainder in the nitrogen budget of intermolt TK shrimp then represents an estimate of their reproductive allocation. Intermolt PC shrimp do not have such a remainder, and this may indicate a reduction in reproductive allocation by these shrimp.

The lower feeding rate measured of intermolt PC shrimp is at least in part the result of the few individuals in both the ovigerous and intermolt PC groups that consumed no food at all during the 24 hour experiment. Still, even when these non-feeding individuals were excluded, intermolt PC shrimp continued to show a lowered feeding rate. Thus the lowered feeding rate should be considered a result of exposure to contaminants in the field rather than the effects of laboratory conditions on a few individuals. All TK shrimp consumed measurable amounts during the experiment. The cessation of feeding in some PC shrimp could be considered part of the expected increase in range predicted when organisms are exposed to contaminants. Although many PC shrimp maintained feeding rates similar to those found in TK shrimp, there was a greater variability in consumption

regardless of reproductive state paired with a general decrease in the feeding rate for intermolt shrimp.

Consumption and growth are two easily measured aspects of shrimp physiology, but these metrics work at very different time scales. Similarly, each separate budget component measures allocation on different time scales. Because growth measures an average rate of production between measurements, it can be a long or short term metric of changes in shrimp metabolism. Measurements must be sufficiently spaced though to show any change over time due to the relatively slow growth rates of shrimp, and the difficulty of measuring growth under controlled conditions. Thus growth is a measure of grass shrimp metabolism with a separation between signal and response that will range from two to several weeks.

Egestion shows a treatment effect that may precede the capture of the shrimp if the egestion rate does not allow clearance of fecal products within the 24 hour acclimation time shrimp were given in these experiments. Although the rate at which a shrimp feeds is close to an instantaneous measure of a shrimp's physiological state, enough time must elapse for the rate to be measured. This could range from a few hours to the full day utilized in these experiments. Thus the results of metabolic measurements are dependent on the time scales, and measuring several metabolic components necessarily means measuring at different time scales.

Rates of feeding and excretion show the strongest response to anthropogenic contaminants found in the field. They are relatively easy to measure and provide controlled results in a limited time frame. Growth and reproductive allocation are more important for predicting population impacts from exposure to toxicants, but the effects of the exposure are masked by the transference of resources from other portions of the nitrogen budget.

While all portions of the nitrogen budget were measured for these shrimp, it was not possible to measure every component simultaneously in a single experiment, nor was it possible to measure every component of every treatment group. Thus the nitrogen budget includes several assumptions and estimates upon which the final results are based. For example, somatic growth and ecdysis could only be measured in intermolt shrimp. Ovigerous shrimp have the same period between molting as intermolt shrimp (Anderson 1985), and the nitrogen content of the exoskeleton is not likely to differ between the two groups, so the estimate of nitrogen allocated to ecdysis for ovigerous shrimp should be a good approximation of actual allocation. The estimate of nitrogen allocated to somatic growth, however, should be considered an upper limit for ovigerous shrimp, because intermolt shrimp are likely to maximize fitness through maximizing potential for growth while ovigerous shrimp will maximize fitness through maximizing reproductive output.

Ecdysis was only measured for intermolt shrimp, and the nitrogen losses to sheds by ovigerous shrimp may have differed. If so, there still would have been little difference in

the overall nitrogen budget due to the relatively small proportion of nitrogen intake allocated to ecdysis.

Several of the components of the budget were measured together, such as feeding, excretion and egestion, but some components such as growth, ecdysis and reproduction were measured separately and represent a different time scale. During the feeding experiment, the shrimp were acclimated without food, and then feeding was measured for one 24 hour period. During the growth experiment, food to excess was supplied continuously for all 28 days. Thus nitrogen consumption is likely to represent a maximum rate that may or may not have been equaled during the longer period of the growth experiment. Reproduction in ovigerous shrimp was measured separately from the other rates and represents actual reproductive output in the field. Thus it may be lower than what it would be under ideal conditions, but also represents field conditions more closely than the other rates. The assumption of a 28-day period between productions of eggs is based on the period of ovigery, equal to the 14-day intermolt period, plus an additional intermolt period of 14 days allowing for vitellogenesis. Reproduction for intermolt shrimp simply represented the remaining nitrogen not allocated to other components of the budget and thus represents an estimate based on several different time scales.

The differences in time scales and assumptions made to construct the nitrogen budgets makes comparisons between components of the budget more qualitative than quantitative. Nevertheless, individual components were measured using identical

methods and therefore quantitative comparisons are possible within each segment of the budgets.

It was not possible to measure growth directly in ovigerous shrimp, or to make a direct measurement of the reproductive allocation of intermolt shrimp, so it is not clear if there is further partitioning of resources between the life history stages. The absence of a remainder in the nitrogen budget for these shrimp may indicate that intermolt PC shrimp are allocating more resources toward growth and away from reproduction. The small but positive remainder for intermolt TK shrimp may indicate evidence of the maintenance of reproductive allocation in these shrimp. Ovigerous PC shrimp may, conversely, partition resources away from growth and toward reproduction, but without a method for measuring growth in ovigerous shrimp, it is impossible to know.

Despite this uncertainty, it is clear that intermolt PC shrimp are able to maintain growth similar to that of intermolt TK shrimp, and ovigerous PC shrimp are able to exceed the reproductive effort of ovigerous TK shrimp. As an ecological and evolutionary strategy it is most important that intermolt shrimp grow as much as possible and ovigerous shrimp carry as many eggs as possible. PC shrimp are able to do this without an increase in consumption through compensatory partitioning of resources.

In conclusion, grass shrimp encountering toxicants in the field increase some metabolic functions while decreasing others. Some results of exposure to toxicants may be indirectly beneficial, while others act as methods for tolerating the toxicant exposure.

The decreased feeding by intermolt shrimp may lower the shrimps' exposure to predation by decreasing the activity time of the shrimp and increasing time in refugia. Decreased excretion in ovigerous PC shrimp allows them to allocate more nitrogen to eggs.

This conclusion supports the general prediction of lowered rates of metabolic processes in response to anthropogenic contamination. The lower rate of NH_4^+excretion found in ovigerous PC shrimp is also indicative of a site effect. By lowering the excretion rate, ovigerous PC shrimp balance the lowered nitrogen intake though feeding. This balance allows for the increased reproductive effort by PC shrimp found in this study and the study by Santiago Bass et al. (2001).

The general trends of lowered metabolic rates combined with an increased range of responses found in this study agrees with the general predictions of dosing studies, but the compensatory partitioning by the shrimp shows that important budgetary components such as growth and reproduction may be maintained despite the presence of contaminants in the environment. Studies focusing on budgetary components such as feeding would find a lowered rate of consumption, while studies examining growth would find no difference between groups. Without understanding the rest of the budgetary allocation, these would seem mutually exclusive findings. To understand the effects of contaminants on an organism it is necessary to perform a comprehensive examination of multiple budgetary components.

Consumption	N	r^2	Slope ± 95% CL
PC ovigerous	34	0.23	0.01 ± 0.01
TK ovigerous	28	0.44	0.01 ± 0.01
PC intermolt	33	0.11	0.02± 0.01
TK intermolt	25	0.52	0.02± 0.01
Excretion			
PC ovigerous	14	0.16	0.005 ± 0.006
TK ovigerous	14	0.25	0.01 ± 0.01
PC intermolt	12	0.05	0.003 ± 0.008
TK intermolt	12	0.52	0.004 ± 0.002
Egestion			
PC shrimp	15	0.004	0.001 ± 0.0006
TK shrimp	28	0.024	0.0002 ± 0.001
Ecdysis			
PC	9	0.24	-0.00002 ± 0.00004
TK	10	0.03	-0.00002± 0.00007
Growth			
PC	17	0.21	-0.002± 0.002
TK	16	0.15	-0.004 ± 0.004
Reproduction			
PC ovigerous	12	0.004	0.0002 ± 0.0008
TK ovigerous	12	0.16	0.0007 ± 0.0003

Table 4.1. Slopes for regression lines of the nitrogen budget components showing 95% confidence intervals and correlation coefficients.

	PC ovigerous	TK ovigerous	PC intermolt	TK intermolt
Consumption	1.51±0.59	1.74±0.30	1.52±0.53	2.14±0.35
Excretion	0.42±0.16	0.97±0.24	0.85±0.20	0.95±0.09
Egestion*	0.44±0.05	0.35±0.05	0.44±0.05	0.35±0.05
Reproduction	0.19±0.04	0.13±0.02	n/a	n/a
Growth**	n/a	n/a	0.17±0.04	0.18±0.06
Ecdysis***	n/a	n/a	0.0008±0.0009	0.0004±0.0003

Table 4.2. Resoure allocation (mg nitrogen (24h)-1) for a standard-sized shrimp of 116.1 mg dry weight. Data are means±95% confidence intervals. Components of the nitrogen budgets for ovigerous and intermolt shrimp from Piles Creek and Tuckerton. Egestion* represents pooled values from combined intermolt and ovigerous shrimp data. Growth** was based on intermolt shrimp only. Ecdysis*** was based on intermolt shrimp only.

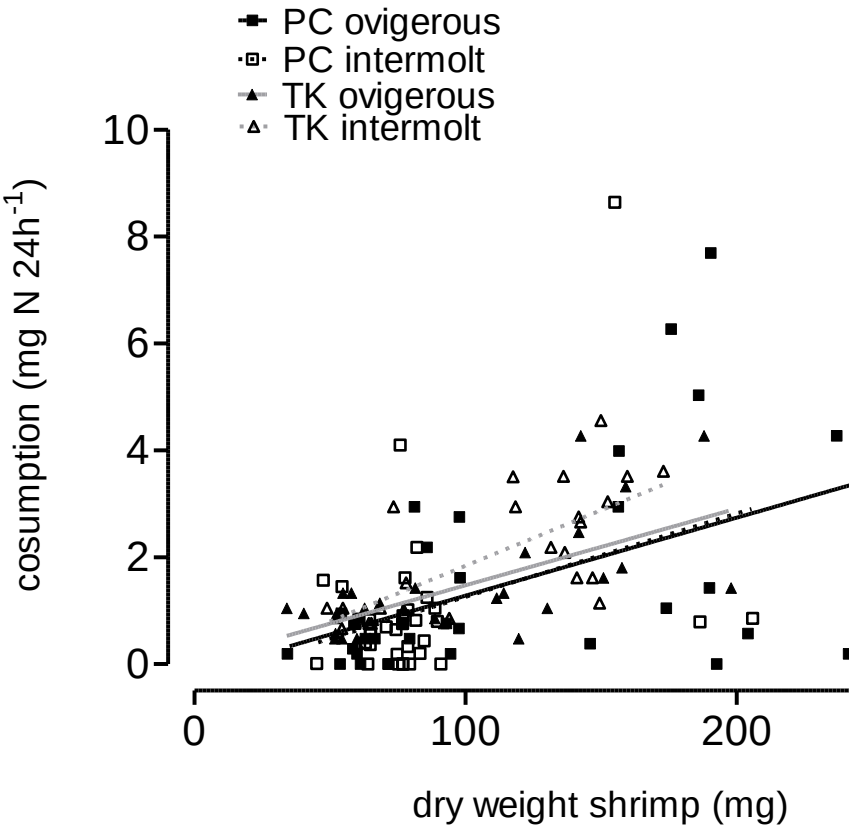


Figure 4.1. Consumption by ovigerous and intermolt Piles Creek and Tuckerton shrimp. All regression lines are statistically parallel with a pooled slope of 0.016 (P=0.86).

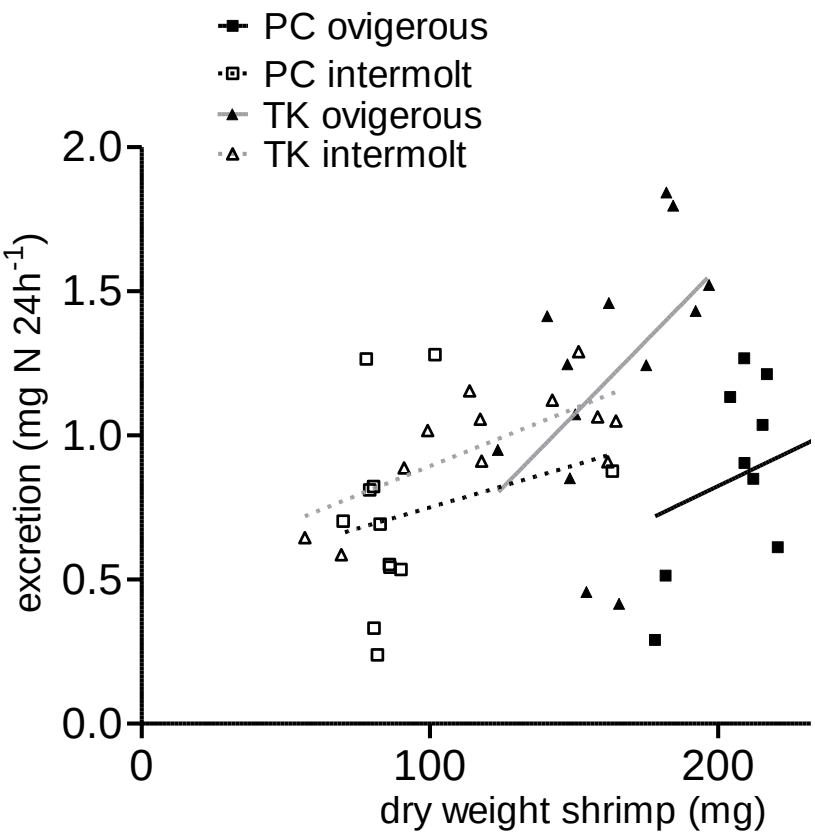


Figure 4.2. Excretion by ovigerous and intermolt Piles Creek and Tuckerton shrimp. All regression lines are statistically parallel with a pooled slope of 0.005 (P=0.58).

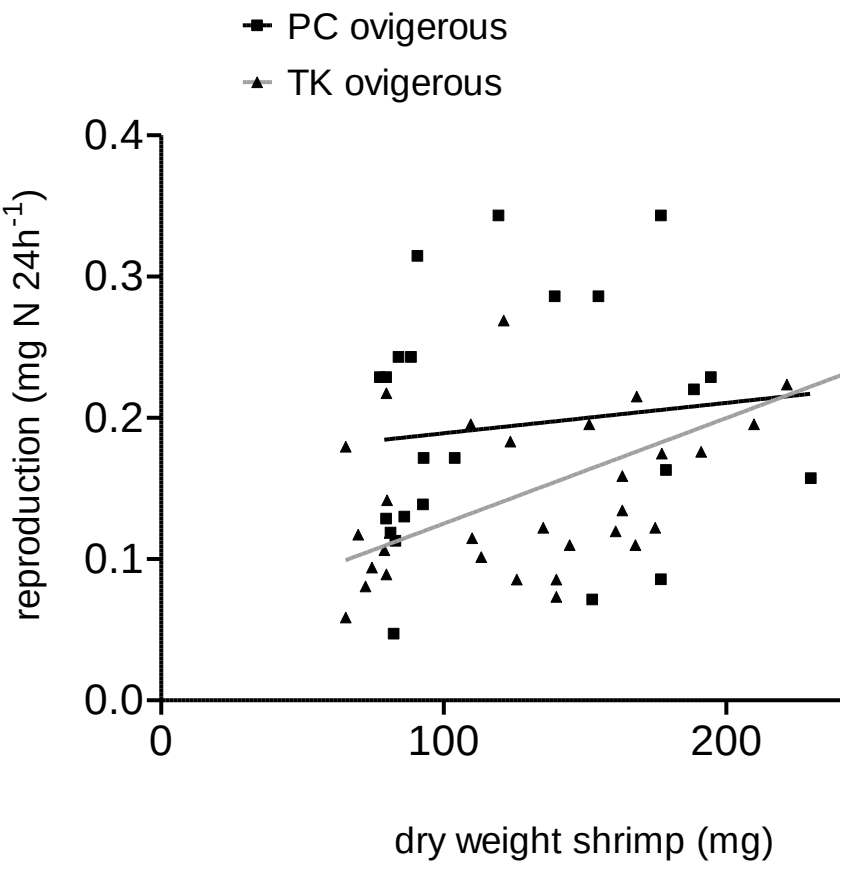


Figure 4.3. Reproduction by ovigerous Piles Creek and Tuckerton shrimp. All regression lines are statistically parallel with a pooled slope of 0.001 (P=0.17).

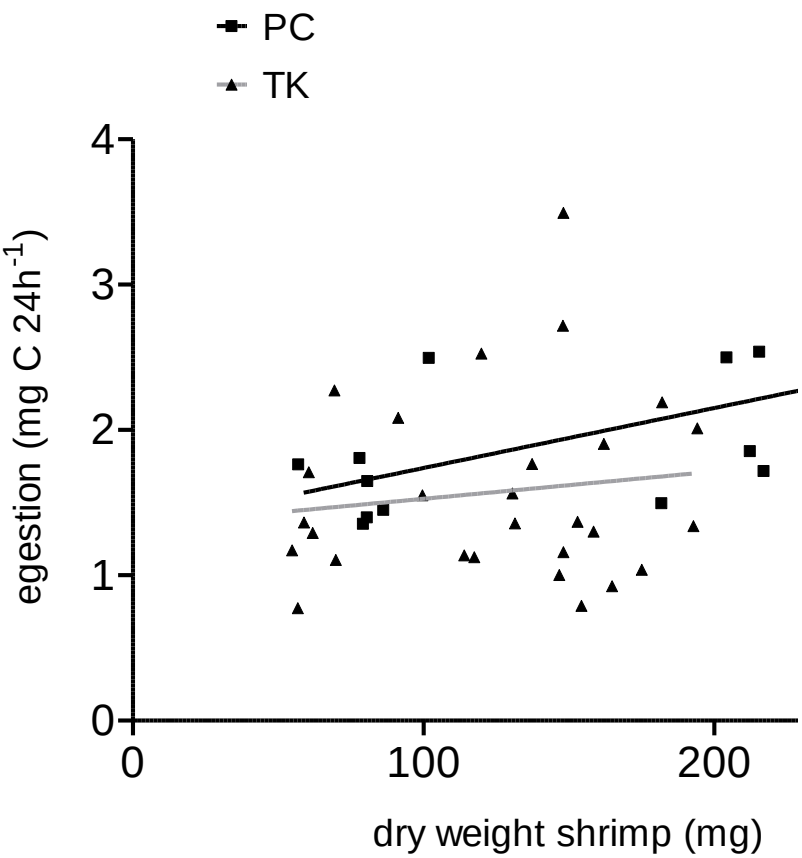


Figure 4.4. Egestion by Piles Creek and Tuckerton shrimp. Intermolt and ovigerous shrimp are grouped together for statistical purposes. The slopes do not differ significantly and have a pooled slope of 0.0001 (P=0.36).

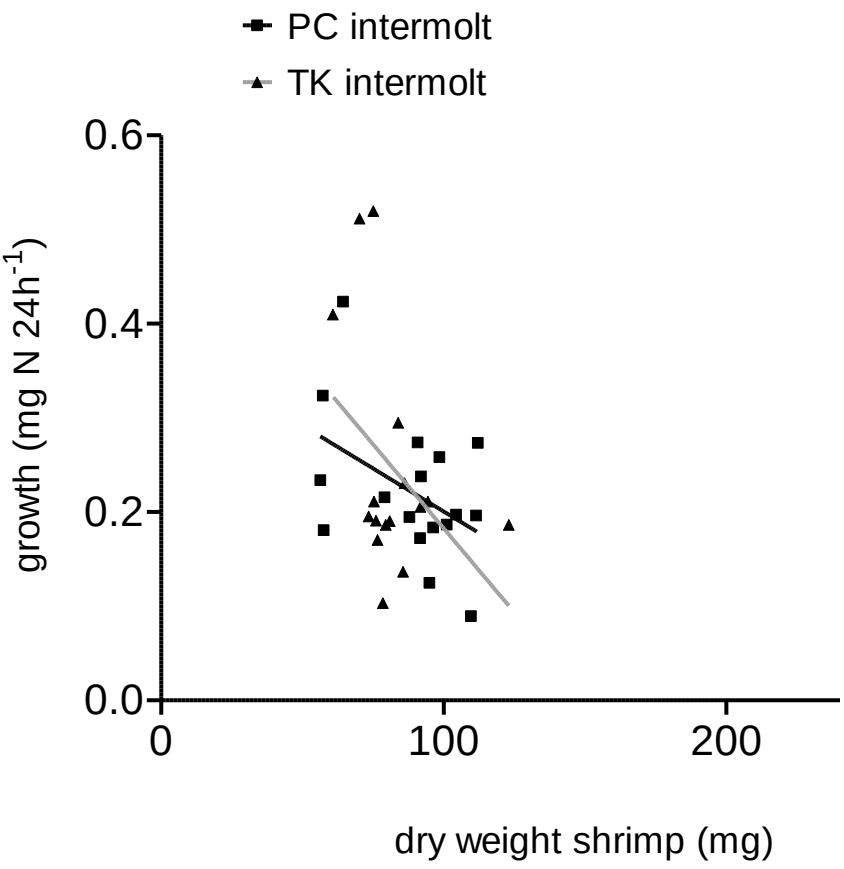


Figure 4.5. Growth by intermolt Piles Creek and Tuckerton shrimp. All regression lines are statistically parallel with a pooled slope of -0.080 (P=0.44).

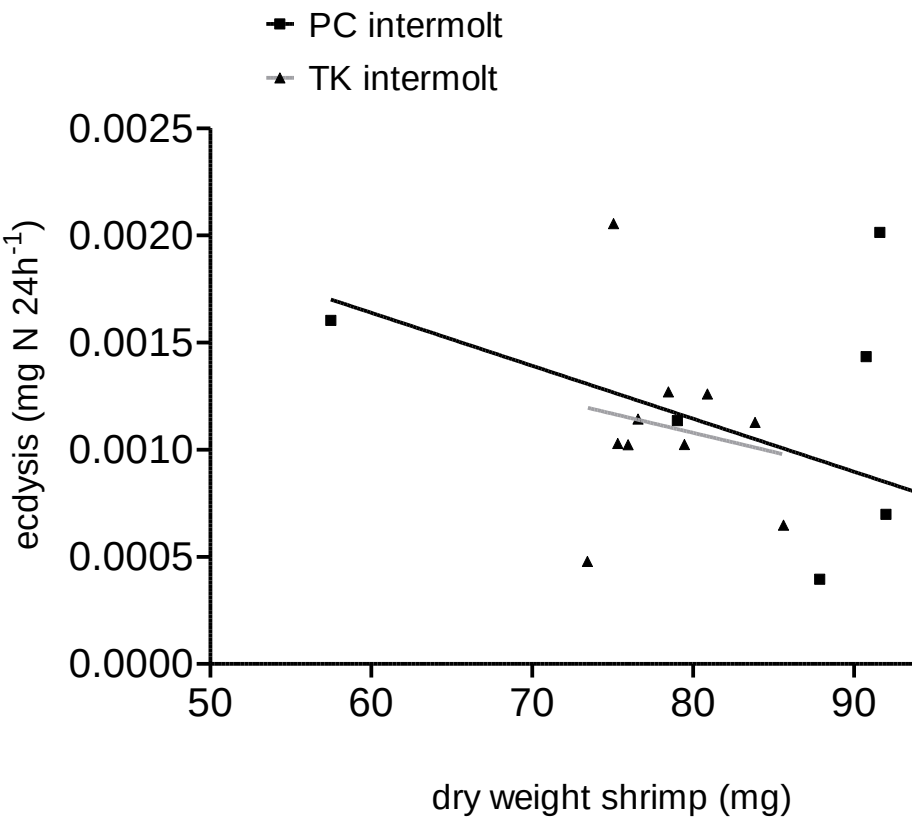


Figure 4.6. Ecdysis by intermolt Piles Creek and Tuckerton shrimp. All regression lines are statistically parallel with a pooled slope of -0.00002 (P=0.89).

Figure 4.7. Nitrogen budget for ovigerous Piles Creek shrimp. Means were calculated using an average shrimp size of 116.1 mg. The error bars show 95% confidence intervals of the mean values. Physiological costs were subtracted line by line in a cumulative fashion from consumption. The ranges of the error bars are also cumulative. Error bars were calculated with the high end of the range representing maximum consumption with minimum costs, and the low end representing minimum consumption with maximum costs. (c=consumption, r=respiration, Pr=reproduction, ex=excretion, Pg=growth, and f=egestion)

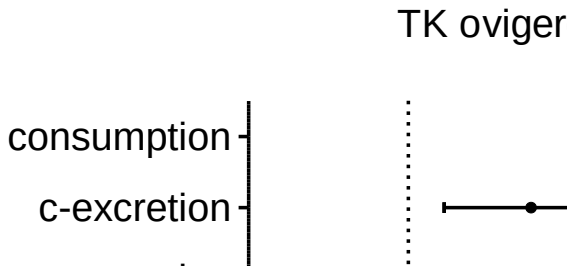


Figure 4.8. Nitrogen budget for ovigerous Tuckerton shrimp. Means were calculated using an average shrimp size of 116.1 mg. The error bars show 95% confidence intervals of the mean values. Physiological costs were subtracted line by line in a cumulative fashion from consumption. The ranges of the error bars are also cumulative. Error bars were calculated with the high end of the range representing maximum consumption with minimum costs, and the low end representing minimum consumption with maximum costs. (c=consumption, r=respiration, Pr=reproduction, ex=excretion, Pg=growth, and f=egestion)

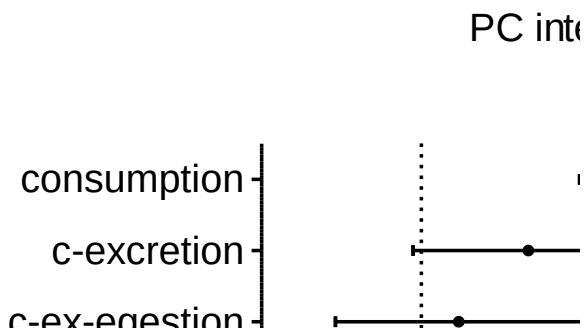


Figure 4.9. Nitrogen budget for intermolt Piles Creek shrimp. Means were calculated using an average shrimp size of 116.1 mg. The error bars show 95% confidence intervals of the mean values. Physiological costs were subtracted line by line in a cumulative fashion from consumption. The ranges of the error bars are also cumulative. Error bars were calculated with the high end of the range representing maximum consumption with minimum costs, and the low end representing minimum consumption with maximum costs. (c=consumption, r=respiration, Pr=reproduction, ex=excretion, Pg=growth, and f=egestion)

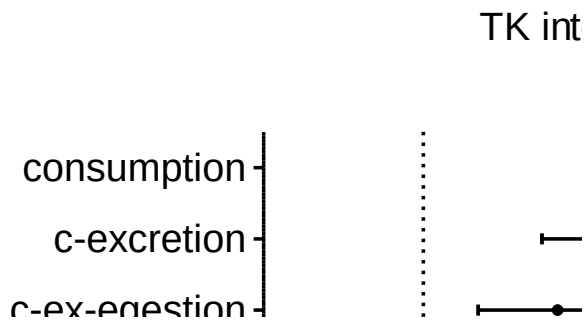


Figure 4.10. Nitrogen budget for intermolt Tuckerton shrimp. Means were calculated using an average shrimp size of 116.1 mg. The error bars show 95% confidence intervals of the mean values. Physiological costs were subtracted line by line in a cumulative fashion from consumption. The ranges of the error bars are also cumulative. Error bars were calculated with the high end of the range representing maximum consumption with minimum costs, and the low end representing minimum consumption with maximum costs. (c=consumption, r=respiration, Pr=reproduction, ex=excretion, Pg=growth, and f=egestion)

Chapter 5

Compensatory partitioning of energy budgets in association with anthropogenic contaminants encountered in the field by the grass shrimp (*Palaemonetes pugio)*

Introduction

The total energy budget of grass shrimp has been well analyzed (Vernberg and Piyatiratitivorakul 1998), but very few studies have looked at how components of the total budget, such as respiration, are affected by exposure to contaminants, and no studies have looked at the effects of contaminants on the entire budget. By measuring the total energy budget of grass shrimp living in contaminated environments, it is possible to determine how important components of the budgets are impacted. Contrasting shrimp from a habitat with low level anthropogenic impacts to shrimp in a highly impacted habitat allows a better understanding of the costs of contamination to an organism.

Energy allocation to somatic growth and reproduction is a general measure of the "health" of an organism and the health of the population. The components of an energy budget are subtle metrics that can show changes of allocation in response to stressors at lower levels than the common endpoint of lethality found in most dosing studies.

The energy budget indicates important elements of an organism's position within an ecosystem. Energy allocated to growth and reproduction affects the population model of an organism. Energy intake dictates how the organism affects lower trophic levels, while allocation to somatic energy content and reproduction affect the availability and quality

of resources for higher trophic levels. Egestion and excretion indicate rates of nutrient recycling, and finally, respiration has a direct bearing on the activity rates and behavior of an organism. These metrics are related to all aspects of an organism's role in an ecosystem and differences in these components associated with contaminants encountered in the field illustrate the costs of tolerance to anthropogenic impacts.

In this study I measured all energy budget components of the grass shrimp *Palaemonetes pugio*. *P. pugio* is common in estuarine environments of the east coast of North America, and is an important species in terms of ecological function. *P. pugio* is one of the most widely distributed and abundant shallow water benthic macroinvertebrates found in the Atlantic and Gulf states (Wood 1967, Odum and Heald 1972, Anderson 1985). Grass shrimp are one of the most important species in estuarine energetics due to their large role in detrital processing (Sikora 1977). In some estuaries they are the primary macroscopic consumer of *Spartina* and *Ulva* detritus, allowing the cycling of detritus into animal biomass (Welsh 1975). *P. pugio* is also a major prey item for a number of estuarine fishes, and is thus indirectly of great recreational and economic importance (Gunter 1945, Darnell 1958, Diener et al. 1974, Anderson 1985, O'Neil 1987, Buckel 1997). Due to its ecological importance *P. pugio* is widely studied, including a large number of both ecological and toxicological studies (Buikema et al. 1980, Wilson 1985, Weber et al. 1996, Wilson 1998, Reinsel et al. 2001).

In this study shrimp were collected from two estuarine marshes with similar physical structure but dissimilar anthropogenic impact. Variations in components of the energy budget were associated with exposure in the field to anthropogenic contaminants.

Materials and methods

Field collection sites

Shrimp were sampled from two New Jersey coastal marshes. Shrimp were collected during their breeding cycle from late June through early September between 2000 and 2005. Shrimp were collected from each site on alternating weeks so that seasonal differences would not obscure site differences. One group was taken from a highly polluted, heavily industrialized salt marsh along the Arthur Kill in the New York/New Jersey Harbor. These shrimp came from Piles Creek, a small marsh creek that runs past active refineries and chemical plants. An abandoned plant that produced photography material is also located nearby. Mercury levels in PC sediment have been found in the range of 10-20 μg/g and lead levels have been found up to 3000 μg/g (Weis and Weis 1989). More recent studies have found mercury contamination has declined to < μg/g (Weis 2002). Other metals include 5.9 μg Cd/g, 623.5 μg Cu/g, and 627.9 μg Zn/g (1987 Khan et al.).

The second group of shrimp was taken from the estuary of the Mullica River near the Rutgers Marine Field Station at Tuckerton, NJ. This estuary is considered one of the least disturbed estuaries in the northeastern United States (Able and DeLuca 1996) and is located within the Jacques Cousteau National Estuarine Research Reserve.

Collection methods

Each week during the breeding cycle ten shrimp were caught from each site in alternation. Shrimp were captured using one meter square umbrella nets baited with ribbed mussel (*Geukensia demissa*) placed in the marsh creeks at the two sites. Mussels used for bait were collected fresh at the collection sites and used immediately after collection. The mussels were cracked and placed in the middle of the nets. The nets were placed in the marsh creeks along the bed, as close as possible to the bank without tipping. Nets were set between the tides. No shrimp were collected at either high or low tides due to their absence at these times. The first shrimp collected coincided with the first ovigerous females found in the nets, and collection continued until ovigerous females were no longer found. The appearance of ovigerous young-of-the-year females in mid to late July was also noted.

Adult ovigerous and intermolt shrimp of similar sizes were selected, placed in five-gallon buckets and taken to the laboratory. Shrimp less than 250 mg wet weight were assumed to be juveniles and excluded from the experiment. Shrimp acclimatized for 24 hours in the laboratory at 20° in water with a salinity of 20. Salinity and temperature were kept constant throughout the experiment. Any shrimp that had softened exoskeletons due to recent shedding or that shed during the holding period and analysis were excluded.

The sex of individuals was noted when possible. Obviously parasitized shrimp were excluded from these experiments.

Consumption, egestion and excretion

Consumption, egestion, and excretion were measured during the shrimp breeding season of 2002 and 2003. Shrimp were caught each week between the beginning of June and the first week in September. A total of 60 shrimp were caught from each site. Each batch of ten shrimp was preselected to include five with eggs and five without eggs for consumption, egestion and excretion analysis. After the 24 hour acclimation period, shrimp were dried with paper towels and then weighed to the nearest milligram.

Shrimp were placed in individual glass fingerbowls in 650 ml of 1μm filtered seawater with salinity of 20. They were provided with food in excess of what they could consume in a 24 hour period. The food consisted of tissue taken from live ribbed mussels that was frozen and then freeze dried. A solid piece of freeze-dried tissue approximately 50mg, weighed to the nearest hundredth of a milligram, was placed in each fingerbowl with the shrimp. The fingerbowls were covered with nylon screening to prevent the shrimp from jumping out. A method blank was included so that in addition to the ten fingerbowls containing shrimp and mussel tissue, there was one fingerbowl that contained only mussel tissue. The method blank was treated identically to the other samples containing shrimp.

After 24 hours, the shrimp were removed from the fingerbowls, dried with paper-towels, and then weighed to the nearest milligram. Fingerbowls that contained dead shrimp, shrimp that had released their eggs, or shrimp that had undergone ecdysis were emptied

and re-washed. Fingerbowls with live, healthy shrimp were carefully examined for any remaining ribbed mussel tissue. The mussel tissue was removed, frozen and freeze-dried for twenty-four hours in a Labonco freeze dryer at -50° in a 5 micron Hg vacuum. The tissue was then weighed to the nearest tenth of a milligram and the consumption rate was determined by difference. The energy content of mussel tissue was determined using micro-bomb calorimetry as discussed below.

The remaining 650 ml of seawater containing both fecal pellets and liquid excretion products was filtered through 47 mm Whatman GF/F glass microfiber filters using a low pressure vacuum pump. Fecal pellets were defined as all substances found in shrimp fingerbowls that did not pass through the filters (0.7 μm nominal pore size). All glassware used was first washed with detergent, and then rinsed with de-ionized water. The glassware was thoroughly rinsed with a solution of 12.5% sulfuric acid, then re-rinsed with de-ionized water, and baked in a muffle furnace at 500° for five hours.

Each filter was also baked at 500° for five hours before use and then sealed. Before use, each filter was weighed to the nearest hundredth of a milligram using a Sartorius microbalance. The filtrate was retained, and a 10 ml sub-sample was frozen for analysis of nitrogen content. Fecal matter was analyzed for CHN content using the methods of Verardo et al. (1989) modified for use with glass fiber filters and an absence of inorganic carbon in the samples. Each filter was thoroughly rinsed with de-ionized water and then placed in a drying oven. After a trial run, no acidification step was used. The filters were weighed to the nearest hundredth of a milligram using a Sartorius microbalance. The

dried filters were cut into four equal pieces and wrapped in tin. The tin sample boats containing the quartered glass fiber filters were kept at 60° for 24 hours or until analyzed.

Excretion

After particulate nitrogen was removed through filtration, a 50 ml sub-sample was collected from the resulting solution. Following the methods of Seitzinger et al. (1997, 2005), total dissolved nitrogen was measured using high temperature combustion of the sample to produce nitric oxide. Total nitric oxide was measured using chemoluminescent detection with an Antek Modell 7000 Total N Analyzer. All aqueous samples were kept frozen until analysis.

Nitrogen losses during the 24 hours of the excretion experiment to gaseous diffusion were not measured due the insignificant loss rate under the experimental conditions. Ammonia in aqueous solution exists as ammonium ions (NH_4^+) and for significant gaseous diffusion they must be converted to ammonia at a pH of 10.5-11.5 and the temperature increased to at least 75ºC (Chidgopkar 1996).

Respiration

Oxygen consumption was measured for resting shrimp. The shrimp were captured during the summer of 2000. The shrimp were placed individually in 200 ml flasks. Before shrimp were added, the dissolved oxygen concentration in each flask was measured using an YSI oxygen meter (model # 57). Each flask was then sealed with a shrimp inside it and left to stand with minimal disturbance for one hour, after which the dissolved oxygen

concentration was remeasured. One blank flask with no shrimp was measured for every ten flasks containing shrimp. The oxygen concentration in blank flasks was never significantly lower at the end of the hour. Oxygen concentrations both before and after were verified to be above the lower limit determined by Burnett and Cochran (1996), below which oxygen consumption is reduced and anaerobic pathways begin to be utilized. CO_2 concentration was not measured, since Burnette and Cochran (1996) found no effect on oxygen uptake in grass shrimp across a wide range of concentrations. After oxygen measurements were taken, each shrimp's length and wet weight were measured.

Somatic Growth

Twenty shrimp from each site were collected in early September 2003. Only intermolt shrimp were selected. Shrimp were dried with paper towels and then weighed to the nearest milligram. Shrimp were placed individually in 500 ml beakers containing 400 ml of 1 μm filtered seawater with a salinity of 20. All beakers were first washed, rinsed with de-ionized water, and then baked at 500° for five hours. Each beaker was covered with an aluminum foil cap, and individually aerated. The beakers were placed in a walk-in incubator maintained at 20°. Once per day the shrimp were fed ~50 mg freeze dried ribbed mussels ground into a coarse powder. The food was the same as used to measure consumption and egestion except for homogenization in a blender. This allowed the mussel tissue to be prepared and freeze dried in a single batch prior to use. The amount of food provided was calibrated to ensure maximum feeding rates at all times. Shrimp were fed to excess, and at no time were shrimp allowed to be without food. Any food remaining from a previous feeding was removed. The water in the beakers was changed

every other day. Each day the shrimp were checked for ecdysis and the shed removed, rinsed with de-ionized water, and saved for later analysis. Any shrimp that could not right itself was removed. After 28 days all shrimp were removed from the beakers and weighed to the nearest milligram.

Ecdysis

Exoskeletons were collected during the growth and feeding studies. Many exoskeletons were rejected for analysis as incomplete due to consumption by the shrimp that had shed them. Exoskeletons were rinsed with de-ionized water and freeze-dried. Multiple samples of 3-5 exoskeletons were used for each calorimetry measurement. The mean energy content of grouped samples was used to calculate the energy content of the most complete exoskeletons by weight. The energy content of the samples was then divided by 14, an estimate based on Vernberg and Piyatiratitivorakul (1998) of the days between molting at 20° C. This derived the energetic cost of molting per 24 hours. Ovigerous shrimp were not included in the measurement of energy lost to ecdysis because shrimp carrying eggs do not molt.

Reproduction

The energy allocated to reproduction was determined through direct measurement of energy allocated to eggs. This was a departure from the standard method (Vernberg and Piyatiratitivorakul 1998) based on the total energy assimilated minus the energy allocated to somatic growth and ecdysis.

The daily energy allocation by ovigerous shrimp to egg production was calculated as the total energy content of individual egg masses divided by 28 days, given the 14-day reproductive period where shrimp are ovigerous, and assuming prior egg production for an additional 14-day intermolt period. A total of 33 ovigerous TK shrimp and 23 ovigerous PC shrimp were captured as above in 2000 and the eggs were removed. The individual egg masses were separated from each shrimp with a scalpel. The egg masses were freeze-dried, then weighed using a Sartorius microbalance. The energy content of egg masses was calculated from the weight of individual egg masses multiplied times an energy conversion factor described below.

CHN analysis

Egestion products were analyzed for CHN content to determine energy content. During CHN analysis all samples were kept in a drying oven until analyzed, to minimize water contamination. Each filter was analyzed for carbon, nitrogen, and hydrogen content using a Carlo/Erba NA-1500 Analyzer gas chromatography instrument. A standard curve was developed using six acetanilide standards ranging from 0.01 to 1mg. No more than 20 filter quarters were run at a single time. Two empty tin boats run as instrument blanks preceded each analysis run. After every six samples an acetanilide check standard was used to monitor accuracy. If the check standard exceeded a 1% deviation from expected results a second check standard was run to verify the deviation. If the nitrogen content continued to deviate by more than 1% from expected, the run was discontinued.

Energy content

Several techniques were used to derive the energetic value of physiological rates (Appendix 1). Consumption was converted to energy intake using micro-bomb calorimetry to measure the energy content of the food. The energy lost to egestion was derived from the CHN content of egestion products. The energetic cost of respiration was derived using indirect calorimetry. A conversion factor was used to convert oxygen consumption into its energetic cost. The excretion rate used a conversion factor for the energetic cost of ammonia excretion. Finally, ecdysis, growth, and reproduction used micro-bomb calorimetry to create energy conversion factors for production.

Oxygen consumption was converted to energy expenditure using a coefficient of 0.0185 J $(O_2 \mu l)^{-1}$. This value assumes that the respiratory quotient (RQ) of the grass shrimp held without food is 0.74 (Brody 1945 and Wolvekamp and Waterman 1960).

Energy lost to ammonia excretion was calculated as a function of the nitrogen content of the resulting filtrate multiplied by the energy conversion for ammonia (0.01697 J μg^{-1} NH_4^+) (Vernberg and Piyatiratitivorakul 1998).

Micro-bomb calorimetry

The energy contents of shrimp somatic tissues, eggs, exoskeletons and the ribbed mussel tissue used as food were measured using a Philipson micro-bomb calorimeter (Gentry Inc., USA). Shrimp and mussels were captured for energy content analysis during the

summer of 2005 and the energetic content of samples was used to convert masses found in previous experiments.

Samples were freeze-dried using a Labconco freeze dryer, weighed to determine relationship of dry to wet weight, then ground with ceramic mortar and pestles that had been baked in a muffle furnace at 500°C for four hours. Sub samples were then made into pellets for analysis. Pellet sizes of 50-100 mg were used when possible, though pellet sizes as low as 30 mg were used for some egg and exuvia samples. Pellet sizes were always equal to or larger than the range of 20-40 mg utilized by Vernberg and Piyatiratitivorakul (1998). The microbomb calorimeter was recalibrated after every ten samples or before each new set of measurements. Calibration and caloric values of samples were calculated using benzoic acid as the standard following the methods of Philipson (1964) and Prus (1975). Calories were converted to joules using the conversion factor of 4.184 joules per calorie.

Proximate biochemical composition

Fecal samples of individual shrimp were too small to be analyzed through standard bomb calorimetry. Rather than use bomb calorimetry on grouped samples, the proximate biochemical composition of samples was determined using CHN analysis. The stoichiometric coefficients of lipids, carbohydrates and proteins allowed their determination with CHN analysis using the techniques of Gnaiger and Bitterlich (1984) (Apendix II). The total energy content of the samples was then determined by summing the energy content of the protein, lipid, and carbohydrate fractions. The methods of

accounting for the energy content of chitin as developed by Riccardi (2000) and Vollenvider (2000) as a correction for the techniques of Gnaiger and Bitterlich (1984) were not used due to the low chitin content in fecal samples obtained from shrimp fed ribbed mussel tissue.

The hydrogen component was calculated, allowing for the residual water fraction of 0.06 total sample weight as determined by Gnaiger and Bitterlich (1984) to account for chemically bound water. Total protein was calculated from nitrogen content using the protein conversion factor of 5.8 as determined by Gnaiger and Bitterlich (1984) for aquatic invertebrates.

Energy budgets

Energy allocation was measured according to the equation developed for grass shrimp energy budgets by Vernberg and Piyatiratitivorakul (1998). Energy allocation is described as $C = R + Pg + Pr + E + Ex + F$ where C is consumption, R is respiration, Pg is the portion of total production allocated to somatic growth, Pr is the production allocated to reproduction, E is excretion, Ex is exuvia, and F is egestion. More properly the equation can be understood as energy assimilation (C-R-E-F) equaling total energy allocated to production (Pg+Pr+Ex).

Statistical analysis

All statistical analysis was performed using GraphPad Prism version 5.02 for Windows (GraphPad Software 2008). All error estimates represent 95% confidence levels for the

data. ANCOVA was used to compare slopes between all groups and the combined slope (weighted average) from each budget component was found. The average slope was then used to calculate the mean values for each treatment group for each component using an average shrimp size of 116.1 mg (Packard and Boardman 1987, 1999).

The total energy budget for each treatment group was derived from the mean rate of consumption minus the mean allocation for each component. The error bars are cumulative showing the 95% confidence intervals.

Results

Consumption

There was no difference in energy intake between ovigerous shrimp, but intermolt TK shrimp had a higher energy intake than all other groups (Figure 5.1 and Table 5.3) TK shrimp showed less variation in consumption than PC shrimp regardless of reproductive condition (Table 5.2) Ovigerous PC shrimp showed a greater range in consumption than ovigerous TK shrimp, with a few individuals consuming more than any TK shrimp. Five ovigerous PC shrimp consumed no measurable amounts during the experiment while all ovigerous TK shrimp consumed measurable amounts. Intermolt PC shrimp also showed a greater range of consumption than intermolt TK shrimp, with some individuals consuming more than any TK shrimp, while seven intermolt PC shrimp did not consume measurable amounts during the experiment. All intermolt TK shrimp consumed measurable amounts.

Egestion

There was no significant difference in egestion by PC and TK shrimp (Figure 5.2 and Table 5.3). There was no significant correlation between shrimp mass and the rate of energy lost to egestion in PC or TK shrimp. The range of egestion was higher in PC shrimp.

The protein content of egesta, as indicated by the nitrogen to carbon ratio, was higher in TK shrimp (0.262±0.007) than it was in PC shrimp (0.226±0.004). Conversely, the hydrogen to carbon ratio was higher in PC shrimp (0.215±0.013) than it was in TK shrimp (0.168±0.011) indicating higher lipid content. Nevertheless, while the stoichiometric differences were statistically significant, the total differences were small and unlikely to be of biological significance.

Respiration

Ovigerous shrimp expended less energy on respiration than did intermolt shrimp, with ovigerous PC shrimp losing the least amount to respiration of any group (Figure 5.3 and Table 5.3). Intermolt shrimp had significantly higher respiration rates with PC intermolt shrimp respiring more than any other group. PC shrimp showed a greater range in respiration than TK shrimp, with intermolt PC shrimp showing the greatest range of any group.

Excretion

Ovigerous PC shrimp lost less energy via excretion than any other treatment group (Figure 5.4 and Table 5.5). Intermolt and ovigerous PC shrimp showed the greatest range of energy lost to ammonia excretion, although ovigerous TK shrimp showed a range that was nearly as great. The lowest rates of ammonia excretion were found in some intermolt TK individuals while the highest rates were seen in PC individuals. Only TK intermolt shrimp showed a significant correlation between mass and energy lost to excretion (Table 5.2).

Growth

Ovigerous PC shrimp had significantly higher somatic energy content than ovigerous TK shrimp ($t=2.19$ $df=22$ $p=0.04$) (Table 5.1). Intermolt PC shrimp similarly had higher energy content than intermolt TK shrimp ($t=2.74$ $df=22$ $p=0.01$). Nevertheless, these differences were small and unlikely to be biologically significant.

There were no significant differences between intermolt PC and TK shrimp in energy expended on somatic growth (Figure 5.5 and Table 5.3). As expected, the two populations displayed a negative slope of shrimp mass to growth (Table 5.2). Nevertheless, shrimp from neither site showed a significant correlation between energy allocated to growth and shrimp mass. The ranges in growth rates were similar with two TK shrimp showing the highest growth rates.

Ecdysis

There was no significant difference in the energy content of shed exoskeletons on a per milligram basis ($p=0.24$ $t=1.22$ $df=17$) (Table 5.1). Nor was there a significant difference between intermolt shrimp in the total energy lost to ecdysis (Figure 5.6 and Table 5.3). Neither TK nor PC shrimp showed any correlation between energy allocated to exuvia and shrimp mass (Table 5.2). Intermolt PC shrimp had the greatest range of energy lost to ecdysis.

Reproduction

There was no difference in the energy content per milligram of PC or TK shrimp eggs ($p=0.20$ $t=1.318$ $df=22$) (Table 5.1). Ovigerous PC shrimp however, had higher weight egg masses and therefore allocated more total energy to reproduction than ovigerous TK shrimp. The two groups had generally similar ranges of energy allocated to reproduction except that two PC shrimp with low reproductive allocation increased the range of PC reproductive allocation.

Energy model

Energy intake was similar for ovigerous shrimp but there were significant differences in proportional allocation of resources as well as total amounts (Figures 7-8 and Table 5.3). Respiration was the greatest energetic cost for all groups, using more than three quarters of the total energetic intake. Energy lost to respiration actually exceeded energy intake for intermolt PC shrimp during the short duration of their measured uptake of oxygen while intermolt TK shrimp lost approximately 95% of energy intake to respiration.

Reproduction received the next highest allocation of energy by ovigerous shrimp with ovigerous PC shrimp allocating approximately half of energy intake to reproduction, and ovigerous TK shrimp allocating approximately a third of intake. Excretion received less than twenty percent of energy intake with ovigerous PC shrimp loosing energy to excretion at roughly half the rate of other groups. Less than ten percent of energy intake was allocated to egestion and somatic growth respectively.

The assimilation rates of ovigerous shrimp were similar with the energy intake balanced by lowered energy allocation to respiration by ovigerous PC shrimp. A similar situation was seen in intermolt shrimp where despite the greater consumption by intermolt TK shrimp and greater respiration by intermolt PC shrimp, differences in assimilation were obscured by the variance of the samples.

All budgets had error bars that overlapped with zero indicating that intake and allocation were roughly balanced.

Discussion

Ovigerous PC shrimp had a higher reproductive allocation than ovigerous TK shrimp despite having similar energy intake. They were able to accomplish this through the compensatory lowering of energy allocated to respiration and excretion. This same compensatory partitioning of resources was not seen in intermolt PC shrimp even though this group had a lower energy intake than intermolt TK shrimp.

PC shrimp had a much wider range of response in consumption, respiration, and excretion. This is a predicted response to contaminant exposure due to individual variability in response (Forbes and Depledge 1996). This variance can be caused by differences in the distribution of contaminants in the field, and by differences in tolerance within a population. The lowered respiration rate in PC ovigerous shrimp is also consistent with the majority of dosing studies that show a lowering of oxygen use in response to contamination (Hutcheson et al. 1985, Kobayashi et al. 1990, 1991, St. Amand et al. 1999, and Anderson et al. 2000). The lower rate of consumption by intermolt PC shrimp in comparison to intermolt TK shrimp is also an expected result of exposure to various toxicants (Maltby 1998). Previous studies have only measured changes in consumption by intermolt shrimp in response to contaminants, and this study found similar results for this group. Several individual PC shrimp with and without eggs ate nothing at all, representing a serious effect of pollutants on a more acutely sensitive portion of the population through a combination of increased variability and overall lowered feeding.

Most studies have avoided working with the effects on shrimp of contaminants encountered under field conditions precisely because of the increased variance. This study was able to find significant treatment effects partially by using a well-defined life history stage and thus reducing variance. The life history stage of ovigerous shrimp is better defined than the more general category of intermolt shrimp, which would include males and females, as well as females in varying states of vitellogenisis. Thus the

ovigerous shrimp can be expected to have a closer relationship between mass and any measured physiological parameter.

The methods in this study were modified from those of Vernberg et al (1999). Most of the modifications such as freeze drying samples rather than using a drying oven should not have lead to significantly different results, but in a few cases the results of this study may depend on differences in the techniques. The shrimp were fed mussel tissue rather than *Artemia* naupli and measurements were taken only 24 hours after capture rather than after a long period of laboratory acclimatization as with Vernberg and Piyatiratitivorakul. More importantly, shrimp in this study were starved twice as long prior to the measurement of respiration, and thus a lower RQ was used to calculate the energy lost following the methods of Wolvekamp and Waterman (1960). Vernberg and Piyatiratitivorakul also allowed shrimp to acclimate for an hour in the flasks prior to respiration measurements, while in this study there was no period of acclimatization.

In the study by Vernberg and Piyatiratitivorakul, 30.6 ± 0.02 mg shrimp respired 53.38 ± 23.63 µl O_2 shrimp^{-1} h^{-1} while in this study 116.1 ± 5.2 mg shrimp respired 203.6 ± 15.9 µl O_2 shrimp^{-1} h^{-1}. If the shrimp in this study had been the same size as those in Vernberg and Piyatiratitivorakul, then they would have respired 82.20 ± 8.59 63 µl O_2 shrimp^{-1} h^{-1} as calculated from the slope of the regression for respiration. The higher respiration in this study may have been due to the lack of a period of acclimatization. Similarly to the budget devised by Vernberg and Piyatiratitivorakul the respiration portion of the budget is at a much different time scale than other portions of the energy

budget such as somatic growth. Respiration was measured over one hour while growth was measured over 28 days, and thus was measured over a 672 times longer period than respiration. The difference in time scale may represent a large variable in the energy budget if shrimp respiration is not uniform over the longer time period.

In general, an energy budget includes several assumptions and estimates upon which the final results are based. The time scale discrepancy between growth and respiration is only the most extreme. All portions of the energy budget were measured for these shrimp, but it was not possible to measure every component simultaneously in a single experiment nor was it possible to measure every component of every treatment group. For example, somatic growth and ecdysis could only be measured in intermolt shrimp.

Ecdysis was only measured for intermolt shrimp, and the energy losses to sheds by ovigerous shrimp may have differed. If so, there still would have been little difference in the overall energy budget due to the relatively small proportion of energy intake allocated to ecdysis.

Ovigerous shrimp have the same period between molting as intermolt shrimp (Anderson 1985), and the energy content of the exoskeleton is not likely to differ between the two groups, so the estimate of energy allocated to ecdysis for ovigerous shrimp should be a good approximation of actual allocation. On the other hand, the estimate of energy allocated to somatic growth should be considered an upper limit for ovigerous shrimp

because intermolt shrimp will maximize fitness through maximizing potential for growth while ovigerous shrimp will maximize fitness by maximizing reproductive output.

Shrimp respiration was measured under resting conditions, without the increased energetic costs of food handling and capture or predator avoidance and thus may represent a lower limit of respiration. On the other hand, without a longer acclimatization period before measuring respiration, shrimp activity levels may have included escape reactions and other active states more similar to what would be found under field conditions.

All shrimp were held without food for 24 hours, so the respiration rate is a function of metabolic requirements without any component representing the respiratory requirements of food digestion. Thus, the respiration rate measured over a single hour could represent either a lower or higher measure of what shrimp would require while engaging in necessary survival activities over a longer time scale.

Respiration by intermolt shrimp roughly equaled or exceeded consumption, indicating that either respiration was higher or consumption was lower than what must occur in the field for intermolt shrimp to survive. This is similar to the results found in other studies where feeding was reduced by intermolt shrimp in response to contaminants without a drop in respiration (Donkin and Widdows 1996, Maltby 1992). The lowered feeding combined with unchanged respiration has led researchers to create population models showing how a population can be eliminated by the resulting loss of “scope for growth”

even without a lowered reproductive rate (Klok and DeRoos 1996). Clearly this has not happened to PC shrimp. In fact, by measuring the physiological rates of ovigerous shrimp as well as the standard intermolt shrimp, this study has shown that reproductive allocation is actually increased in response to living in a contaminated habitat, at least in this case.

Finally, the energy intake shown in this study represents a short-term measure of consumption by shrimp that had been fasting for 24 hours. When presented with constant food over a longer time period, shrimp are likely to consume at a slightly lower rate. Thus, the shrimp growth rate during the 28 days of the growth experiment is likely to be related to a lower rate of consumption than that found in the 24 hour feeding experiment.

Taken together, these estimates indicate that the results of the energy budget are likely to be contingent upon the experimental design. Nevertheless, the relative differences in allocation between the two treatment groups arose under conditions constant within each experiment, and thus represent a good estimate of the relative impact of anthropogenic contaminants on the components of the energy budgets of grass shrimp.

Differences in methodology and experimental design make comparisons with other studies difficult, but it is possible to look at some similarities and differences that this study has with previous work.

The somatic energy content of PC and TK shrimp was quite similar to the 17.06 ± 1.62 joules mg^{-1} found by Vernberg and Piyatiratitivorakul (1998) (Table 5.1). The mean shrimp size was quite different than in this study as discussed previously indicating that energy content is relatively uniform for grass shrimp regardless of size.

A lowered respiration rate in PC shrimp is expected from previous dosing studies, which generally show reduced respiration in shrimp exposed to contaminants. There is no clear reason, however, why the decreased respiration is confined to ovigerous shrimp. Perhaps there is an increased benefit to reduced activity and hence reduced respiration in ovigerous shrimp, although fanning with the pleopods is a necessary activity for providing oxygenated water to the eggs. Thus ovigerous shrimp would be expected to expend more energy through respiration than intermolt shrimp in all situations. Nevertheless, the reduced energetic costs in ovigerous PC shrimp allow for increased reproductive output. Reduced respiration in ovigerous PC shrimp can be seen as part of a general response to stressors, in which the shrimp use compensatory partitioning of energy allocation to allow the maintenance of investment in production. This could also explain the reduced excretion seen in ovigerous PC shrimp, though once again, this reduction is seen without a parallel reduction of excretion in intermolt PC shrimp.

Energy lost through fecal production in this study appears to be slightly lower than that found by Vernberg et al (1998), though comparison is complicated by Vernberg and Piyatiratitivorakul reporting energy loss at maximal rates predicted by temperature. The differences may also be a function of the different methods used to measure the energy

content. By using the technique of Bitterlich and Gnaiger (1984), this study did not measure the direct combustion of the material, but several proxy measures designed to sum to a total energy measure. If the residual water estimate developed by Bitterlich and Gnaiger is low, there is a dramatic reduction in the measured lipid content, and the total energy content would thus be substantially lower.

Despite similar energy intake for ovigerous PC and TK shrimp, and lower energy intake by intermolt PC shrimp, PC shrimp were able to match the somatic growth of TK shrimp while increasing their reproductive allocation. (Figures 7-10). Ovigerous PC shrimp allocated a proportionally greater amount of energy intake to reproduction while intermolt PC and TK shrimp allocated similar amounts of energy intake to growth despite higher respiratory costs by intermolt PC shrimp.

The measured reproductive allocation relies on an estimate of twenty-eight days between productions of egg masses. If eggs can be produced in back-to-back molting cycles of 14 days, then the reproductive energy allocation would be doubled, however, no indication of such continuous reproductive output was seen under laboratory conditions in this study. Nor do field observations support the possibility of continuous egg production. There are always non-ovigerous females present, indicating some time between egg cycles.

Another source of possible error could be that the estimate is based on insufficient accounting of all energy outputs. For example, no measurement of energy losses to

dissolved organic matter was made; nevertheless, such losses should be minimal in large crustaceans such as grass shrimp. Furthermore, any dissolved proteinaceous compounds would have already been included in the energy allocation to excretion.

If one examines the mean values shown in the energy budget figures it appears that PC and TK shrimp are running energy deficits. The error bars indicate however, that zero values are within the 95% confidence limits and thus there is a good probability that the budgets balance input and output as one would expect.

Nevertheless, in Vernberg and Piyatiratitivorakul (1998) intermolt shrimp at less than 17°C showed negative reproductive allocation, so such results can be expected under certain experimental conditions. At the uniform temperature conditions of 20°C used in this study, a positive reproductive allocation would be expected. Vernberg demonstrates that any slight shift of allocation toward somatic growth and away from reproductive allocation can result in negative estimates of reproduction. One could assume that the converse could also be the case. A shift toward reproduction could lead to a negative estimate of somatic growth.

What is quite clear from this study is that PC shrimp do indeed show negative effects from living in highly polluted Piles Creek. The depressed respiration and excretion in ovigerous PC shrimp and the reduced feeding in intermolt PC shrimp are expected results of living in such an environment. Nonetheless, through compensatory partitioning of energy resources, PC shrimp are able to achieve parity with TK shrimp in energy

allocated to growth and exceed TK shrimp in allocation to reproduction. This explains the higher production by PC shrimp found by Santiago Bass et al (2001), while hinting at the underlying mechanism.

The best evidence for compensatory partitioning is seen in the lower rates of respiration found in the ovigerous shrimp from both field sites. This appears to work in conjunction with a lowering of respiratory activity due to exposure to contaminants. Respiration is generally depressed after exposure to contaminants, as has been found in other studies of shrimp toxicant dosing (Anderson et al. 1974, Hutcheson et al. 1985, and St-Amand et al. 1999), but this appears to work in combination with lowered respiration found in ovigerous shrimp from both sites. Thus, ovigerous shrimp exposed to contaminants have greatly reduced energy allocation to respiration, when compared to intermolt shrimp not exposed to contaminants. This allows ovigerous PC shrimp to have increased reproductive allocation to that of ovigerous TK shrimp despite similar rates of consumption.

There must be a cost to a lowered rate of oxidative metabolism, because it requires lowered activity rates. PC shrimp are shielded from the effects of intraspecific competition, as all shrimp entering the creek would encounter similar toxicants, causing a similar reduction in feeding and oxidative metabolism. PC shrimp are also shielded from the effects of predation, which would tend to select against organisms with less ability to avoid predators due to lower oxidative metabolism. As Weis et al. (2001) have shown, *Fundulus heteroclitus*, a major vertebrate predator of grass shrimp, has severely

compromised hunting ability due to the presence of mercury and other heavy metals at Piles Creek. Thus, PC shrimp are able to tolerate and even thrive in the heavily polluted Piles Creek, due in part to compensatory partitioning, which allows reproductive expenditures to surpass those found in less severely impacted habitats. The shrimp are also shielded from the negative effects of such a trade off in energy allocation due to the trophic cascade effect. The negative effect of predation has largely been removed by the severe central nervous system impairment of their major vertebrate predator.

Compensatory partitioning of energy resources should be seen as one of the mechanism for tolerance to anthropogenic impacts, but it should not be seen as evidence that grass shrimp are so tolerant of contaminants that they are immune to contaminants' effects. Large differences can be seen between the energy budgets of shrimp from the two sites. This leads to changes in the function of these shrimp within an ecosystem. The reduced feeding by intermolt PC shrimp may reduce their role as detrital processors; while at the same time allocation to growth and reproduction produces large amounts of shrimp biomass. This increased animal biomass is in turn not available to vertebrate predators due to the effects of contaminants on their central nervous systems. Thus what appears to be a healthy shrimp population is not able to carry out one of its main ecological roles as a primary link between organic detritus and commercially valuable vertebrate biomass.

Energy content	PC ovigerous	PC intermolt	TK ovigerous	TK intermolt
(dry shrimp Joules mg $^{-1}$)	16.05±0.46 n=12	16.98±1.12 n=12	15.42±0.43 n=12	15.53±0.33 n=12
(dry eggs Joules mg $^{-1}$)	21.81±0.51 n=12		21.26±0.48 n=12	
(dry exuvia Joules mg $^{-1}$)		0.11 ± 0.01 n=9		0.09 ± 0.01 n=10

dry ribbed mussel tissue (Joules mg $^{-1}$)	15.10±0.28 (n=12)

Table 5.1. Energy conversions used for converting sample masses to energy content. All values were obtained using micro-bomb calorimetry. The energy contents are by dry weight (mg) with 95% CI.

Consumption	N	r^2	Slope ± 95% CL
PC ovigerous	34	0.22	0.43 ± 0.29
TK ovigerous	28	0.44	0.38 ± 0.17
PC intermolt	33	0.26	0.81± 0.51
TK intermolt	25	0.52	0.54± 0.22
Respiration			
PC ovigerous	23	0.64	74 ± 0.25
TK ovigerous	26	0.18	0.35 ± 0.32
PC intermolt	23	0.19	0.54 ± 0.49
TK intermolt	27	0.09	0.25 ± 0.33
Excretion			
PC ovigerous	14	0.16	0.08 ± 0.12
TK ovigerous	14	0.27	0.11 ± 0.12
PC intermolt	12	0.05	0.05 ± 0.15
TK intermolt	12	0.52	0.07 ± 0.05
Egestion			
PC shrimp	15	0.004	0.002 ± 0.025
TK shrimp	28	0.024	0.003 ± 0.014
Ecdysis			
PC	9	0.08	0.002 ± 0.001
TK	10	0.001	0.001± 0.014
Growth			
PC	17	0.21	-0.06± 0.07
TK	16	0.15	-0.12 ± 0.16
Reproduction			
PC ovigerous	12	0.004	0.02 ± 0.23
TK ovigerous	12	0.16	0.08 ± 0.13

Table 5.2. Slopes for regression lines of the energy budget components showing 95% confidence intervals and correlation coefficients.

	PC ovigerous	TK ovigerous	PC intermolt	TK intermolt
Consumption	76.92±17.15	93.20±8.20	82.21±16.66	104.20±9.10
Respiration	61.18±12.12	75.46±11.18	119.1±20.90	98.54±10.96
Reproduction	40.52±5.93	30.06±2.8	n/a	n/a
Excretion	7.14±3.04	16.85±3.81	14.68±3.42	16.43±1.57
Growth*	n/a	n/a	5.20±1.19	5.49±2.06
Egestion**	3.29±1.34	2.23±0.36	3.29±1.34	2.23±0.36
Ecdysis***	n/a	n/a	0.25±0.06	0.19±0.05

Table 5.3. Components of the energy budgets for ovigerous and intermolt shrimp from Piles Creek and Tuckerton. Resource allocation (Joules (24h)-1) for a standard-sized shrimp of 116.1 mg dry weight. Data are means±95% confidence intervals. Growth* was based on intermolt shrimp only. Egestion** represents pooled values from combined intermolt and ovigerous shrimp data. Ecdysis*** was based on intermolt shrimp only.

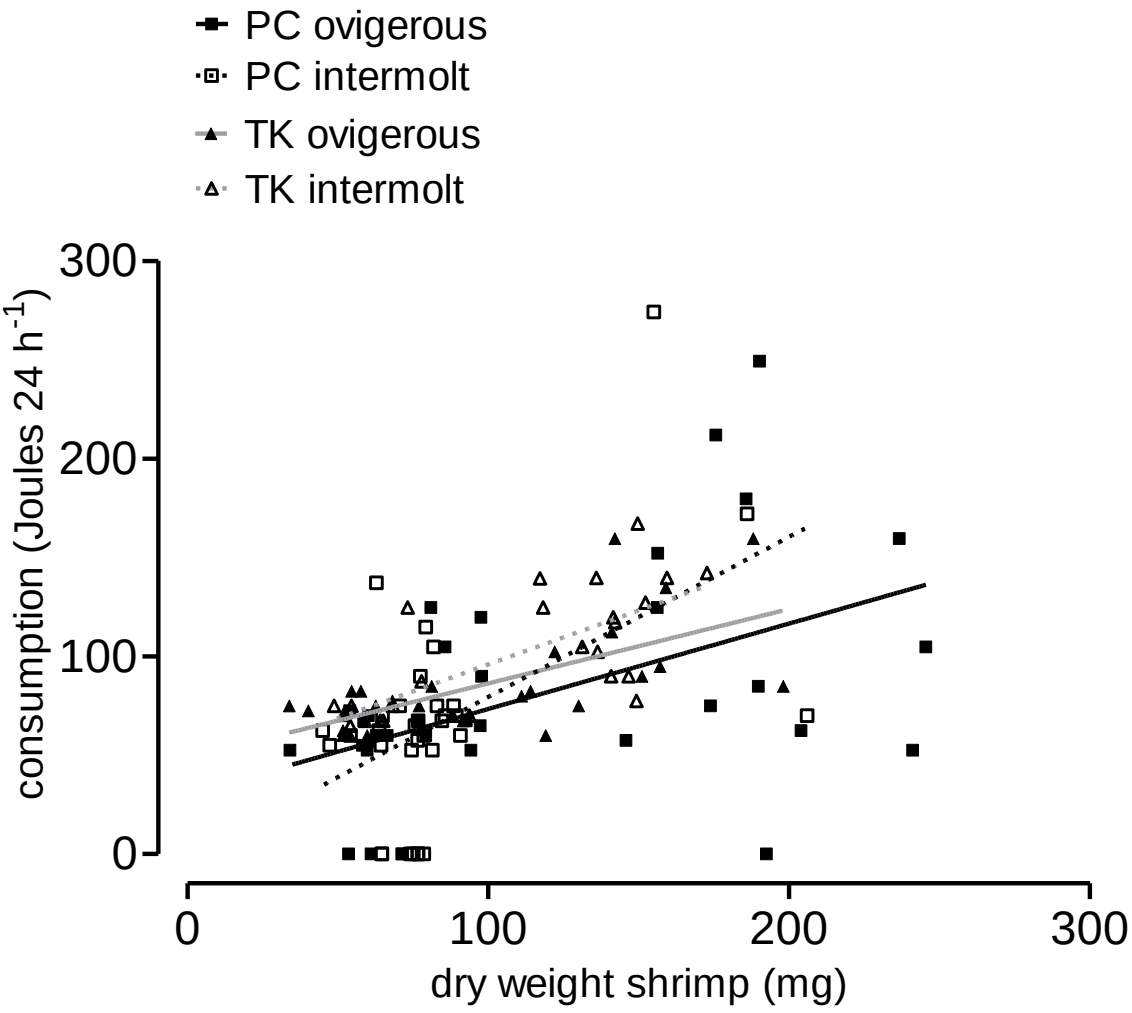


Figure 5.1. Consumption by ovigerous and intermolt Piles Creek and Tuckerton shrimp. All regression lines are statistically parallel with a pooled slope of 0.489 (P=0.36).

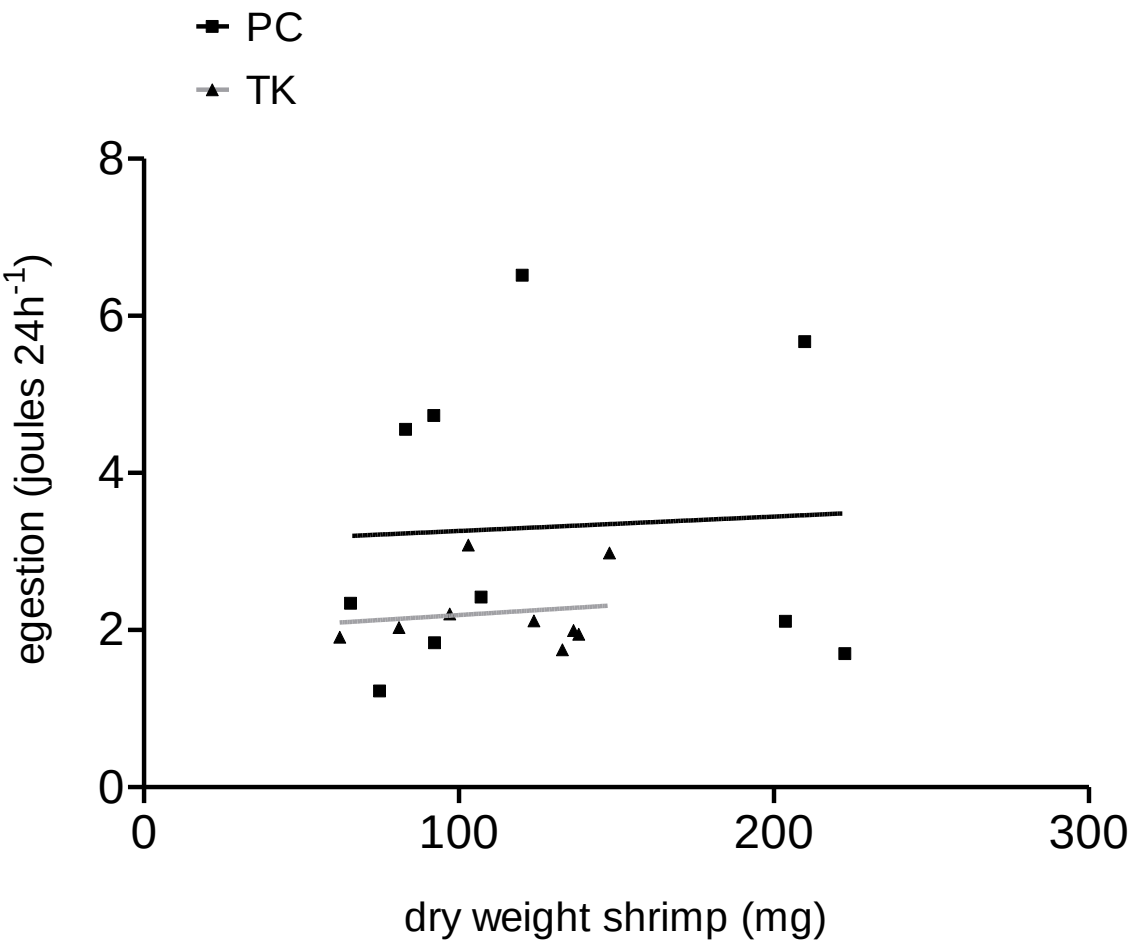


Figure 5.2. Egestion by Piles Creek and Tuckerton shrimp. Intermolt and ovigerous shrimp are grouped together for statistical purposes. The slopes do not differ significantly and have a pooled slope of 0.002 (P=0.97).

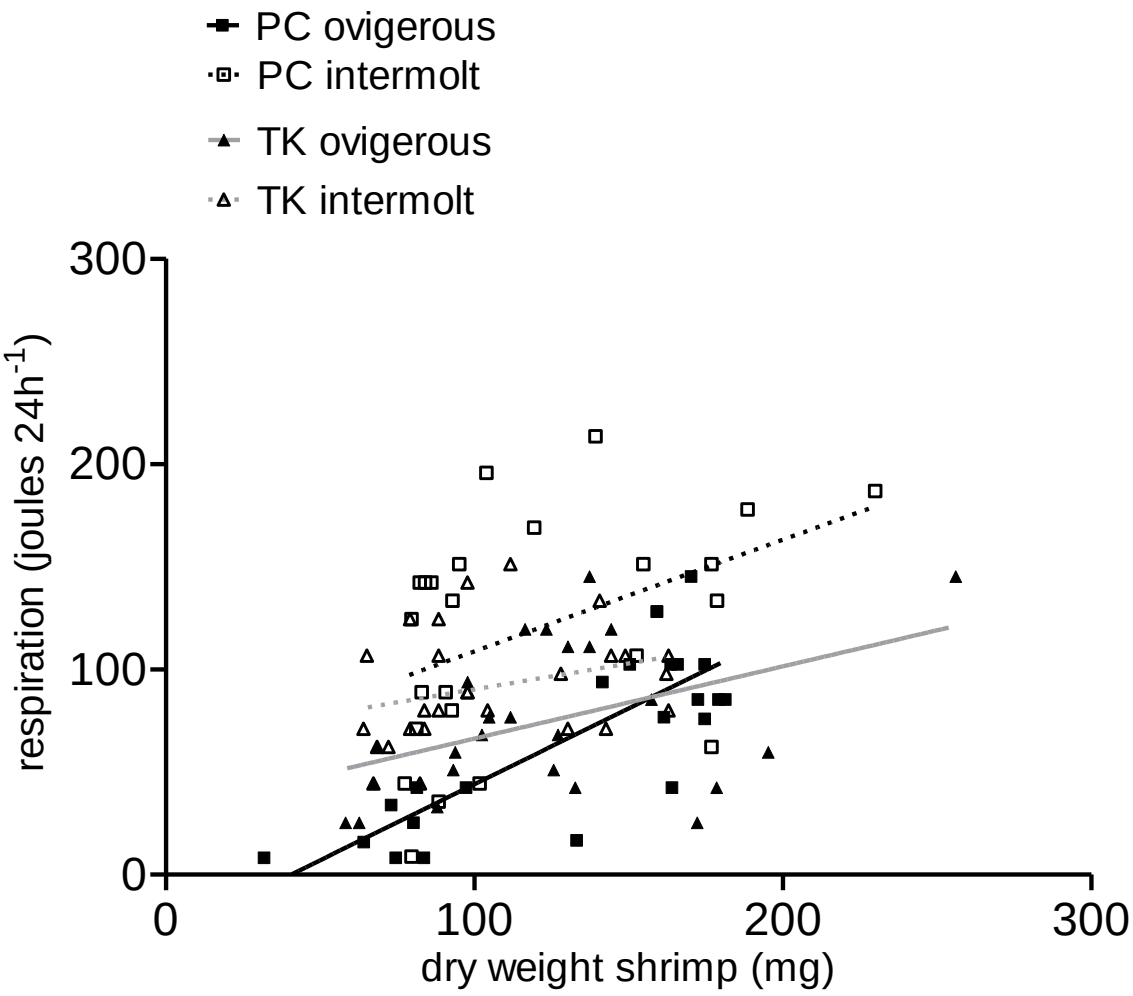


Figure 5.3. Respiration by ovigerous and intermolt Piles Creek and Tuckerton shrimp. All regression lines are statistically parallel with a pooled slope of 0.495 (P=0.23).

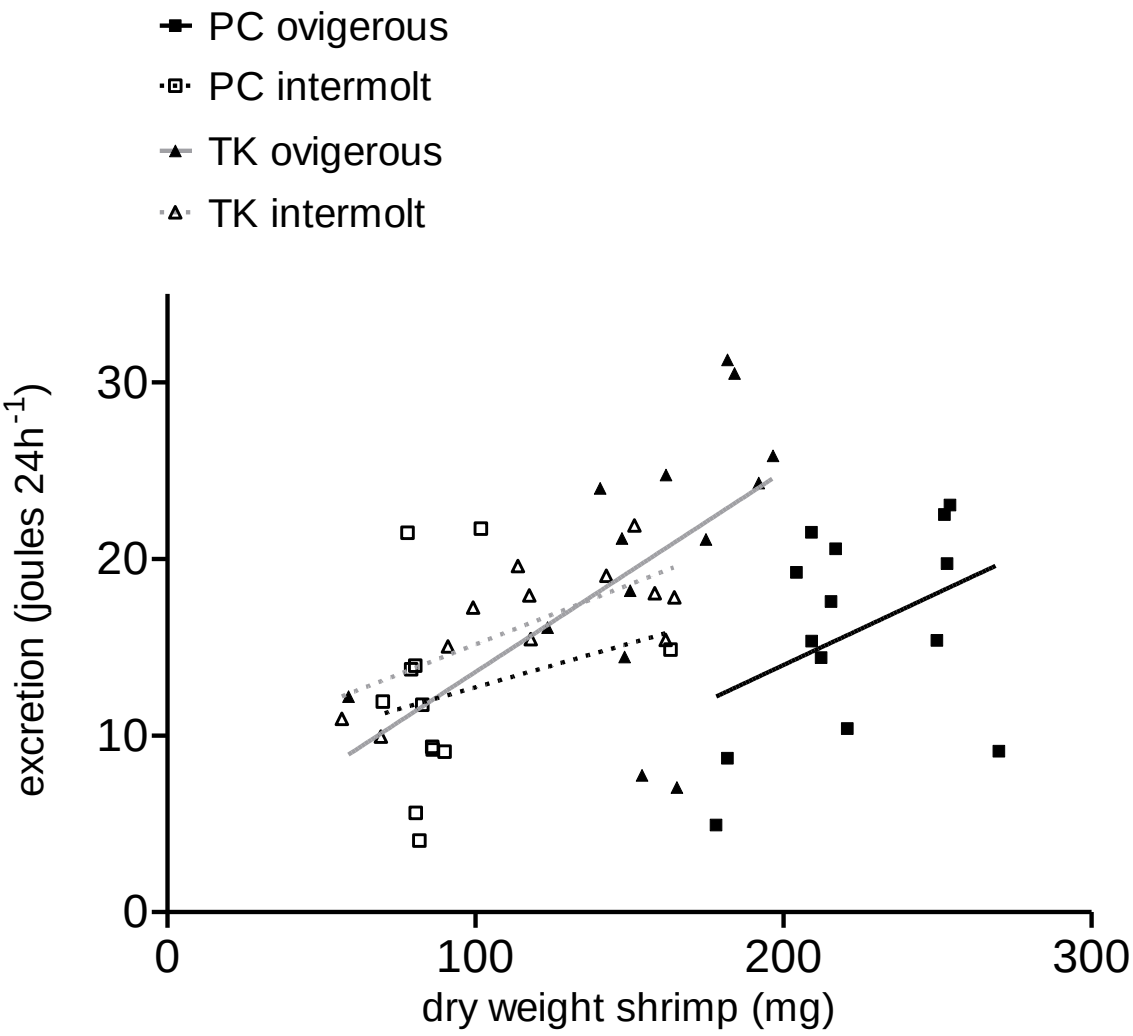


Figure 5.4. Excretion by ovigerous and intermolt Piles Creek and Tuckerton shrimp. All regression lines are statistically parallel with a pooled slope of 0.084 (P=0.84).

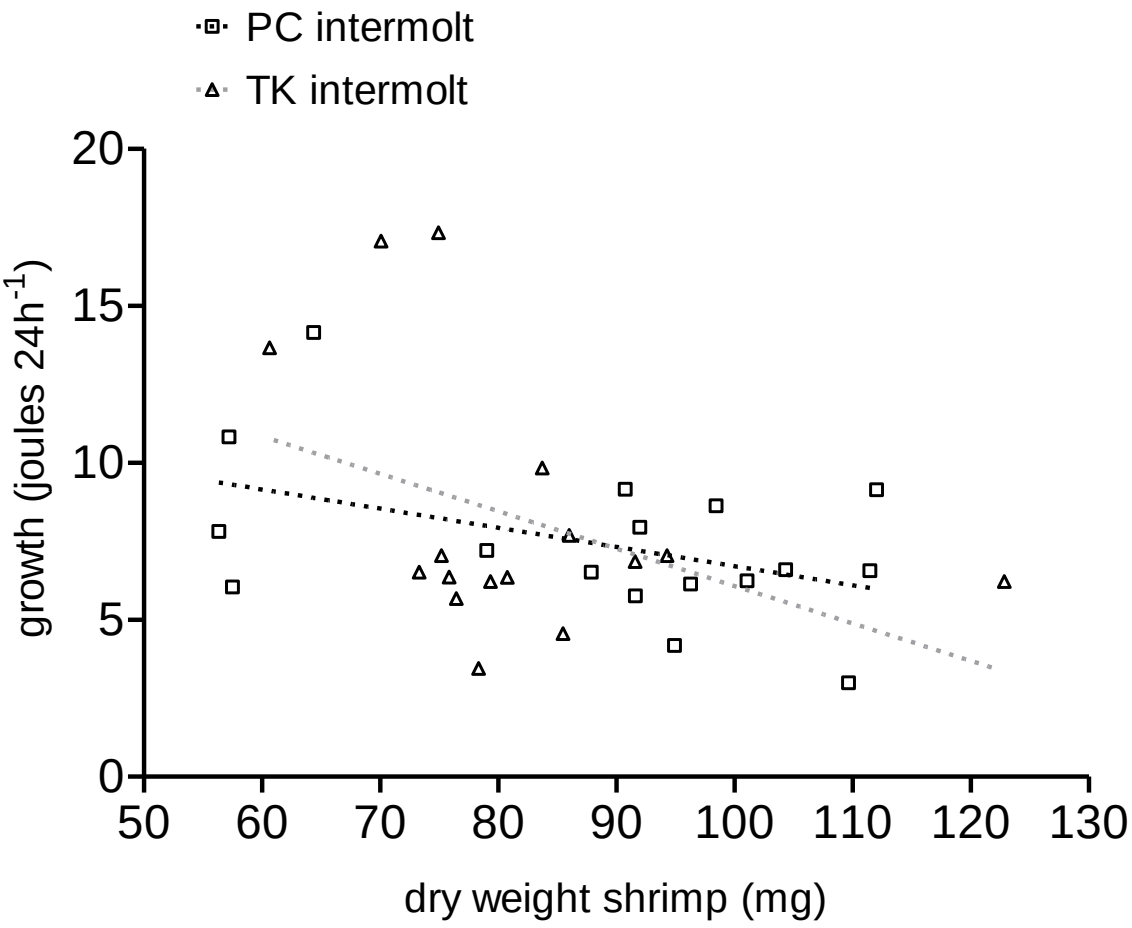


Figure 5.5. Growth by intermolt Piles Creek and Tuckerton shrimp. All regression lines are statistically parallel with a pooled slope of -0.080 (P=0.44).

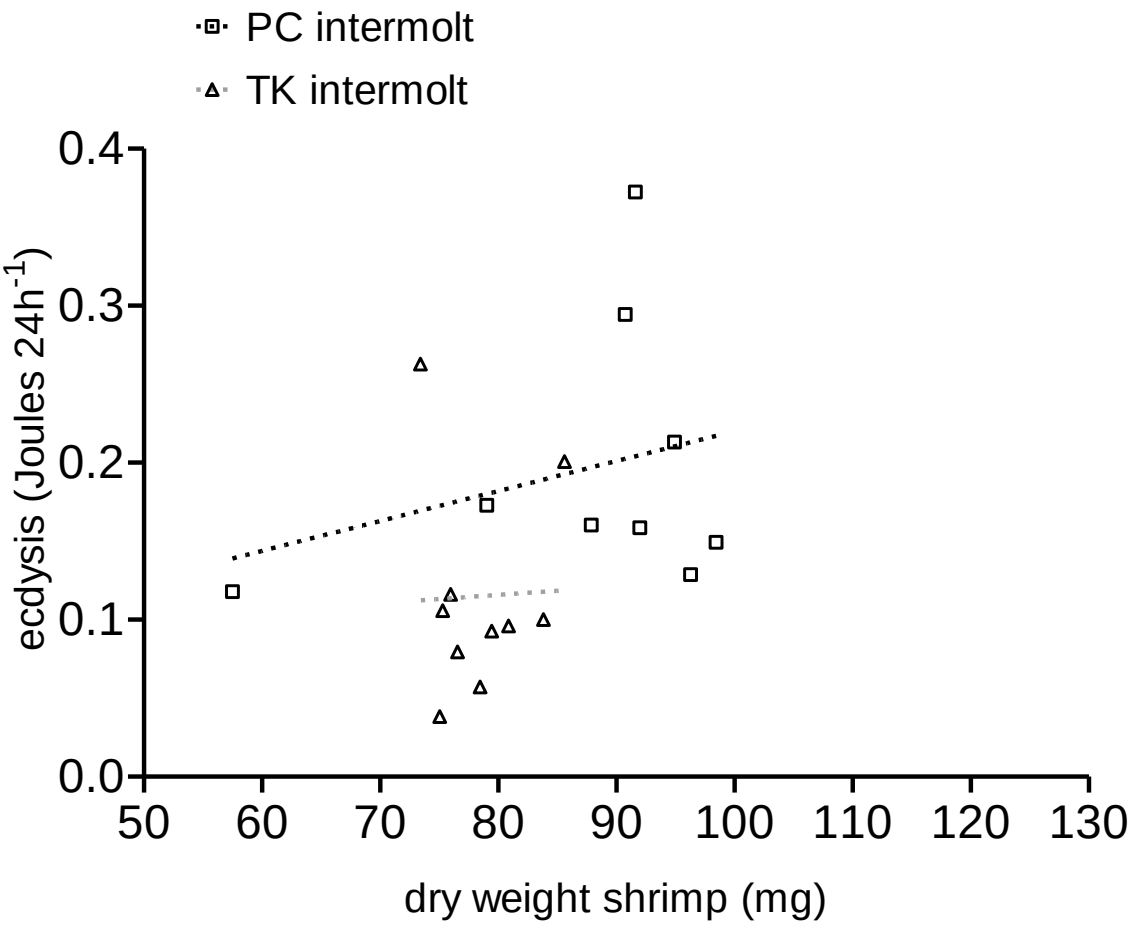


Figure 5.5. Ecdysis by intermolt Piles Creek and Tuckerton shrimp. All regression lines are statistically parallel with a pooled slope of 0.002 (P=0.84).

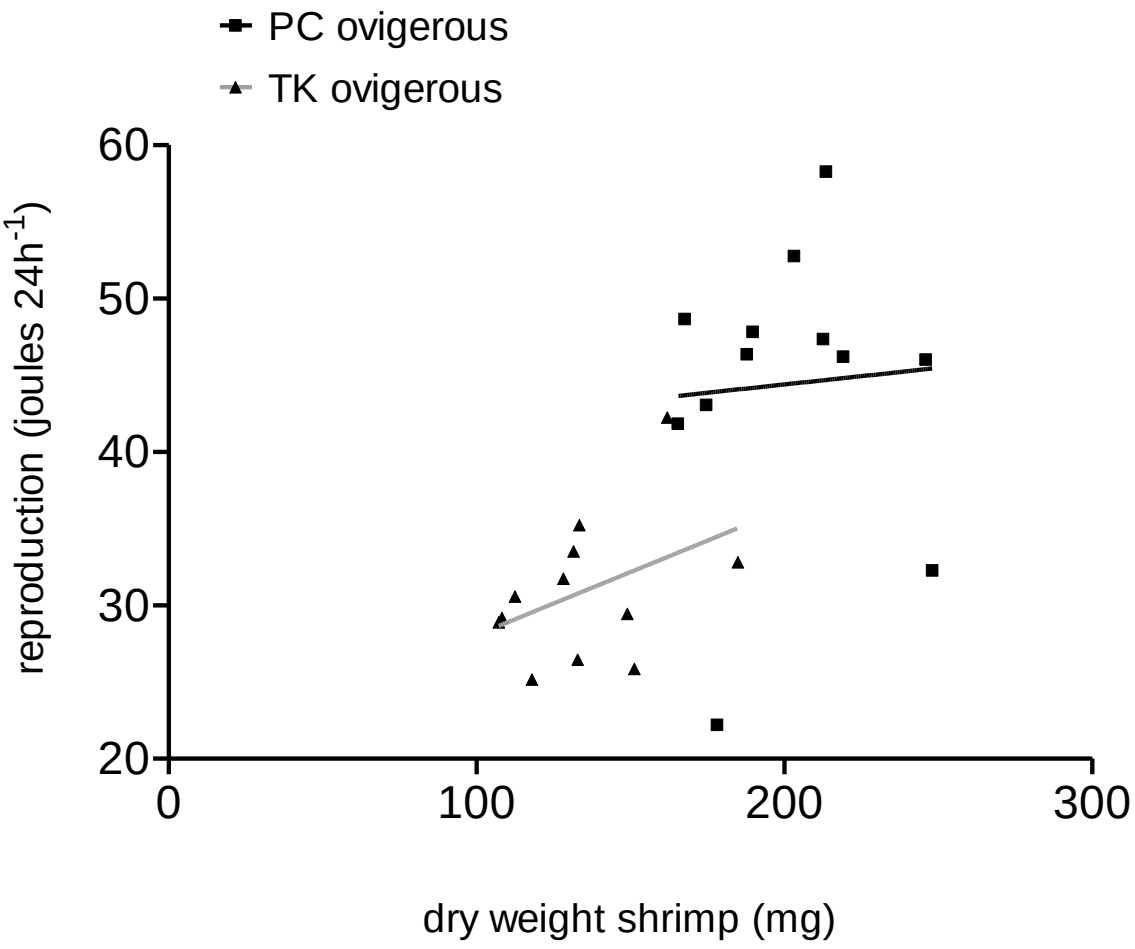


Figure 5.6. Reproduction by ovigerous Piles Creek and Tuckerton shrimp. All regression lines are statistically parallel with a pooled slope of 0.046 (P=0.64).

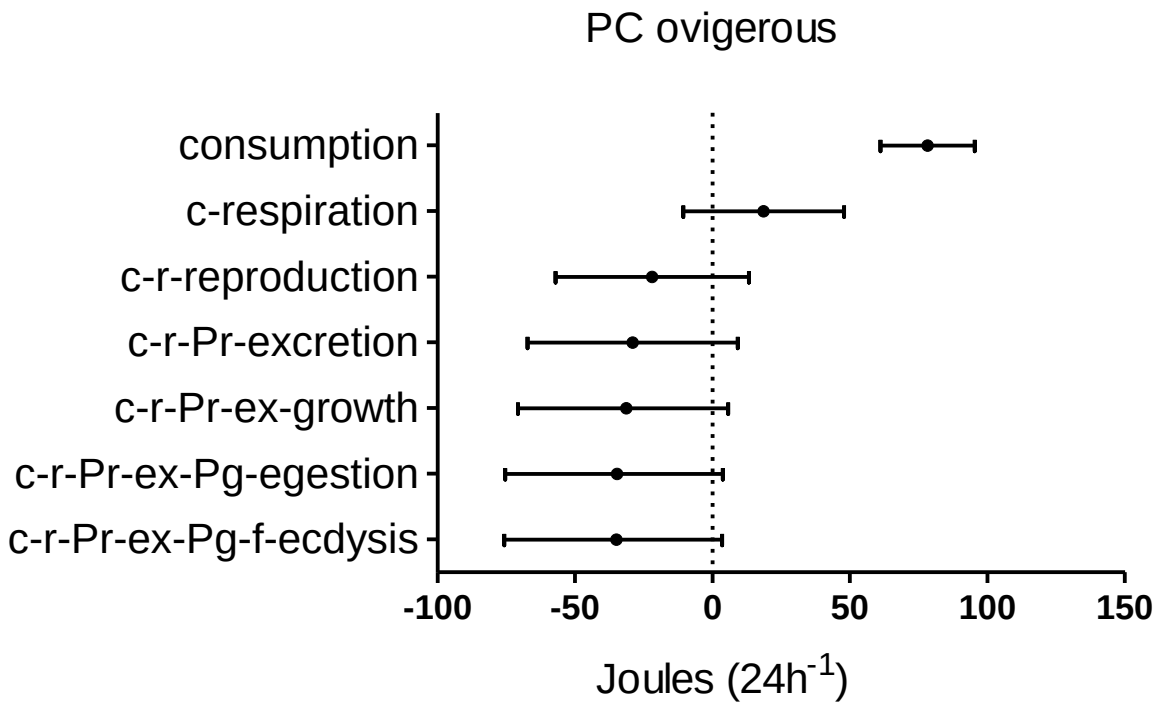
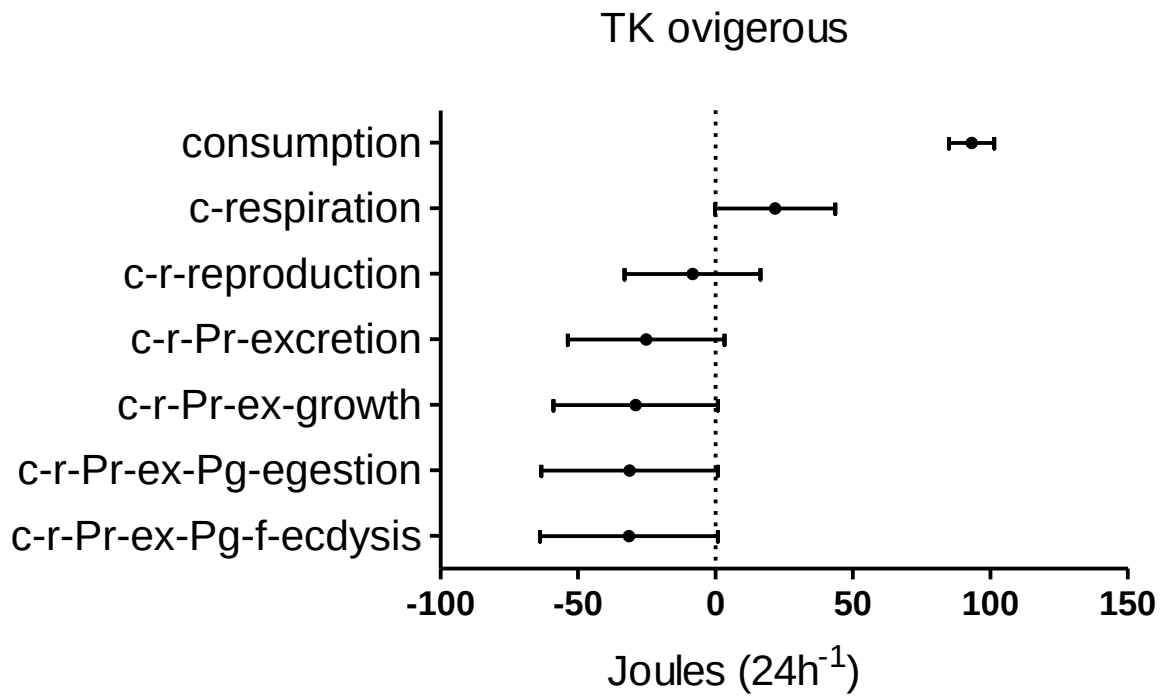
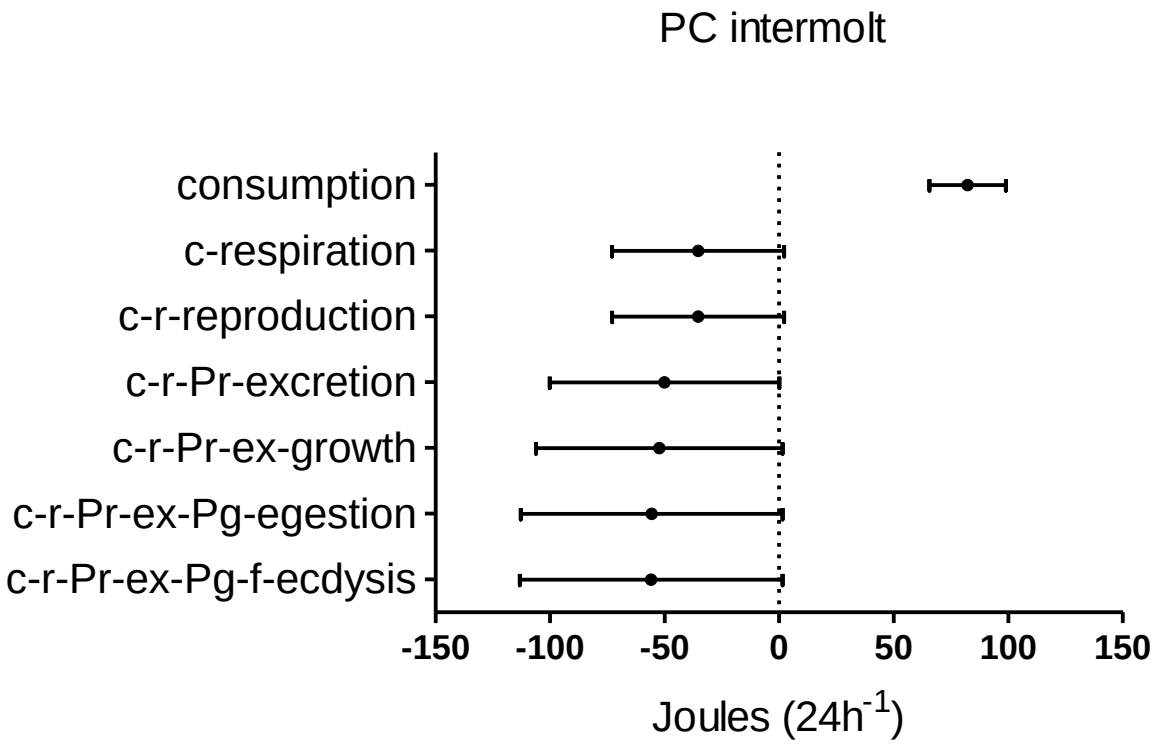


Figure 5.7. Energy budget for ovigerous Piles Creek shrimp. Means were calculated using an average shrimp size of 116 mg. The error bars show 95% confidence intervals around the mean values. Physiological costs are subtracted line by line in a cumulative fashion from consumption. The ranges of the error bars are also cumulative. Error bars were calculated with the high end of the range representing maximum consumption with minimum costs, and the low end representing minimum consumption with maximum costs. (c=consumption, r=respiration, Pr=reproduction, ex=excretion, Pg=growth, and f=egestion)



Figures 5.8. Energy budget for ovigerous Tuckerton shrimp. Means were calculated using an average shrimp size of 116 mg. The error bars show 95% confidence intervals around the mean values. Physiological costs are subtracted line by line in a cumulative fashion from consumption. The ranges of the error bars are also cumulative. Error bars were calculated with the high end of the range representing maximum consumption with minimum costs, and the low end representing minimum consumption with maximum costs. (c=consumption, r=respiration, Pr=reproduction, ex=excretion, Pg=growth, and f=egestion)



Figures 5.9. Energy budget for intermolt Piles Creek shrimp. Means were calculated using an average shrimp size of 116 mg. The error bars show 95% confidence intervals around the mean values. Physiological costs are subtracted line by line in a cumulative fashion from consumption. The ranges of the error bars are also cumulative. Error bars were calculated with the high end of the range representing maximum consumption with minimum costs, and the low end representing minimum consumption with maximum costs. (c=consumption, r=respiration, Pr=reproduction, ex=excretion, Pg=growth, and f=egestion)

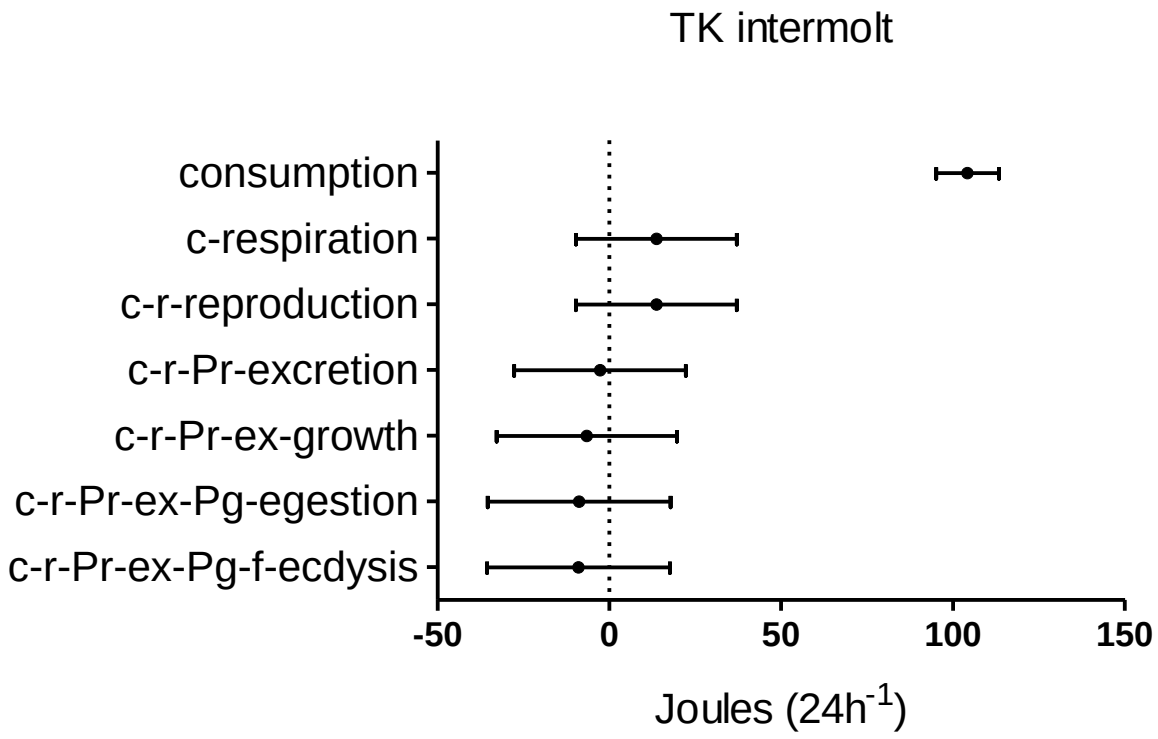


Figure 5.10. Energy budget for intermoltTuckerton shrimp. Means were calculated using an average shrimp size of 116 mg. The error bars show 95% confidence intervals around the mean values. Physiological costs are subtracted line by line in a cumulative fashion from consumption. The ranges of the error bars are also cumulative. Error bars were calculated with the high end of the range representing maximum consumption with minimum costs, and the low end representing minimum consumption with maximum costs. (c=consumption, r=respiration, Pr=reproduction, ex=excretion, Pg=growth, and f=egestion)

Chapter 6

Conclusions

This study showed compensatory partitioning of physiological resource budgets by *P. pugio* in association with anthropogenic contaminants. Intermolt shrimp from Piles Creek, a site with severe anthropogenic impacts, had a lower rate of consumption than intermolt shrimp from a marsh creek near Tuckerton, yet were able to have a similar rate of growth. Ovigerous PC shrimp had similar consumption to that of intermolt TK shrimp yet were able to allocate more to reproduction through the transfer of resources away from other portions of the budget such as respiration and ammonia excretion. Compensatory partitioning is seen most clearly in the ovigerous PC shrimp. Ovigerous PC shrimp had the lowest rates of respiration and excretion of any group, while maintaining their allocation to eggs at rates surpassing ovigerous TK shrimp.

Reduced metabolic rates are predicted results for organisms in contact with contaminants. Thus, production would be expected to be lower in shrimp from a contaminated habitat than in shrimp from an uncontaminated habitat. Nonetheless, Santiago Bass et al. (2001) found PC shrimp to be larger than TK shrimp with a higher reproductive output. The larger size and reproductive rate in PC shrimp is an apparent contradiction of previous studies. The authors found no increase in growth rates among PC shrimp and explained the increased size and reproductive output as a result of predation by *Fundulus heteroclitus*. They did not, however, show how PC shrimp were able to have rates of production and reproduction equal to or higher than TK shrimp. This study supports the finding of Santiago Bass et al. (2001), while revealing that it is compensatory partitioning

that allows grass shrimp to tolerate the conditions found at Piles Creek. PC shrimp are able to maximize somatic growth and reproduction, with total allocation of resources equal or exceeding that of TK shrimp.

The reduced respiration by ovigerous PC shrimp allowed them to compensate for their increased egestion without an increase in consumption. While ovigerous PC shrimp had the lowest rate of respiration of any group, ovigerous TK shrimp also showed reduced respiration in comparison to intermolt shrimp from the two sites. It is not clear why ovigerous shrimp had the lowest respiration, nor was it an expected result. Ovigerous shrimp would be expected to have a higher resting respiratory rate because they must constantly fan their pleopods to oxygenate their eggs. Nonetheless, the reduced respiratory expenditure by ovigerous shrimp allowed them to allocate more resources to other components of their physiological resource budgets.

Intermolt shrimp displayed increased respiration, and evidenced resource losses to respiration similar to resources gained through consumption. Intermolt PC shrimp were not able to compensate for their reduced consumption yet still had a similar allocation to growth as intermolt TK shrimp.

The reduced consumption and increased respiration by PC intermolt shrimp indicates lower reproductive potential in these shrimp compared to intermolt TK shrimp. This is similar to the results found in other studies, in which ingestion was reduced by intermolt shrimp in response to contaminants, but without a reduction in respiration (Donkin and

Widdows 1996, Maltby 1992). The lowered rate of consumption combined with unchanged respiration found in previous experiments has lead researchers to create models showing how a population can be eliminated by the resulting loss of "scope for growth," even without a lowered reproductive rate (Klok and DeRoos 1996). Clearly this has not happened to PC shrimp, however, the models were based only on intermolt shrimp. Compensatory partitioning by ovigerous shrimp has not been found by previous studies because they did not examine this life history stage. This may partly explain the apparent contradiction.

Ammonia excretion was reduced by ovigerous PC shrimp in comparison to ovigerous TK shrimp, with other treatment groups showing no differences. The reduction in nitrogen lost to excretion by ovigerous PC shrimp allowed them allocate additional resources to reproduction in the same manner that reduced respiration allowed increased carbon allocation to reproduction. The energy lost in each case was a direct function of the elemental losses, and so the reduced losses of carbon and nitrogen by ovigerous PC shrimp led to combined energy savings.

Ecdysis showed no difference between treatment groups for carbon and nitrogen or energy, but the majority of individual sheds showed higher allocation by intermolt PC shrimp. An increased rate of ecdysis may represent another method for shrimp to rid themselves of toxins, particularly heavy metals. Nevertheless, the increase was not significant for the three budgets, and further study would be needed to draw firm conclusions.

Growth rates were equal for intermolt PC and TK shrimp, with all three budgets showing no significant differences. This is unexpected because intermolt PC shrimp had low assimilation rates and showed no evidence of compensatory partitioning of the resource budgets, yet they were still able to allocate similar resources to growth as intermolt TK shrimp. Even though intermolt TK shrimp also had low assimilation rates, they had more resources available than intermolt PC shrimp, yet growth rates were not significantly different between the two groups.

Only in the nitrogen budget was the confidence interval for intermolt TK shrimp above zero indicating additional resources for reproductive allocation. The large confidence intervals for all budgets made estimates of reproductive allocation for intermolt shrimp impossible for all budgets where zero was included in the confidence intervals.

Reproductive allocation was directly measured for ovigerous shrimp through analyzing the resources contained in the eggs. This provided a more accurate measure of reproduction than the traditional estimates and allowed realistic confidence intervals to be used rather than simply calculating estimated reproduction based on the means.

The apparent absence of reproductive allocation by intermolt shrimp may represent a form of compensatory partitioning in its own right. Intermolt shrimp may maximize their potential for growth through a reduction in reproductive allocation.

Compared with the range of response seen in TK shrimp for most budget components, PC shrimp displayed an increased range of response. This was an expected result, but was not uniform for all components of all budgets. For example, the egestion component of PC shrimps' carbon and nitrogen budgets showed a lower range of response than that seen in TK shrimp. On the other hand, in the egestion component of the energy budget, PC shrimp showed a greater range of response. It is unclear why the budgets differed in the variance of the egestion component. Egestion was measured in a similar way for all budgets, but energy was calculated including hydrogen as well as nitrogen and carbon. The inclusion of the carbon to hydrogen ratio to measure lipid content may have led to the differing results.

The contaminants found at Piles Creek appear to cause an expected lowering of metrics of physiological resource allocation, but the lowering of resource allocation was not uniform. Intermolt PC shrimp decreased consumption and increased respiration while ovigerous PC shrimp did the opposite. This highlights the need for life history specific measures of anthropogenic impacts. What appears to be a severe impact on one life history stage may be tolerated by another.

To be considered tolerant, an organism in a contaminated environment must be able to maintain growth and reproduction. Ovigerous PC shrimp exceed the reproductive allocation seen in ovigerous TK shrimp through a reduction in allocation to respiration and excretion, while intermolt PC shrimp have a growth rate similar to that of intermolt TK shrimp despite reduced feeding. Unlike ovigerous PC shrimp, however, intermolt PC

shrimp do not show clear evidence of compensatory portioning through reduced respiration or excretion. Thus some questions remain unresolved concerning the partitioning of shrimp resource budgets. How are intermolt PC shrimp able to maintain allocation to growth with reduced resources available? Do ovigerous shrimp show similar allocations to growth and ecdysis as intermolt shrimp? What is the measured reproductive allocation for intermolt shrimp? Is there a tradeoff between intermolt and ovigerous shrimp with ovigerous shrimp allocating more resources to reproduction and intermolt shrimp allocating more resources to growth?

These questions can only be answered by further studies using different methods such as a measure of growth by ovigerous shrimp through some proxy such as mRNA or other measure of protein synthesis. Similarly, reproduction in intermolt shrimp would need to measure vitellogenisis in female shrimp and sperm production in male shrimp. Further studies are also needed to determine how depuration products, such as protein bound metals, are proportioned among excretion products, ecdysis, and eggs.

By measuring the physiological budget allocation of Piles Creek shrimp, the present study demonstrates how it is possible for shrimp to thrive in what appears to be a sub-optimal habitat through physiological tolerance, in the form of compensatory partitioning of physiological resources. When comparing the present results to previous studies, it is clear that a more complete understanding of resource allocation in physiological budgets leads to better understanding of the impacts of contaminants. Laboratory dosing studies need to examine more than a single physiological effect from a particular contaminant to

make predictions about what may happen in the field. Also, a contaminant that appears to have a severe impact on one life history stage may show less severe consequences when another life history stage is examined. Laboratory models of contaminant impacts must be supported by field studies to verify predictions.

Finally, what is the future for shrimp living in contaminated estuaries along the Eastern Seaboard? Many acute toxicants such as the organic forms of mercury have declined over time with better regulation (Weis et al. 2001). How will this change the function of the Piles Creek system? The reduction of mercury is likely to indirectly benefit the grass shrimp until contaminant levels at Piles Creek are reduced enough to allow normal feeding rates by *Fundulus heteroclitus.* The impact of lower feeding by vertebrate predators due to contaminants acts in conjunction with the growth and reproductive allocation by the shrimp. Vertebrates with their complex central nervous systems might be more likely to demonstrate negative results longer than shrimp. This scenario would be upset, however, if more selective contaminants with low vertebrate toxicity, such as mosquito control agents, were introduced into the system. A negative impact on shrimp in the absence of a negative impact on the predators could quickly reduce the population of shrimp.

Nevertheless, for the foreseeable future, PC shrimp can be expected to have a high abundance from the combination of increased reproductive output and decreased predation. Unfortunately, the decreased feeding by intermolt shrimp, and decreased

feeding by vertebrate predators means that the high abundance of shrimp does not translate into increased ecological function.

Appendix I

Shrimp resource budgets

The resource budgets are based on the balanced energy equation of Winberg (1960). This equation provides a simple measure of productivity or growth potential combined with gamete production expressed by the equation "P=Ab-R-U", where P is productivity or growth potential combined with reproductive expenditures, Ab is energy absorbed from food, U is energy excreted by ammonia, and R is energy respired, measured by oxygen consumption. In this form of the equation P is not normally measured directly but is derived from the other variables (Wang and Stickle 1987). In this form P is the aggregate energy available for production. The components for P may also be separated to examine energy available for reproduction as well as growth.

This equation has been expanded to separate production into somatic growth and reproduction (Vernberg and Piyatiratitivorakul 1998). The equation has also been expanded to separate the components of excretion and egestion. The expanded equation is C= Pg+Pr+R+E+Ex+F, where C is consumption, Pg is resource allocation for somatic growth, Pr is resource allocation for reproduction, R is oxidative metabolic costs, E is losses to ammonia excretion, Ex is allocation to ecdysis, and F is losses to feces (egestion). The costs have been broken down into a more detailed accounting that that seen in Winberg's budget. The P of Winberg's equation has been broken down into growth, reproduction, and ecdysis portions of production. The component U, listed as ammonia excretion in Winberg's formula, represents the components of excretion. In the

formula by Vernberg and Piyatiratitivorakul excretion has been broken down into the components of ammonia excretion (E), and unabsorbed egested resources (F).

In theory, all components of this equation may be measured directly. Nevertheless, it is common for the energy expense of reproduction to be measured as the energy remaining from consumption after subtraction of the other variables. This is the method used in this study for inferring the value of Pr as an estimate of reproductive allocation. For the purposes of comparison between the two populations, Pr is also measured directly by examining the content of the egg masses from gravid shrimp from the two populations. This provides a more accurate measure of relative reproductive expenditure between the two populations. The cost of egg production is the primary reproductive cost in grass shrimp, with relatively little expenditure on sperm production.

The resource content of an egg mass is an instantaneous measure of allocation that must be integrated into the entire period of a reproductive cycle. The cost of oogenesis is represented by the resource content of the eggs plus the increased respiratory costs. The respiratory costs are included in R, so the resource value of an egg mass represents the costs in resources of the entire two week shrimp reproductive cycle. By using the inferred value of Pr it is possible to establish the rate of reproduction expenditure, while measuring the resource content of the eggs gives the direct cost of a given brood. The value of Pr/day should be similar to the resource value of the eggs divided by 28 to give a maximum daily cost. This assumes alternating cycles of reproduction and vitellogenisis in adult females. The resource content of the eggs provides a measurement of the cost of

reproduction per gravid female in a given population. Because this cost can be measured directly, it provides a more accurate comparison of reproductive costs between the two populations than an inferred value of Pr.

Appendix II

Conversion factors for shrimp

Conversion factors	PC shrimp	TK shrimp
mg wet shrimp/mg dry shrimp	4.131 ± 0.21 (n=50)	4.666 ± 0.13 (n=50)
dry weight/ash free dry weight	1.246 ± 0.011 (n=18)	1.257 ± 0.017 (n=18)
mm shrimp/mg dry weight	0.305 ± 0.028 (n=50)	0.387 ± 0.031 (n=50)
mg wet eggs/mg dry eggs	2.74 ± 0.19 (n=28)	3.11 ± 0.28 (n=25)

Details of methods assumptions and constants used for determining energy content.

Respiration: RQ and oxycaloritic coefficient.

RQ for starved grass shrimp taken from (Wolvercamp and Waterman 1960) for *Palaemon serratus*.

The oxycaloritic coefficient of 0.0183 is based on the work of Brody (1945) for starved shrimp assuming no carbohydrate utilization. The range of possible values from 0.0203 to 0.0183 represents the differences in carbohydrate, lipid, and protein utilization between starved and well fed shrimp. According to Wolvercamp and Waterman (1960) *P. pugio* does not utilize carbohydrates under starvation conditions, and uses carbohydrates as the primary energy source when fed.

Egestion: Proximate biochemical composition determination of energy content.

Assumptions taken from Gnaiger and Bitterlich (1984)

Assumption 1. All nitrogenous compounds other than proteins (DNA etc.) are counted as energetically similar to protein.

Assumption 2. The protein content of aquatic organisms can be determined using a conversion of 5.8 times the nitrogen content by weight.

Assumption 3. Residual water after drying equals 0.06 the total sample mass. This is important for determining the energy content because the C/H ratio determines the lipid content.

Assumption 4. C/H ratio for carbohydrate equals 0.140 and C/H ratio for lipid equals 0.147

Using these assumptions and equations provided, the following conversion equations were derived:

mg lipids=(mg C /ash free sample weight)*(((mg H*1-residual water)/(mgC))/0.147)

mg carbohydrates=(total organic C-(5.8*mg N)-mg lipids

mg protein=5.8*mg N

Calories=(5.5* protein)+(4.1*carbohydrates)+(9.3*lipids)

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Curriculum Vitae

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Education

Rutgers University, Ph.D. 2009
State University at Albany, Albany, NY. Undergraduate and Graduate Classes in Biology
The New School for Social Research, New York, NY. M.A. Philosophy, May, 1993
Sarah Lawrence College, Bronxville, NY. B.A. Liberal Arts, May, 1989

Professional Positions

Laboratory Technician. James J. Howard Marine Sciences Laboratory NOAA/NMFS organic analysis laboratory 1998.

Research Assistant. The New York State Museum, Aquatic Biology Laboratory. May-September 1996. Duties included: field collection of samples from Lake Champlain and other Adirondack lakes, slide mounting of specimens, analysis of water samples (biotic and abiotic parameters), and developing estimates of benthic and planktonic populations.

Volunteer Laboratory Technician. The New York State Museum, Aquatic Biology Laboratory. January 1995-May 1996. Duties included: field collection of samples, preparation of samples, identifying samples to species, and cataloguing specimens.

Teaching Positions

Vertebrate zoology laboratory instructor. Led two laboratory sections, taught species identification and lead field trips. Organized laboratory course materials and designed handouts. Spring, 2004

Invertebrate zoology adjunct. Presented class lectures, organized syllabus, and organized all testing and grading. 2003

Invertebrate zoology laboratory instructor. Gave laboratory lectures, ran dissections and identification classes. Organized laboratory course materials and designed handouts. Fall, 2000-2003

Limnology teaching assistant. Led students on field trips, ran laboratory dissections and identification classes. Spring, 2002

Ornithology teaching assistant. Led students on field trips, ran laboratory dissections and identification classes. Spring, 2001

Genetics recitation instructor. Led three sections of recitation classes focused on mathematics of genetics, also some discussion of molecular techniques. Fall, 1998, Spring, 1999, Spring, 2000

General biology laboratory instructor. Led two sections of general biology laboratory. Fall, 1999

Publications

Abstracts:

Stout, Joseph, L. Walrath, and C. Seigfried 1996. Diversity of Chironomid Larvae as a Function of Dissolved Oxygen. Program and Abstracts: The New York Natural History Conference IV, April 24-27, 1996: A Forum For Current Research. The University of the State of New York, State Education Department, Albany, NY

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