JUVENILE *EPILOBOCERA SINUATIFRONS GROWTH RATES AND ONTOGENETIC SHIFTS IN FEEDING IN WILD POPULATIONS*

by

KAUAOA MATTHEW SAM FRAIOLA

(Under the direction of Alan P. Covich)

Abstract

Freshwater amphibious crabs are understudied and found in tropical streams of many parts of the world and have the potential to influence stream and terrestrial communities. In this thesis I investigate basic biology and ecology aspects of the freshwater amphibious crab *Epilobocera sinuatifrons* in headwater streams of the Luquillo Experimental Forest, Puerto Rico. I measured growth rates of juvenile crabs given high and low quality food resources, and investigated ontogenetic shifts in feeding using natural abundances of stable isotopes δ^{13}C and δ^{15}N in crab tissue from two streams. Crabs are slow growing, with no differences in growth rates on different quality foods. Stable carbon and nitrogen stable isotopes suggest that crabs are increasing their trophic position as they grow larger in the wild, possibly the result of foraging on land as adults. These studies suggest that *E. sinuatifrons* are long lived and may require a range of foods to survive.

INDEX WORDS: Food webs, Growth, Ontogenetic shift, Life-history omnivory, Tropical streams, Freshwater crabs, Epilobocera sinuatifrons, Puerto Rico, Stable isotopes

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DEDICATION

This thesis is dedicated to my family, friends, Puerto Rico, and La Buruquena.

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Chapter 1

INTRODUCTION

In many insular tropical streams decapods play a major role in processing terrestrially derived carbon and can attain high biomass (Crowl et al. 2001; Smith et al. 2003). Macroconsumers in tropical streams, such as omnivorous shrimp, have also been shown to affect multiple trophic levels from basal food resources such as leaf detritus to secondary consumers, such as aquatic insect abundances (Rosemond et al. 1998; Wright and Covich 2005). Of the common decapod crustaceans found in tropical headwater streams, we know very little about the ecology of freshwater crabs and their roles in these food webs (Covich and McDowell 1996; Dobson 2004). However, freshwater crabs have the potential to be important seed predators, and predators of other animals, as well as important processors of terrestrial plant material in streams.

Freshwater crabs are found in tropical lotic systems all around the world. The strong influence of marine and land crab species in other ecosystems (Gutiérrez et al. 2006; Lindquist and Carroll 2004; Green et al. 1997) suggests that freshwater crabs may be strong interactors in their own environments. Dobson (2004) suggested that freshwater crabs in Africa can be important processors of leaf litter. This role for freshwater decapods contrasts with the temperate view of stream food webs, in which aquatic insects are important players along with crayfish and amphipods in the processing of terrestrially derived carbon (Wallace 1997; Huryn and Wallace 1987; Graça 2001). Unlike temperate aquatic insects, most freshwater crabs spend some time outside of the water in the surrounding riparian zone (Okano et al. 2000; Johnson and Robson 2005; Dobson 2004).

In Puerto Rico, the freshwater crab *Epilobocera sinuatifrons* (Pseudothelphusidae) is amphibious and adults are readily observed at night on the forest floor in the riparian zone of the head water streams (Covich and McDowell 1996). None of the life cycle of *E. sinuatifrons* is spent in marine waters and it has no larval stage, like the shrimps that co-occur in these streams. Juvenile crabs directly develop from the attached eggs which are held under the telson of the female. Juveniles of this species are restricted to riverine habitats where they feed on plant detritus and aquatic insects. Adults live and forage both within the stream as well as in the surrounding riparian forest.

This study investigated the effects of different food resources (plant and animal) and combinations of food resources on the growth rate of juvenile *E. sinuatifrons* from Puerto Rico. In addition, the effects of food (microbially conditioned leaf litter) origins (native versus non-native) on the growth rates of juvenile crabs were also examined using controlled laboratory experiments. This thesis also explored the extent of life-history omnivory that these crabs exhibit, using observational techniques in the field as well as carbon and nitrogen stable isotopes analyses. The following two chapters will be submitted for publication in the journals *Freshwater Biology* and *The Caribbean Journal of Science*.

E. sinuatifrons is a popular local food in Puerto Rico and experiences increased fishing pressure due to limited habitat as forests are cleared for development of agriculture and housing. Historically, crab fisheries all around the world are prone to collapse. It is hoped that this thesis will help to strengthen our knowledge of *E. sinuatifrons* in Puerto Rico and help resource managers sustain these populations for future generations. It is also hoped that this new information will help us better understand how stream ecosystems function in Puerto Rico and other tropical areas around the world.

CHAPTER 2

EVIDENCE OF ONTOGENETIC SHIFTS IN FEEDING OF A FRESHWATER CRAB, *EPILOBOCERA SINUATIFRONS*, PUERTO RICO

Fraiola, K.M.S. and A.P. Covich to be submitted to *Freshwater Biology*, 07/30/06

Abstract

Zoobenthic macroinvertebrates are important components in the functioning of stream ecosystems. Many species shift their mode of foraging with age and alter their consumption of either plant or animal foods. This study is the first to document ontogenetic changes in diet among size and age classes of an amphibious freshwater crab (*Epilobocera sinuatifrons,* Pseudothelphusidae). Older, larger *E. sinuatifrons* are hypothesized to increase their carnivory as they become amphibious and forage outside the stream on a higher proportion of terrestrial animal food resources than juveniles. Juveniles are hypothesized to forage only on aquatic insects and on conditioned leaf litter within the stream channel. N-15 analysis demonstrated that ontogenetic diet shifts occurred and differed in two first-order forested streams in the Luquillo Experimental Forest, Puerto Rico that had experienced different disturbance histories from hurricanes, floods, droughts and prior landuses. These disturbances altered terrestrial vegetation and supplies of leaf-litter detritus and fruitfall used by crabs as a source of carbon. C-13 provided some evidence of changes in food resources among four different crab size classes. Analyses of standardized trophic position of different size classes indicate that adult crabs (> 75mm in carapace width- CW) function at higher-trophic positions than juveniles (< 25mm CW). These results also indicate that despite foraging more often on animal prey, adult crabs consume a higher proportion of the fruits of Sierra Palms (*Prestoea acuminata* var. *montana)* and Tabonuco (*Dacryodes excelsa,* Burseraceae) in their diets relative to crabs in rain forests where these tree species are less abundant.

Introduction

Benthic macroinvertebrates (e.g. crustaceans and aquatic insects) are important in freshwater ecosystem processes because they perform multiple functional roles as primary and secondary consumers as well as detritivores during their different life-history phases (Covich et al., 1999; Covich et al., 2004). These ontogenetic shifts in foraging are important in altering how different sizes and types of food resources are used as energy sources by a wide range of freshwater consumers (Werner & Gilliam, 1984; Cummins et al., 1989; Wallace et al., 1997; Garcia-Berthou, 2002). For example, some species of aquatic insects such as caddisflies display shifts in diet with age (Basaguren et al., 2002). Decapod crustaceans such as crayfishes are known to vary in their degree of omnivory and are well studied in temperate-zone stream ecosystems (e.g., Usio, 2000; Parkyn et al., 2001; Usio & Townsend, 2002; Bondar et al.,2005; Rudnick & Resh, 2005; Stenroth et al., 2006). In tropical streams, the role of shrimps is becoming better understood in terms of their roles in processing leaf detritus and as active grazers and predators (Covich & McDowell, 1996; Crowl et al., 2001; Larned et al., 2003; Mantel & Dudgeon, 2004; De Souza & Moulton, 2005; Crowl et al., 2006).

Although freshwater crabs are commonly distributed in many tropical streams, their roles in these food webs remain less well defined (Covich & McDowell, 1996; Dobson, 2004). Dobson et al. (2002) proposed that freshwater crabs (*Potamonautes* sp.) in Kenyan highland streams may play a major role in processing terrestrially derived coarse particulate organic matter, such as leaves from riparian forests. In Puerto Rico, *Epilobocera sinuatifrons* is the only species of freshwater crab found in the headwaters. This species is endemic and widely distributed in Puerto Rico and in a few localities in St. Croix (Chase & Hobbs, 1969; Rivera, 1996). To date, there has been relatively little information published about the ecology of *E. sinuatifrons* or related crabs in the Caribbean (Zimmerman & Covich, 2003). Individuals smaller than 30 mm carapace width (CW) are found in leaf litter and under rocks with in active stream channels while gravid females (>50 mm CW) forage in wet leaf litter on the forest floor

(Rivera 1996). *E. sinuatifrons* has direct development of young and a single female carries hundreds of eggs and hatchlings under her telson until they are large enough to be independent (4 mm CW). Besides *E. sinuatifrons,* there are 5 other endemic species of *Epilobocera* with 3 species in Cuba (*E. gertraudae, E. cubensis, and E. armata*), and 2 species in Hispaniola (*E. haytensis and E. gilmanii*).

Understanding the full range of food resources used by these crabs is especially difficult because gut content analysis has proven ineffective for distinguishing food items to characterize food preferences or degree of omnivory. The only information regarding the diet of these crabs has been based on direct observations of feeding in headwater streams (A.P. Covich, pers. comm.), without quantification of food availability. Other studies (Henry, 2000; March & Pringle, 2003) demonstrated that mayflies in these headwater streams may be an important food resource for *E. sinuatifrons*. Few data on the distributions and diets of freshwater crabs are available, in part because comprehensive study of both juveniles and adults requires intensive sampling in a wide range of aquatic and terrestrial habitats.

These freshwater crabs are hypothesized to undergo an ontogenetic shift in trophic position: as crabs increase in age and size, they presumably increase their consumption of more and larger animal prey. Juveniles are expected to feed at lower trophic positions, primarily on coarse particulate organic matter, leaves, and aquatic insects. Adults are likely to be more mobile within the stream channel and to feed at higher trophic positions on shrimps and crabs. Moreover, as crabs mature they can be amphibious and feed on a wide array of additional terrestrial prey. Their enlarged carapace retains moisture around their gills and they can use atmospheric oxygen when foraging in wet leaf litter on the forest floor. To investigate this ontogenetic-shift hypothesis, direct observations of crab feeding in different habitats were combined with $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis of tissue samples of crabs from four size classes.

Materials and methods

Study site

The Luquillo Experimental Forest (Caribbean National Forest), is located at latitude 18°18' N, longitude 65°47' W and consists of over 11,000 hectares of steeply sloped rain forest in northeastern Puerto Rico. It is the only tropical rainforest site within the U.S. National Science Foundation's Long-Term Ecological Research Program. The site is also a United Nations Biosphere Reserve and has been under intensive ecological study for more than 50 yrs. Montane streams in northeastern Puerto Rico are characterized by rapid runoff from steeply sloped forest. Average annual precipitation of 3600 mm is slightly seasonal, with peak flows during May through December. Droughts and hurricanes occur infrequently with recurrence intervals of extreme low flows varying from between 5 to 10 yrs and hurricane-derived floods varying between 10 to 30 years (Larsen, 2000; Covich et al., 2003; Covich et al., 2006). Annual discharge in streams draining the forest is highly variable and closely follows rainfall (García-Martino et al., 1996; Schellekens et al., 2004). Water temperatures range annually from 18 to 26 °C.

This study took place in two first-order streams in the Luquillo Experimental Forest, in the Rio Espiritu Santo drainage basin, at elevations ranging from 200 to 350 m during June – December 2005. The two unnamed study streams (designated A and B in this study) are downslope from the University of Puerto Rico's El Verde Field Station and drain small catchments composed of secondary forest.

Sampling protocol

Nine timed field surveys were conducted at night at each stream from June – December 2005. A 100 m stretch of stream was searched for 4 hrs within the active stream channel and within a 30 m zone on each side of the channel. Crabs were captured by hand or dip net to avoid trap bias and to collect individuals of all sizes both in the stream and on land. Trapping methods only captured large adults. Two legs were removed from individuals with a carapace width (CW) > 8 mm and only the muscle tissue was used for isotope analyses. Small crabs (< 8 mm CW) were placed in aquaria in a nearby lab where they were allowed to clear their guts so that the entire body could be used if leg tissue was insufficient for analysis. Additional larger crabs (12 – 18 mm CW) were also allowed to clear their guts so that they could be used to calculate a correction factor for those crabs in which the whole body was needed. Aquatic insects (mayflies and caddisflies) were collected from stream by collecting leaf packs and washing mayflies off the leaves into a 500 µm sieve. Mayflies and caddisflies were kept in river water for 24 hrs in order to clear their guts before drying and analysis for stable isotopes.

Microhabitat data were recorded along with other information on the sex, whether eggs or fully developed young were present, and activities when captured (feeding, walking, hiding, etc.) for every crab captured. If a crab was encountered feeding, the food it was eating was taken, identified and recorded. Crabs were separated into four size classes: I, < 25 mm CW; II, 25 – 50 mm CW; III, 50 – 75 mm CW; and IV, > 75mm CW. Size classes were chosen such that they seperated crab size ranges into four even size ranges and followed the natural shift of crabs from aquatic to terrestrial habitat that was observed in the field at a threshold of 29 mm CW.

Isotope analysis

All samples were dried at 60° C until constant weight. Samples were combusted to CO_2 and N_2 and analyzed in a Carlo Erba NA 1500 CHN analyzer connected to a Finnagan Delta C mass spectrometer. Stable isotope signatures were calculated as follows:

$\delta^{15}N$ (‰) = $[(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}]$ X 1,000, where R is $^{15}N/^{14}N$ or $^{13}C/^{12}C$.

The standard was PDB (Pee Dee Belemnite) carbonate for carbon and atmospheric N_2 for nitrogen.

To adjust for stream-specific variation in $\delta^{15}N$, an index was used for standardization based on Branstrator et al. (2000):

Trophic elevation = $[(\delta^{15}N_{\textit{E. sinuatifrons}} - \delta^{15}N_{\text{mayfly larvae}})/2.54]$

where 2.54 is the trophic position increment (i.e., enrichment factor). This value is the mean enrichment factor from a meta-analysis by Vanderklift and Ponsard (2003). This value is lower than the mean value of 3.4 used previously by other investigators (Minagawa & Wada, 1984, Post 2002) and therefore, our estimates of crab trophic elevation represent an upper estimate.

Processing samples and Correction factor

A total of 76 crabs was used in this analysis. Tissues used for stable isotope analyses were taken from crab legs, except for 31 of the smallest crabs (4.0 – 10 mm CW). For the smallest size crabs, the whole body was needed in order to have enough tissue (1.5 mg) for stable isotope analysis. To ensure that carbon from the carapace did not influence the results, inorganic carbon from the carapace was removed with a 20% HCl solution. Leg tissue was used whenever possible to have comparable materials. To avoid any potential effects of different adult tissues between sex and sizes (gonads), whole crab bodies were not used for the 36 larger crabs (>11 mm CW).

To correct for the use of the whole body (carapace, pinchers, and legs), a correction factor was calculated by comparing isotopic signature of the legs and body of six other crab samples. The crabs used for this determination ranged in size from 12 – 17 mm and were the smallest size possible to run legs and body separately. For each crab a correction factor was calculated as the difference between the estimated value of the whole body and of the legs. To estimate the $\delta^{15}N$ and $\delta^{13}C$ of the whole body of a crab we measured the $\delta^{15}N$ and $\delta^{13}C$ of the legs/pinchers (appendages) and carapace of a crab separately and estimated its whole body isotopic concentrations as being half way between these two points. This assumption was set arbitrarily. The difference in ‰ $\delta^{15}N$ between leg tissue and the estimated whole body isotopic concentrations ranged from -0.60 to + 0.98 ‰ $\delta^{15}N$ and averaged + 0.12 ‰ $\delta^{15}N \pm 0.12$. For $\delta^{13}C$ the difference ranged between + 0.09 and + 0.66 and averaged + 0.35 ‰ $\delta^{13}C \pm 0.1$. The averages were used as the correction factor for 31 crabs in which the whole body had to be used in order to obtain enough tissue for analysis. Statistical analyses were then run with and with-out the correction factor in order to show the effects of the correction factor or the statistical results.

Data Analysis

One way ANOVA with PROC GLM in SAS (Statistical Analysis Software) 9.1 to analyze the least squared means was used to test for an effect of crab size class on trophic elevation. Post ANOVA a least squared means multiple comparison test was used to look at differences between size classes. These analyses were conducted separately on each of the two streams. PROC GLM was used to account for uneven sample sizes between crab size classes. For all tests a significance level of 0.05 was used.

Results

Habitat Observations

A total of 73 crabs was observed and collected in both streams (Table 2.1). Crabs were found in both the active channel and surrounding forest of both streams, and ranged in size from 4 to 105 mm CW. The sizes of crabs caught within the active channel ranged from 4 – 105 mm CW and those captured on land ranged from 29 to 103 mm CW (Table 2.1). Of the crabs captured, 13 % were observed feeding, five were feeding on seeds and nuts, one on freshwater shrimp, and three on fine detritus (Table 2.2). The crab observed feeding on shrimp was within the largest size class (IV), whereas the crabs feeding on seeds ranged from size class II to IV, and those feeding on fine detritus were in size classes I (Table 2.2). The seeds were, in order of increasing size, *Guarea* sp., *Prestoea montana*, and *Dacryodes excelsa*. Smaller crabs (size class II) were observed feeding on smaller seeds (*Guarea* sp, Table 2.2), and larger crabs were observed feeding on larger seeds (*P. montana* and *D. excelsa*, Table 2.2).

Trophic elevation and $\delta^{13}C$

Mean δ^{15}N values for the crabs ranged from 3.09 to 6.00 ‰ and generally increased with increasing crab size (Table 2.3) as did trophic elevation which ranged from -0.15 to 0.93 levels (Table 2.4). The mean δ^{15}N values of crab size classes from stream A ranged from 3.0 to 6.8 ‰ δ^{15}N with a mean trophic elevation ranging from -0.15 (I) to 0.45 (III) levels, and ordered thus I<II<IV<III. There was a significant effect of crab size class on trophic elevation of crab tissue ($F_{3,28} = 4.45$, $P = 0.011$) in Stream A. Stream B also showed a significant effect of crab size class ($F_{3,42} = 12.18$, $P = 0.0001$) its mean δ^{15}N values per size class ranged from 1.04 to 6.3 ‰ while its mean trophic elevations ranged from 0.09 (I) to 0.93 (IV) levels, and were ordered thus I<II<III<IV (Figure 2.3). Mayfly larva δ^{15}N values in this study ranged from 3.60 to 5.17 ‰

(Table 2.3). Mayfly $\delta^{15}N$ values from stream A ranged from 4.69 to 5.17 ‰ with a mean of 4.84 ± 0.12 ‰, whereas in Stream B they ranged from 2.55 to 3.60 ‰ with a mean of 2.86 ± 0.25 ‰ (Table 2.3). Mean Seeds captured from feeding crabs $\delta^{15}N$ values ranged from -1 to 2.68 ‰ (Table 2.3). Mean Conditioned leaves from the river and biofilm $\delta^{15}N$ were from -1.26 to 0.76 and 1.19 to 5.22 ‰ (Table 2.3).

Mean $\delta^{13}C$ values for the crabs ranged from -24.02 to -25.24 ‰ (Table 2.3) and also increased with increasing crab size and trophic elevation (Table 4). The mean $\delta^{13}C$ values of crab size classes from streams A and B were with in similar ranges (Stream A: -24.02 to -25.24 and Stream B: -24.10 to -25.13 ‰). The $\delta^{13}C$ values in the following order for stream A: II<I<III<IV; and I<II<III<IV for Stream B. There was a significant effect of crab size class on $\delta^{13}C$ values of crab tissue ($F_{3,28} = 4.57$, $P = 0.010$) in Stream A as well as Stream B ($F_{3,42} = 3.92$, $P = 0.015$). In Stream A, size classes I and II were statistically different from size class IV, and size class III was not statistically different from any other size classes (Table 2.6). In stream B size classes I, II, and III were not statistically different from one another but they were all statistically different from size class IV (Table 2.6). Mean mayfly larva $\delta^{13}C$ values from streams A and B were -26.60 and -27.07 ‰ (Table 2.3). Seeds captured from feeding crabs $\delta^{13}C$ values ranged from -28.46 to -26.32 ‰ (Table 2.3). Mean Conditioned leaves from the river and biofilm $\delta^{13}C$ were from -30.68 to -29.02 and -32.44 to -31.07 ‰ (Table 2.3).

On average across both streams, crabs > 75mm in carapace width were elevated 0.48 levels above conspecifics < 25mm (Table 2.3). In both streams there was a general trend of increasing trophic elevation from I through III, but for size class IV in stream A the value decreased by 0.30 levels from III, while it increased by 0.26 levels in stream B (Figure 2.1). In stream B the trophic levels of size classes II through IV were not statistically different (Table

2.5). In stream A, size classes I and II, II and IV, and III and IV are not statistically significant from one another in trophic elevation (Table 2.5). The streams also differed in the overall trophic elevation of size classes with stream B having higher trophic elevation values than A in all size classes (Figure 2.1). We did not compare size classes between streams statistically because we lacked sufficient replication.

Discussion

The significant elevation in trophic position and increasing values of $\delta^{13}C$ as crab size supports our hypothesis that *E. sinuatifrons* is an omnivore during its foraging. These crabs changed their diet and acquired more of their nutrition from higher trophic levels as they grew larger. The similar trends to increased carnivory observed across size classes in the two study streams indicate that crabs in both these populations under went some degree of ontogenetic shift. However, the dissimilarity in actual trophic elevation values of size classes from the two streams also indicate that foraging by crabs from these two streams differed in proportions and types of plant and animal foods.

The range of $\delta^{13}C$ and $\delta^{15}N$ values for crabs of this study encompassed that of the only known published value for *E. sinuatifrons* (no crab size data) from a nearby river based on a single crab specimen reported by March and Pringle (2003). The Rio Sonadora is a larger river (13 m channel width) with a more open canopy than the closed canopy of the two first - order study streams (1 to 2 m channel width). The $\delta^{13}C$ and $\delta^{15}N$ values of the mayflies and leaves in the two small study streams were also similar to those reported by March and Pringle (2003) from the Rio Sonadora. Biofilm $\delta^{15}N$ values in these two streams were similar to those values reported by March and Pringle (2003), however they were slightly more enriched in $\delta^{13}C$.

The low to negative trophic elevations of size class I in both streams suggest that juvenile crabs were feeding at or below the same trophic position as mayfly larvae in the two streams. At this size class their $\delta^{13}C$ values suggest that they may have fed on the same foods as mayfly larvae. Mayfly larvae in these streams have been categorized as collector–gather insects (Pringle et al., 1993; March, 2001). The observation that crabs of the smallest size class (I) fed on fine particular organic matter (FPOM) on stream bottoms further supports the interpretation that these small crabs have a similar trophic position as collector gathers. Preliminary laboratory studies with fluorescent labeled FPOM showed that juvenile crabs will collect and feed on FPOM as early as 4 mm (CW), but are not able to capture aquatic insects (such as mayfly larvae) until they are ≥ 8-12 mm (Fraiola, In review). Similar shifts from easy to capture and ingest foods (such as detritus and algae) to more difficult, but protein-rich, prey has been shown in blue crabs in estuaries (Rosas et al., 1994).

The progressive increase in the trophic elevation and $\delta^{13}C$ values above that of mayflies as crabs increased in size from II to IV in both streams indicates that crabs were feeding on different foods at the same trophic position as mayflies (e.g. smallest crabs, mayflies, and other macro invertebrates) or above (e.g. shrimps, juvenile crabs, or terrestrial animals). Observations that juvenile crabs readily feed and survive on live mayfly larvae in the lab once the crabs are above > 8 – 12mm CW (Fraiola, unpublished data; Zimmerman & Covich, personal communication) is consistent with the stable isotopes analyses. The additional observations from this study that crabs (largest size class IV) prey on freshwater atyid shrimp further indicates specifics foods in their diets such as animal prey are likely important throughout their life history. However, the trend toward increased carnivory with size and maturity and increased trophic position is likely to be widespread for those tropical freshwater crabs that can selectively forage over a large area of forest floor. In contrast, Bondar et al. (2005) found that crayfish in temperate streams did not undergo ontogenetic shifts to higher trophic positions or feed on different food resources other than allochthonous detritus. Whereas in temperate lakes France (1996) found strong isotopic evidence for a shift from feeding on epilithic algae to terrestrial organic matter as crayfish grew larger. The strong chelae of adult crabs allow them to capture larger animal prey as well as to crush hard seeds found on the forest floor.

Other evidence that aquatic insects might be a food resource for juvenile *E. sinuatifrons* includes: 1) aquatic insects are abundant in some microhabitats; 2) juvenile crabs are present in all stream microhabitats and can be very abundant; and 3) juvenile crabs share many of the same habitats as aquatic insects (McDowell & Covich, 1996; Zimmerman & Covich, 2003; and Ramirez & Hernandez-Cruz, 2004). Leaf packs are a common microhabitat which often contain high densities of chironomids and mayflies, fewer caddisflies and small crabs (Zimmerman & Covich, 2003; March et al., 2002; Ramirez & Hernandes-Cruz, 2004). Mantel and Dungeon (2004) documented extensive predation by an omnivorous shrimp on a variety of aquatic insects, including chironomids, caddisflies, and mayflies in Hong Kong streams. Some African species of freshwater crabs actively consume animal prey when they are small and medium in size (Williams, 1965).

The decrease in trophic elevation from size class III to IV in stream A may be a result of larger adult crabs feeding more on plant material in stream A, such as seeds. Larger adult crabs were observed feeding on large plant seeds, like *Prestoea acuminate*, and *Dacryodes excelsa,* only in Stream A (Table 2). These tree species and their seeds were more numerous at Stream A relative to Stream B (Fraiola, personal observation). This difference in seed abundance and diversity may also explain why size classes in Stream A had a consistently lower trophic elevation than Stream B. Consumption of seeds and seedlings by land crabs in other areas, such as Costa Rica and Christmas Island, can have strong effects on the terrestrial plant communities (Lindquist & Carroll, 2004; Green et al., 1997). However, the $\delta^{13}C$ values of the largest size classes are very similar. This similarity suggests they consume many similar food resources. Studies of crayfish have shown variability based on food availability, individuals fed on more plant material in some habitats but not others (Parkyn et al., 2001). The relative importance of fruits and seeds from riparian and forest plants to *E. sinuatifrons* remains an interesting but unresolved question.

E. sinuatifrons is not only able to consume many different plant foods when on land, but there are also numerous species of invertebrates and vertebrates that inhabit the litter on the forest floor (Reagan & Waide, 1996). For example, there are 34 species of land snails that live in the leaf litter on the floor of the Luquillo Experimental Forest, some larger species can reach densities of up to 5 individuals m^{-2} (Reagan et al., 1996). Some species, such as *Gaeotis nigrolineata* (Bulimulidae) and *Nenia ridens* (Clausiliidae), are frequently associated with Sierra Palm leaf litter (Secrest et al., 1996) where palm fruits are also seasonally abundant. Crabs are readily found foraging in wet leaf litter on forest floor at night and likely consume land snails and other litter invertebrates. Snails are commonly used as prey by freshwater and marine crabs (Vermeij & Covich, 1978; Vermeij, 1987, 1993). Examples of land crabs (*Grecarcinus lateralis,* Gecarcinidae) predating on land snails (genus *Cerion*) have been reported in the Bahamas and the Florida Keys (Quensen & Woodruff, 1997).

The presence of individuals both in the active channel and up to 30 m away from the channel (associated with burrows) on land within the riparian forest show that these crabs are amphibious and are capable of traveling considerable distances. This ability to travel and forage over large areas of land may not only be beneficial due to increased food resources, but it may also aid in the dispersal of adults among populations within and between watersheds.

The release of juveniles with seasonal environmental factors, such has high rainfall and discharge, has been thought to be important in the dispersal of some species of juvenile freshwater crabs (Okano et al., 2000; Johnson & Robinson, 2005). The presence of the smallest juvenile crabs (4 mm CW) during the months of May, June, July, August, and December in this study, along with the observation of Zimmerman and Covich (2003), indicate that reproduction in this species of crab may be occurring all year. This lack of seasonality in reproduction may be a result of a lack of major variability in climatic variables such as rainfall and temperature in Puerto Rico.

In this study we found strong support for the hypothesis that the freshwater crab *Epilobocera sinuatifrons* relyed more on foods from higher trophic positions as they grew larger. Fruits and seeds from plants in the riparian and adjacent forests are likely to be important to the diet of larger crabs. Any disturbances or alterations of the plant communities resulting from land-use change in the catchment may alter crab foraging behavior. Parkyn et al (2001) showed that changes in habitat (native forest to pasture) and consequently, altered availability of food resources in New Zealand streams, affected food choice by crayfish more than age or size. Stream and riparian habitats in the Luquillo Experimental Forest experience numerous disturbances such as hurricanes, floods, droughts, and previous agricultural land uses (Thompson et al., 2002; Covich et al., 2003; Beard et al., 2005). Some disturbance events, such as drought and tree falls, are known to alter abundance of potential terrestrial prey (Alvarez & Willig, 1993; Bloch & Willig, 2006). Reductions in volumes of stream pools can concentrate aquatic prey (Covich et al., 2003) and may increase crab predation during drought on shrimp and insects.

Effects of extreme drought on pool sizes and locations, litter production, land- snail abundance and crab foraging are likely to be linked to long-term changes in crab populations.

Different degrees of disturbance and legacies of past events potentially increase vulnerability of crab populations to unregulated harvesting for food resources by local communities. Future research is needed to consider the potential effects of disturbances and feeding by adult *E. sinuatifrons* on seeds and seedlings as well as on invertebrate communities in the rain forest adjacent to streams. Sustaining populations of this endemic species will require additional understanding of growth rates, survivorship and life history in a wide range of habitats.

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Table 2.1. Catch statistics from field surveys, with number of crab's observed in stream (within wetted perimeter) and forest habitats (outside of wetted perimeter), the size range in mm carapace width (CW), and the number (No.) of crabs caught feeding. n = number of samples.

Habitat	n	CW (mm) min	CW (mm) max	No. feeding
Stream A				
Stream	20	5.1	97.2	2
Forest	12	49	102.8	3
Stream B				
Stream	38	4.3	104.8	3
Forest	8	29	101	2
Summary	78	4.3	105.8	10

Table 2.2. Summary of the 10 crabs observed feeding by food type, stream, and size class. There were two food categories: 1) Seeds from *Dacryodes excelsa*, *Prestoea montana*, *Guarea* spp, and a Liana; 2) Animal and detritus, *Atya lenipes*, *Epilobocera sinuatifrons*, and fine particles. * = same crab; φ = unidentified taxa; and γ = material of particles unknown, picked off substrate.

	Stream A				**Stream B**			
Seeds	I	II	III	IV	I	II	III	IV
D. excelsa	--	--	1	1	--	--	--	--
P. montana	--	--	1*	--	--	--	--	--
Guarea Spp.	--	--	1*	--	--	1	--	--
Liana$^{\varphi}$	--	1	--	--	--	--	--	--
Animal and other								
A. lenipes	--	--	--	1	--	--	--	--
E. sinuatifrons	--	--	--	--	--	1	--	--
Fine particles$^{\gamma}$	1	--	--	--	2	--	--	--

Table 2.3. Table of the mean $\delta^{13}C$ and $\delta^{15}N$ values for all 4 size classes I (< 25mm), II (26 – 50mm), III (51-75mm), and IV(>76mm), mayfly larvae, conditioned leaves from the stream, Biofilm, and seeds from each stream plus or minus the standard error (se). N = number of samples. $\delta^{13}C$ values with the same superscript letter are not statistically different (P>0.05).

Crab size classes and resources	N	$\delta^{13}C$		$\delta^{15}N$	
		mean	*se*	*mean*	*se*
Stream A					
I	8	-25.24^{a}	0.41	4.47	0.38
II	9	-24.88^{a}	0.21	5.12	0.26
III	5	$-24.65^{a,b}$	0.23	6.00	0.24
IV	10	-24.02^{b}	0.11	5.22	0.13
Mayfly Larvae	3	-26.60	0.15	4.84	0.12
Guarea sp.	1	-28.53	---	2.36	---
D. excelsa	1	-27.80	---	-1	---
P. montana	2	-26.32	0.07	2.19	0.09
Leaves	3	-29.02	0.20	0.76	0.23
Biofilm	3	-31.07	0.83	5.22	0.72
Stream B					
I	32	-25.12^{a}	0.16	3.09	0.19
II	3	-25.13^{a}	0.18	4.42	0.42
III	2	-24.63^{a}	0.02	4.57	0.93
IV	9	-24.10^{b}	0.06	5.23	0.25
Mayfly Larvae	4	-27.07	0.13	2.86	0.25
Guarea sp.	1	-28.46	---	2.68	---
Leaves	3	-30.68	0.06	-1.26	0.21
Biofilm	3	-32.44	1.40	1.19	0.60

Table 2.4. Table of the mean trophic elevation values for all 4 size classes I (< 25mm), II (26 – 50mm), III (51-75mm), and IV(>76mm) for each size class from each stream plus or minus the standard error (se). n = number of samples. Size classes with the same superscript letter are not statistically significant (P>0.05).

	Crab Size Class			
Stream	**I**	**II**	**III**	**IV**
A	-0.15 ± 0.15^{a}	0.11 ± $0.10^{a,b}$	0.45 ± $0.09^{b,c}$	0.15 ± $0.05^{b,c}$
n	8	9	5	10
B	0.09 ± 0.07^{a}	0.61 ± 0.16^{b}	0.67 ± 0.37^{b}	0.93 ± 0.10^{b}
n	32	3	2	9
Summary	0.04 ± 0.06	0.23 ± 0.09	0.52 ± 0.10	0.52 ± 0.08

Table 2.5. Results of the Post-ANOVA least squared means comparison test testing for differences in trophic elevation between size classes (I, II, III, and IV) in Stream A and B. Showing the P values.

Stream A	**I**	**II**	**III**	**IV**
I	--	0.0839	0.0012	0.0424
II	0.0839	--	0.0414	0.7664
III	0.0012	0.0414	--	0.0644
IV	0.0424	0.7644	0.0644	--
Stream B				
I	--	0.0324	0.0479	< 0.0001
II	0.0324	--	0.8706	0.2242
III	0.0479	0.8706	--	0.0644
IV	< 0.0001	0.2242	0.3943	--

Table 2.6. Results of the Post-ANOVA least squared means comparison test testing for differences in $\delta^{13}C$ between size classes (I, II, III, and IV) in Stream A and B. Showing the P values.

Stream A	**I**	**II**	**III**	**IV**
I	--	0.3184	0.1663	0.0014
II	0.3184	--	0.5749	0.0153
III	0.1663	0.5749	--	0.1233
IV	0.0014	0.0153	0.1233	--
Stream B				
I	--	0.9832	0.4087	0.0017
II	0.9832	--	0.5	0.0615
III	0.4087	0.5	--	0.4035
IV	0.0017	0.0615	0.4035	--

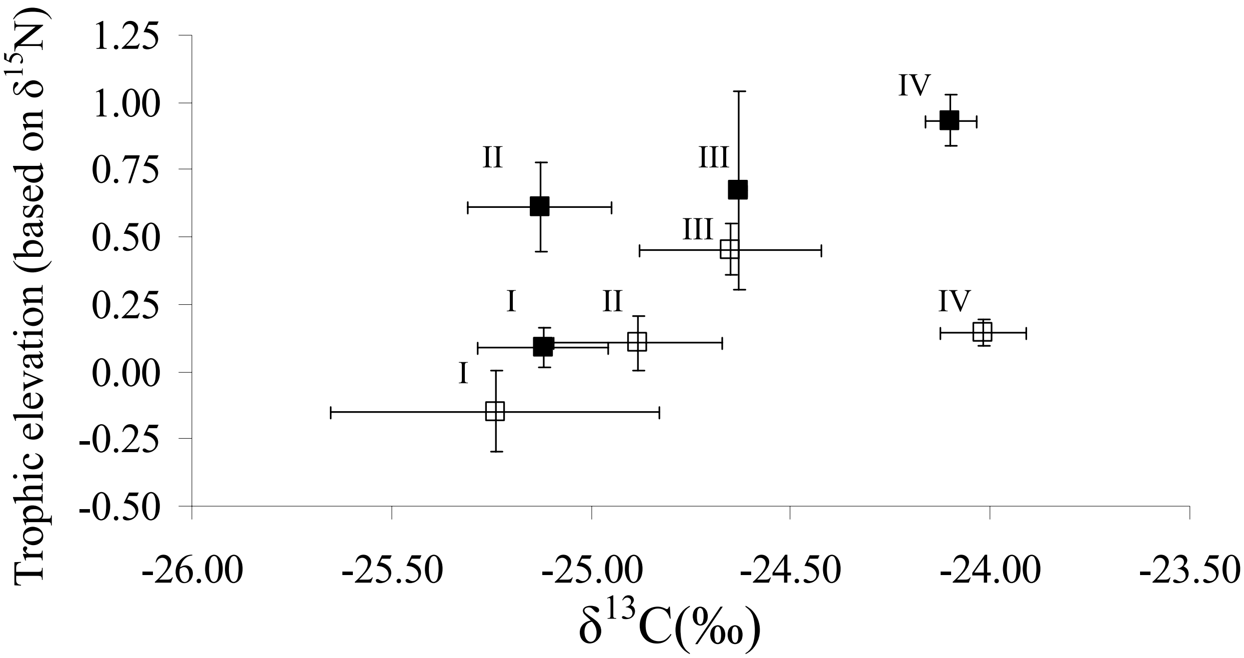

Figure 2.1. Relationship between trophic elevation and $\delta^{13}C$ of *Epilobocera sinuatifrons* for stream A (open circles) and Stream B (closed circles) based on equation 1 using the mean $\delta^{15}N$ values of mayflies from each stream and standard error bars. For each size class: I (< 25 mm) = 12.5 mm; II (26-50 mm) = 37.5 mm; III (51-75 mm) = 62.5 mm; and IV (> 76mm) = 87.5 mm.

CHAPTER 3

THE EFFECT OF FOOD QUALITY ON THE GROWTH RATE OF JUVENILE FRESHWATER CRAB (*EPILOBOCERA SINUATIFRONS*), USA[1]

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[1] Fraiola, K.M.S. and A.P. Covich. To be submitted to *Caribbean Journal of Science*, 07/30/06

Abstract

Riparian tree species on stream food webs are interconnected because of the importance of terrestrial plant material as a source of carbon to stream food webs. Both juvenile and adult crabs link terrestrial and aquatic components of riparian zones because as omnivores they consume a wide range of plant and animals foods and transport nutrients across habitat boundaries. Freshwater crabs are found in lotic habitats in many tropical areas around the world and are important to leaf litter processing in these aquatic habitats. Effects of leaves from different riparian tree species on growth rates of juveniles of the freshwater amphibious crab *Epilobocera sinuatifrons* were investigated. Comparative studies were also conducted on the effects of a mixed diet of plant and animal food resources relative to growth when juveniles were fed either only plant or only animal foods. Leaf litter from two native species and two non-native species were used as sources of food for comparisons of juvenile-crab growth rates on different species. Mean juvenile crab growth rate across all four leaf species was 0.019 mg $mg^{-1}day^{-1}$ when fed four different leaf species. Crabs did not grow significantly faster on a combination of plant and animal foods than on either food type alone. There was also no difference in growth rates when feeding on different species of plant. These are the first recorded instantaneous growth rates for the amphibious freshwater crab *E. sinuatifrons*. Our results show that it is slow growing and may take seven years to reach maturity.

Introduction

Interactions between terrestrial and aquatic components of watersheds and the effects of introduced species on stream food webs are active areas of study in temperate ecosystems (e.g., Lenat and Crawford 1994; Stenroth and Nyström 2003; Roy et al. 2003). These terrestrial – aquatic linkages are especially important because riparian plant material is a source of energy for detritivores in forested headwater streams (Wallace et al. 1997; Graça et al. 2001). Decreasing the amount of leaf litter available in headwater streams changes species abundances, biomass, and production of aquatic insects in a headwater stream of North Carolina (Wallace et al. 1997; Wallace and Hutchens 2000). Aquatic insects are important in the processing and cycling of the carbon within streams and as a food subsidy for terrestrial organisms in temperate-zone streams (Wallace et al. 1997; Nakano and Murakami 2001). Less documented are the roles of non-insect detritivores such as decapod crustaceans in both temperate (Usio 2000; Parkyn et al. 2001; Usio and Townsend 2002) and tropical catchments (Covich 1988; Crowl et al. 2001). This study focuses on freshwater crabs in rainforest headwaters where leaf litter is aseasonal and diverse (Thompson et al. 2002) but where non-native riparian vegetation is becoming widely dispersed and changing the timing and quality of litter inputs (e.g., O'Connor et al. 2000).

Changes in the timing and amounts of riparian leaf litter following introductions and establishment of non-native, invasive plants can have major effects on stream food webs once these new plant species become abundant. Relatively few studies have examined the importance of these invasive riparian plants on stream food webs in temperate-zone watersheds (Valdovinos 2001; Graça et al. 2001) and fewer studies have been conducted in insular tropical watersheds where invasive species are increasingly prevalent (Chong et al. 2000; O'Connor et al. 2000; Larned et al. 2003).

In many insular tropical streams, decapods such as freshwater shrimps play a major role in processing terrestrially derived carbon in streams and can attain high biomass (Crowl et al. 2001; Smith et al. 2003; De Souza and Moulton 2005; Crowl et al. 2006). Preliminary evidence suggests that juvenile freshwater crabs (*Epilobocera sinuatifrons*) in Puerto Rico utilize riparian inputs of leaf litter and aquatic insect larvae as food (Zimmerman and Covich 2003). Freshwater crabs are widely distributed in many tropical streams, although their foraging behavior is not well known (Covich and McDowell 1996; Dobson 2004). The natural abundances and feeding preferences of these crabs may be important in defining their role in processing organic matter derived from a wide range of riparian-produced foods. Omnivorous crabs are likely to be well adapted to feed widely on naturally diverse foods so that the addition of non-native species of riparian vegetation or of animal food may not significantly alter their growth rates.

The objectives of this study were first to measure the growth rate of juvenile *E. sinuatifrons* on indigenous and non-indigenous riparian leaf litter. The second objective was to compare growth rates on: a mixed diet of leaf litter from different species, a single species of riparian leaf litter, and on a control food (commercial crab food). Consequently, this study first tested the null hypotheses that growth rates are the same regardless of the source of leaves from different riparian tree species. A second null hypothesis we tested was that growth rates were the same regardless of consumption of a mixed diet of plant and animal material vs. a diet of only plant or animal foods.

Methods

Study Site

This study took place in the Luquillo Experimental Forest in northeastern Puerto Rico, latitude 18^{o} 18' N, longitude 65^{o} 47' W. This secondary, mature Tabonuco forest is dominated by *Dacryodes excelsa* (Burseraceae) Cecropia *scheberiana* (Moraceae) and *Prestoea acuminata* var. m*ontana* (Palmae) in riparian zones. Mean annual precipitation is 3600 mm, and stream temperatures typically range between 20 ˚C and 26 ˚C with little seasonal change. Headwater streams are heavily shaded and have low nitrogen and phosphorus concentrations resulting in limited primary production (Covich and McDowell 1996).

Epilobocera sinuatifrons is an amphibious crab that is commonly found in the upper reaches of stream networks in Puerto Rico (Rivera 1996; Zimmerman and Covich 2003). Both juveniles and adults are found in streams, although some adults also maintain burrows away (> 50 m) from stream channels and forage on land. Juveniles are found in a variety of microhabitats including leaf packs, under rocks and in gravel, riffles and pools (Zimmerman and Covich 2003).

The riparian forest community in Puerto Rican streams is changing rapidly in terms of species composition (Heartsill-Scalley and Aide 2003). The African Tulip tree (*Spathodea campanulata*) was introduced to Puerto Rico and now is the most widespread tree on the island (Franco et al. 1997). Another introduced tree, Rose Apple (*Syzygium jambos*), is among the top 10 most frequently found species in forests in Puerto Rico (Franco et al. 1997). These introduced trees are dominant in the forests and along the riparian zones of many Puerto Rican streams (Lugo et al. 2001; Heartsill-Scalley and Aide 2003; Lugo 2004; Brown et al. 2006). Because leaf litter input from *S. campanulata* and *S. jambos* may affect the abundance and biomass of freshwater crabs in streams, it is important to evaluate crab feeding rates on different sources of leaf litter and the consequences for individual crab growth rates.

Feeding Studies and Growth Rates

Freshly senesced leaves from two native species, *D. excelsa*, *C. scheberiana*, and two non-native species, *S. campanulata*, and *S. jambos* were collected from the forest floor for these experiments. Leaves were then incubated in-stream for 1 week, to allow for conditioning by microbes. To standardize leaf area, intact leaves were cut into circles with 2.26 cm^2 area using a cork borer. We minimized differences in initial leaf mass by standardizing leaf thickness and avoiding holes and mid-vein sections. Mayfly larvae were captured from streams and added as a distinct food treatment to microcosms soon after capture.

For the growth and leaf decomposition experiment, we set up 46 microcosms (59 cm · 43 cm · 15 cm) positioned randomly, similar to procedures used by Wright and Covich (2005). The growth experiment ran for 52 days from May 25 – July 15 2005. Each microcosm was aerated and covered with shade cloth to minimize light intensity, algal growth, and water temperature, and each contained 0.70 L of stream water collected from Rio Espiritu Santo, with a clay tile for shelter. Water depth was maintained about 2 - 3 cm so that the top of the shelter was above the waters, allowing crabs to climb out of water. Water temperature during the experiment was kept at 27° C ± 1.5, a typical temperature of mid-elevation streams in Puerto Rico, which is slightly warm than the environment they were collected, but within the range of stream temperatures they are known to inhabit.

Juvenile crabs of approximately 9 mm carapace width (CW) were collected by hand from the stream. This size was chosen based on preliminary data that showed crabs less than 5 – 6 mm were unable to capture mayflies in laboratory microcosms. Two native leaf species were used (*D. excelsa* and *C. scheberiana*) and two non-native species were used (*S. campanulata* and *S. jambos*) for investigating the native versus non-native leaf-litter food hypothesis. Three treatments were used, *D. excelsa* leaf discs, mayfly larvae, and a combination of mayfly larvae and *D. excelsa* leaves, for the single- versus mixed-diet study. For both of the previous comparisons commercial crab food (Hikari Tropical Crab Cuisine) was used as a “control”, because of its high nutritional value. There were six replicates per treatment. Crabs were fed 3 leaf disks of 2.26 cm^2 once a week. Leaf discs were removed after approximately one week to accommodate fastest consumption rates, so that the crabs would have food constantly. Water was changed once a week for all treatments, and leftover food (leaves and crab food) removed. Crabs supplied with mayflies as their food treatment were given 30 mayflies initially. Mayfly treatments were checked every day to determine if each crab had consumed any insects. If so, more mayflies were added to maintain a continuous availability of this food type. Dead or emerged mayflies were removed during feeding.

The control food treatment was initially 0.035 g of commercial crab food enriched in calcium. The initial amount of food given to crabs was chosen based on the manufactures recommendations. The amount of the control food was reduced to 0.015 g during the second half of the experiment after observing uneaten amounts of food. This reduced amount was sufficient for crabs to feed *ad libitum*.

Crab growth rates were calculated using the equation:

Growth = $\ln(M_f - M_0)/t$,

where M_0 = initial dry mass and M_f = final dry mass, and t = time (number of days grown). Initial crab weight was based on a carapace width to dry mass regression, $\ln(M_0)$= 0.3267(carapace width) + 0.4863 (R^2= 0.953). The regression was based on 15 juvenile crabs from the natural stream. At the end of the experiment, the crab's dry weight was measured. If a crab molt was left in the water with the crab so that it could be consumed. This was done because preliminary lab studies had shown that juvenile *E. sinuatifrons* consumed their carapace after molting. Crabs that died or escaped were replaced and measurements re-started.

Analyses

One way ANOVA with PROC GLM in SAS (Statistical Analysis Software) 9.1 with a significance level of 0.05 was used to analyze test for an effect of the treatment on growth rates in each comparison. The first comparison was between the mean growth rates of crabs in treatments, leaves, mayfly larvae, combination, and control. A second comparison was made between the leaf treatments, *D. excelsa*, *C. sheberiana*, *S. campanulata*, *S. jambos*, and control.

The mean growth rates in the "leaves" and "control" treatments from the first comparison were the same used in the second comparison, where the "leaves" treatment was *D. excelsa*. PROC GLM in SAS 9.1was used because of uneven sample sizes between leaf species treatments.

Results

Effects of Types of Leaf Litter

Initial crab carapace width ranged from 7 mm – 13 mm (Table 3.1). There was no statistically significant interaction between initial crab size and treatment ($F_{4, 14} = 2.16$, $P=0.127$) across the five treatments compared (two native and two non-native leaf species, and control food). Daily growth rates were retrieved from a total of 19 individuals, five from control-food treatment and 14 across all four riparian species of leaf litter. The growth rates among the leaf species ranged from -0.03 to 0.08 mg mg^{-1} day^{-1} (Figure 3.1), and for the control-food treatment it was 0.02 mg mg^{-1} day^{-1}. With mean growth rates increasing in this order *C. scheberiana* (native), *S. jambos* (non-native) < *D. excelsa* (native), < control food < *S. campanulata* (non-native). There was no statistically significant interaction between treatments (leaf species and control food) and crab growth rates ($F_{4, 14} = 0.25$, $P=0.904$).

Effects of Mixed plant and Animal Diet

Initial carapace width ranged from 7.2 mm – 12.7 mm (Table 3.2). There was no statistically significant interaction between initial crab size and treatment ($F_{3,12} = 2.19$, $P=0.142$) across the four treatments compared (control food, leaves (*D. excelsa*), mayfly larvae, and leaves + mayfly larvae). Daily growth rates were measured for a total of 16 individuals, five from control food, three, from *D. excelsa*, and four each from the mayfly larvae and combination leaf and mayfly treatments (Table 3.2). The growth rates for the control-food treatment and leaf treatment were the same as the previous section. The growth rates among the leaf, mayfly larvae, and leaf and mayfly larvae treatment ranged from -0.03 to 0.08 mg mg^{-1} day^{-1}, with the mean growth rates in the following order, leaf < control food < mayfly larvae < leaves + mayfly (Figure 3.2). There was no statistically significant interaction between these four treatments and treatments and crab growth rates ($F_{3,12} = 0.33$, P=0.8024).

Molt and Related Statistics

Of all the crabs raised in both experiments four crabs molted at least once and of those, three were restricted to feeding on *S. campanulata* (African Tulip) and one on control food. One of the crabs in the *S. campanulata* (African Tulip) treatment molted twice. The molt frequency for the control - food treatment was 39 days $molt^{-1}$(Table 3.3). For the *S. campanulata* treatment it ranged from 21 - 29 days $molt^{-1}$ and averaged 24 ± 2 days $molt^{-1}$ (Table 3.3). The change in carapace width per molt for the *S. campanulata* treatment ranged from 0.5 – 1.7 mm/molt and averaged 0.9 ± 0.3 mm $molt^{-1}$. For the control-food treatment it was 1.4 mm $molt^{-1}$. The growth in mm per day for the crabs that molted ranged from 0.02 – 0.06 mm $molt^{-1}$ and averaged 0.04 ± 0.01 mm $molt^{-1}$ for *S. campanulata*, and 0.03 mm/day for the control-food treatment (Table 3.3). The number of growth rate replicates per treatment was low and uneven due to deaths and escapes.

Discussion

In this study both the combination treatment (mayflies and *D. excelsa* leaves) and the control food treatment were nutritionally diverse. The lower crab growth rate on the control food treatment may have been a result of poor water quality from initial over feeding. The large range of growth rates among the different leaf species suggests that there may be some differences in food quality among the different species. Differences in the physical and chemical properties among the riparian species may account for the variation observed in mean growth rates of juvenile crab growth rates on different leaf species. These different attributes of leaves can limit the rate at which a stream detritivore can consume the food, and the nutritional value of the food (Webster and Benfield 1986; Cummins et al. 1989). Wright and Covich (2005) showed that the high lignin content in *C. scheberiana* and thick cuticle on *D. excelsa* leaves slowed microbial colonization of these leaves, and resulted in a reduced rate of microbial colonization and higher measures of leaf toughness. This higher toughness may prevent small juvenile crabs from shredding large portions of leaves and might result in low growth rates exhibited by these treatments. Future studies should look into the leaf chemistry of both *S. jambos* and *S. campanulata*, for they are make up a large proportion of the riparian tree species at lower elevations in Puerto Rican forests.

The observed similarity among growth rates on leaf litter from different species indicate we are unable to reject our null hypothesis. Nor were we able to reject the null hypothesis that juvenile *E. sinuatifrons* growth rates would not differ on a mixed diet of plant and animal food than on either plant or animal alone. Although there are no previously published growth rates for *E. sinuatifrons,* higher growth rates on mixed diets of plant and animal tissue have been reported for juvenile mud crabs (Pinheiro et al. 2005 and citations there in). How do the growth rates of *E. sinuatifrons* measured in this study compare to other stream organisms? Recent studies of an abundant filter feeding atyid shrimp that inhabits the same headwater streams was shown to grow at about the same rate, and its longevity is estimated to be at least 8 years (Cross and Covich in prep). Our crab growth rates were also within the range of growth rates for tropical omnivorous freshwater shrimp and temperate crayfish (Table 3.4).

The slow growth of these freshwater crabs in Puerto Rican streams indicates that this species is vulnerable to unregulated overharvesting. This species and most other crab species are eaten in many tropical regions and their distributions may be declining as a result of their slow growth (Rivera 1996; Maitland 2002; Obara et al. 2004). Based on the average daily growth rate from across all treatments in this study (0.02 mg mg^{-1} day^{-1}) I estimate the age of reproducing adult *E. sinuatifrons* is about 7 years, assuming crabs start from an initial dry mass of 0.005 g and reach a final dry mass of 60 g with a constant growth rate. This is a conservative estimate given that growth rates of animals after slow as they mature. It is also important to understand the life history and ecology of juvenile crabs within the stream ecosystem given the role that high densities of both juvenile and adult crabs may have as herbivores, detritivores, and predators (Fraiola et al. In press; Zimmerman and Covich 2003). These amphibious omnivores may have major impacts on stream and riparian food webs because they consume a wide range of terrestrially and aquatically produced foods. Some land crabs have been shown to have a significant impact on the forest plant community (Green et al. 1997). The subsequent reduction in crab abundances by introduced ants has caused a dramatic shift in the forest community (O'Dowd et al. 2003).

In summary, juvenile *E. sinuatifrons* grow slowly regardless food types. This slow growth makes *E. sinuatifrons* susceptible to over fishing as has been the case with other slow-growing crab species. From this study we did not have enough evidence to statistically show that juvenile growth rates differ among a mixture of plant and animal foods versus either plant or animal tissue alone. We also did not have enough evidence to show that juvenile growth rates differed when feeding on different species of plants.

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Table 3.1. Minimum (min) and maximum (max) initial crab carapace width (CW). Mean carapace width (standard error). N = number of replicate per treatment. Means with the same superscript letter are not statistically significant different (P>0.05).

		CW (mm)				
Treatment	N	min	max	Mean		
D. excelsa	3	9.4	19	10.5^a	±	0.7
C. scheberiana	3	7.9	13.5	11.8^a	±	1.7
S. campanulata	5	7.2	12	9.1^a	±	0.8
S. jambos	3	8.5	11.1	10.4^a	±	0.4
Control	5	7.2	10.8	8.8^a	±	0.4

Table 3.2. Minimum (min) and maximum (max) initial crab carapace width (CW). Mean carapace width (standard error). N = number of replicate per treatment, leaves (*Dacryodes excelsa*), mayfly larvae, and combination (leaves + mayfly larvae). Means with the same superscript letter are not statistically significant different (P>0.05).

		CW (mm)		
Treatment	n	min	max	Mean
Leaf	3	9.4	19	10.5^{a} ± 0.7
Mayfly larvae	4	8.1	12.7	9.9^{a} ± 1.0
Combination	4	7.9	9.7	8.2^{a} ± 0.5
Control	5	7.2	10.8	8.8^{a} ± 0.4

Table 3.3. Molt frequency of crabs. Initial carapace width (CW) in mm, change in carapace width (ΔCW) per molt, and the crab growth (CG) in $\Delta CW \cdot day^{-1}$ calculated as ΔCW/MF. n = number of replicates.

	Initial CW (mm)		MF			
Treatment	n	CW ± se	n	Days ± se	Δ CW (mm)	CG (mm/day)
Crab food	2	8.3 ± 1.1	2	28 ± 11	1.4 ± 0	0.058 ± 0.02
African Tulip	3	10.5 ± 1.3	4	27 ± 4	1.2 ± 0.25	0.041 ± 0.01

Table 3.4. Published individual instantaneous growth rates of different stream decapods including this thesis. All growth rates are presented in units mass per day and were calculated based on the equation $\ln(M_f - M_0)/t$, where M_f = final mass, M_0 = initial mass, and t = time grown. AFDM = ash free dry mass and d = days.

Organism	**Individual growth**	**Reference**
Macrobrachium haianense	0.002 - 0.004 AFDM/d	Mantel and Dudgeon 2004
Caridina cantonensis	0.041 - 0.004 AFDM/d	Yam and Dudgeon 2006
Cambarus bartonii	0.0045 - 0.0008 AFDM/d	Huryn and Wallace 198
Epilobocera sinuatifrons	0.03 - 0.08 mg/d**	K. Fraiola Thesis

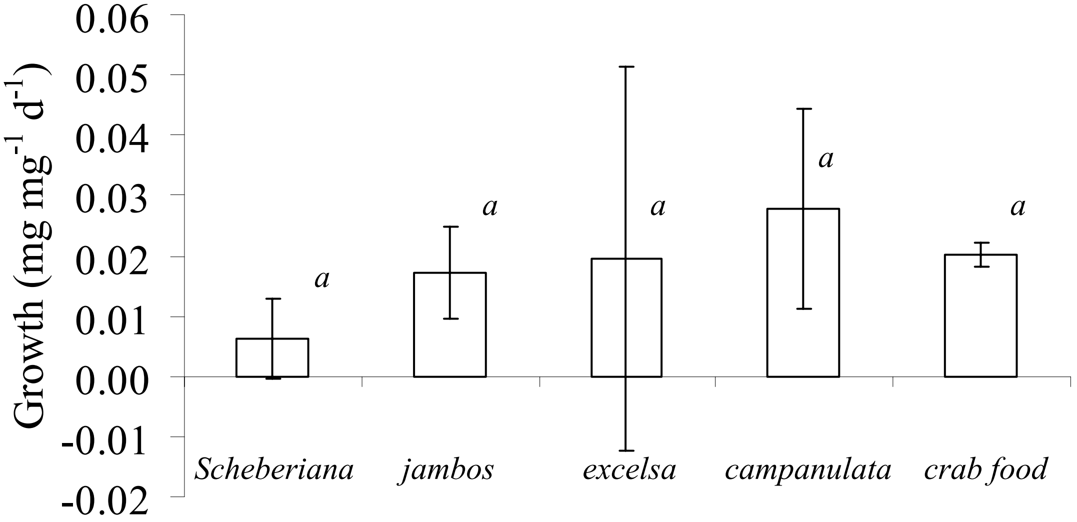

Figure 3.1. Mean juvenile crab daily growth rates when feeding on different leaf species: *Dacryodes excelsa* (native), *Cecropia scheberiana* (native), *Spathodea campanulata* (non-native), *Syzygium jambos* (non-native), and crab food. With standard error bars for leaf treatments. Means with the same letter are not statistically significant different (P>0.05).

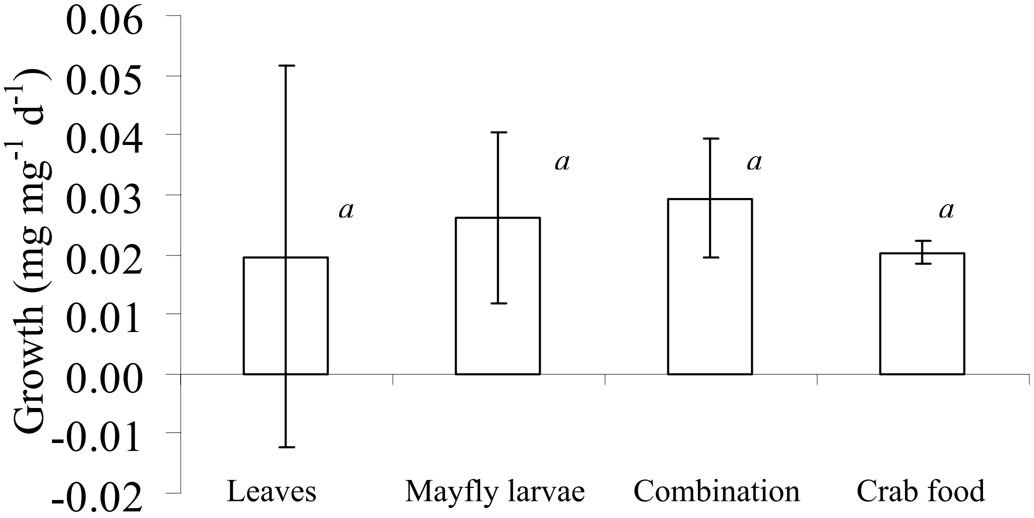

Figure 3.2. Graph of the mean juvenile crab daily growth rates when feeding on leaves (*Dacryodes excelsa*), mayfly larvae, combination (*D. excelsa* leaves and mayfly larvae), and crab food with standard error bars. Bars with the same letter are not statistically significant different ($P>0.05$).

Chapter 4

CONCLUSION

Our study suggests that juvenile *E. sinuatifrons* grow slowly regardless food type and that they feed on a wide variety of plant and animal material over the course of their life span. This broad feeding niche and slow growth has several implications for their role in stream food webs in Puerto Rico and other tropical ecosystems as well as enhanced management of their populations.

Because *E. sinuatifrons* feeds on a wide variety of plants and animals in the stream as well as adjacent forests and riparian zones, this omnivorous species can affect both terrestrial and aquatic communities, as well as ecosystem functions. For example, in streams their utilization of basal food resources such as fine particular organic matter and coarse particulate organic matter (e.g. leaf fragments) as juveniles means that they can play an important role in organic matter break-down and carbon flow in these stream ecosystems. As adults, their predation on other macro-consumers, such as filter feeders (atyid shrimps) means they can have a top down effect on how carbon is processed in these stream ecosystems. In the future, it would be interesting to study how the densities and size distributions of *E. sinuatifrons* within streams, as well as adjacent terrestrial areas, change along the stream gradient. Measuring the instantaneous growth rates of differently size individuals would also be important to consider in different streams throughout the species' range of distribution. A combination of size distributions, densities, and size-specific growth rates would make it possible to calculate the annual production of crabs. Rates of productivity are essential for understanding the role of organisms in food webs and ecosystem processes (Huryn and Wallace 1987).

Our observations of *E. sinuatifrons* feeding on a wide variety of seeds on land means they have the potential to impact the terrestrial plant community via seed and seedling predation such as is known for some species of land crabs in the Indian Ocean (O'Dowd et al. 2003). *E. sinuatifrons* also has the potential to affect animal communities on the forest floor where they forage, similar to species of land crabs in the Caribbean (Quensen and Woodruff, 1997). Future studies need to consider the complete role of *E. sinuatifrons* in terrestrial ecosystems as a seed/seedling predator as well as a predator of animals such as land snails in the leaf litter.

Their utilization of a broad range of plant and animal materials from terrestrial and aquatic ecosystems may also make crabs more susceptible to changes in habitat due to natural disturbances such as hurricanes because the forest floor can dry out and become inaccessible to adult crabs. Furthermore, less seeds and leaf litter production can be greatly reduced for many months following a major natural disturbance. Human disturbances such as deforestation can also result in reduced production of leaf litter and seeds. Future studies looking into the importance of omnivory as well as life-history omnivory to crab growth and survival are needed for comparisons in different locations. The two streams in Puerto Rico indicate the importance of local variability in food resources.

The slow growth rates combined with their relatively large size means it may take *E. sinuatifrons* 6 to 8 years to reach sexual maturity. This requirement may make them susceptible to over fishing, like other slow-growing crab species. Despite the lack of a commercial fishery, pressure from artisanal and recreational fisheries may be high, given the extremely high density of people on Puerto Rico and the easy access to forested head water streams via the extensive net work of roads in Puerto Rico. A current study looking into the effects that road crossings have on stream food webs as well as their value and connection to the people of Puerto Rico will help us to better understand the potential effects of harvesting on crab populations.

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APPENDICES

Appendix A.

1 = yes or female; 0 = male; -- = no or not present in a particular habitat.

ID	Stream	Capture Date	Gender	Carapace width (mm)	Size class	Within active channel	In Leaf pack	Under rock	On Gravel	Distance from active channel (m)	Gravid	Young	Number of young	Notes
1	A	07/16/05	--	5.1	I	1	--	1	--	--	--	--	--	Under rock
2	A	07/16/05	--	5.7	I	1	--	1	--	--	--	--	--	Under rock picking fine detritus from stream bottom
3	A	07/06/06	--	7.5	I	1	1	--	--	--	--	--	--	Leaf pack
4	A	07/07/06	--	7.5	I	1	1	--	--	--	--	--	--	Leaf pack
5	A	07/16/05	--	12.7	I	1	1	--	--	--	--	--	--	Leaf pack
6	A	07/12/05	--	13.7	I	1	--	1	--	--	--	--	--	Under rock
7	A	07/16/05	--	15.3	I	1	--	--	1	--	--	--	--	Standing in stream
8	A	07/16/05	--	20.6	I	1	1			--	--	--	--	Leaf pack
9	A	07/19/05	--	26.1	II	1	--	1		--	--	--	--	In gravel pull a liana seed into a crevice
10	A	07/12/05	--	28.5	II	1	--	--	1	--	--	--	--	In crevice
11	A	07/17/05	--	31.4	II	1	--	1	--	--	--	--	--	Wet under rock in stream
12	A	07/16/05	0	35	II	1	--	1	--	--	--	--	--	In stream under rock
13	A	07/18/05	--	35.1	II	1	--	1	--	--	--	--	--	Under rock
14	A	12/27/05	--	39.1	II	1	--	1	--	--	--	--	--	In stream under rock
15	A	07/16/05	0	41.8	II	1	--	--	1		--	--	--	Walking in stream
16	A	07/15/05	--	44.8	II	1	--	1	--		--	--	--	In stream under rock
17	A	07/18/05	0	48.7	II	--	--	--	--	15	--	--	--	15 m from stream
18	A	07/19/05	1	51.9	III	--	--	--	--	4	--	--	--	4 m from stream walking with two Tabonuko fruit
19	A	12/27/05	--	52.5	III	1	--	1	--		--	--	--	In stream under rock
20	A	07/19/05	0	57.1	III	--	--	--	--	2	--	--	--	2m from stream, eating a Guarea seed and holding a palm nut
21	A	01/06/06	--	59.3	III	1	--	1	--	--	--	--	--	In stream under rock
22	A	07/19/05	1	60.8	III	--	--	1	--	10 - 20	--	--	--	10 - 20m from main stem under large rock over looking pool
23	A	01/06/06	--	77.6	IV	1	--	1	--	--	--	--	--	In stream under rock
24	A	07/19/05	0	78.7	IV	--	--	--	--	2	--	--	--	2 m from stream under boulder
25	A	07/19/05	1	86.2	IV	--	--	--	--	1	--	--	--	1 m from stream, not gravid
26	A	07/19/05	1	89.3	IV	--	--	--	--	9	--	--	--	9 m from stream, not gravid
27	A	07/19/05	1	89.9	IV	--	--	--	--	1	--	--	--	1 m from stream, not gravid
28	A	07/18/05	0	92.7	IV	--	--	--	--	4	--	--	--	4 m from stream holding two Tabonuko seeds

29	A	07/18/05	1	94	IV	--	--	--	--	30	--	--	--	20 - 30 m from walking around
30	A	07/19/05	1	95.7	IV	--	--	--	--	2	--	--	--	2 m from stream picking through the leaf litter
31	A	07/19/05	1	97.2	IV	1	--	--	1		--	--	--	Walking in stream, caught large atya shrimp
32	A	07/19/05	1	102.8	IV	--	--	--	--	2	--	--	--	2 m from stream seems weak has big white patches on her shell
33	B	01/06/06	--	4.3	I	1	--	--	1	--	--	--	--	Picking fine detritus off stream bottom
34	B	01/06/06	--	4.5	I	1	--	--	1	--	--	--	--	Picking fine detritus off stream bottom
35	B	07/12/05	--	4.6	I	1	--	--	1	--	--	--	--	Dry in gravel
36	B	07/12/05	--	4.7	I	1	1	--	--	--	--	--	--	In leaf pack in water in stream
37	B	07/12/05	--	4.7	I	1	1	--	--	--	--	--	--	In leaf pack in water in stream
38	B	07/12/05	--	4.7	I	1	1	--	--	--	--	--	--	In leaf pack in water in stream
39	B	01/06/06	--	4.9	I	1	1	--	--	--	--	--	--	In leaf pack in water in stream
40	B	01/06/06	--	5	I	1	1	--	--	--	--	--	--	In leaf pack in water in stream
41	B	07/12/05	--	5.1	I	1	--	1	--	--	--	--	--	Under rock in stream
42	B	07/12/05	--	5.1	I	1	--	1	--	--	--	--	--	Under rock in stream
43	B	01/06/06	--	5.1	I	1	1	--	--	--	--	--	--	In leaf pack in water in stream
44	B	01/06/06	--	6.1	I	1	1	--	--	--	--	--	--	In leaf pack in water in stream
45	B	07/12/05	--	6.8	I	1	1	--	--	--	--	--	--	Dry leaf pack in crevice
46	B	01/06/06	--	8.4	I	1	1	--	--	--	--	--	--	In leaf pack in water in stream
47	B	07/12/05	--	8.6	I	1	--	--	1	--	--	--	--	Dry gravel bed under rock
48	B	07/12/05	--	9.9	I	1	--	--	1	--	--	--	--	In stream on dry gravel near water
49	B	07/12/05	--	9.9	I	1	--	--	1	--	--	--	--	In stream on dry gravel near water
50	B	07/12/05	--	10.9	I	1	--	--	1	--	--	--	--	On dry gravel bed
51	B	01/06/06	--	12	I	1	1	--	--	--	--	--	--	In leaf pack in water in stream
52	B	07/12/05	--	13.6	I	1	--	1	--	--	--	--	--	Under rock
53	B	07/14/05	--	14.3	I	1	--	--	1	--	--	--	--	At waters edge, in a pool
54	B	07/12/05	--	15.5	I	1	--	--	1	--	--	--	--	In crevice on a dry gravel bed
55	B	07/12/05	--	15.6	I	1	--	--	1	--	--	--	--	In dry gravel bed
56	B	07/12/05	--	16	I	1	--	--	1	--	--	--	--	In stream on dry gravel bed, under rock
57	B	07/12/05	--	16.4	I	1	--	--	1	--	--	--	--	Within gravel bed
58	B	07/12/05	--	17.7	I	1	1	--	--	--	--	--	--	Under dry leaf pack
59	B	07/12/05	--	17.7	I	1	1	--	--	--	--	--	--	Under dry leaf pack
60	B	07/14/05	--	18.2	I	1	--	--	1	--	--	--	--	Near pool on dry land
61	B	07/12/05	--	18.7	I	1	1	--	--	--	--	--	--	Inside dry leaf pack wedged in a crevice
62	B	07/12/05	--	22	I	1	1	--	--	--	--	--	--	Under a dry leaf pack
63	B	07/12/05	--	23.3	I	1	1	--	--	--	--	--	--	Inside a wet leaf pack in the stream
64	B	07/12/05	--	23.5	I	1	--	--	1	--	--	--	--	In a dry gravel bed under a rock

65	B	07/20/05	--	29.1	II	--	--	--	--	5	--	--	--	5 m from side channel on hill
66	B	07/13/05	--	34	II	--	--	--	--	3	--	--	--	Was eating a Guarea seed on land
67	B	07/13/06	0	47.8	II	--	--	--	--	2	--	--	--	2 meters from stream, tried to eat a smaller crab walking near by
68	B	07/20/05	1	66.5	III	--	--	--	--	15	--	--	--	15 m from side channel
69	B	07/21/05	1	70.2	III	1	--	1	--		--	--	--	Under boulder in water fall (small)
70	B	07/20/05	0	82	IV	--	--	--	--	15	--	--	--	15 meters from stream
71	B	07/13/05	1	87.8	IV	--	--	--	--	40	1	1	220	40 m from stream
72	B	07/13/05	1	91.5	IV	1	--	--	--	5	--	--	--	In pool 5m from main channel
73	B	07/16/05	1	94.3	IV	1	--	--	1		1	1	--	On rocks in a pool
74	B	07/14/05	0	95.2	IV	--	--	--	--	6	--	--	--	6m from stream
75	B	07/13/05	1	97.2	IV	1	--	1		--	1	--	--	Under boulder in riffle
76	B	07/14/05	0	100.4	IV	1	--	--	1	--	--	--	--	In a pool, which I caught many other crabs from in previous nights
77	B	07/13/05	0	100.5	IV	--	--	--	--	10	--	--	--	10 m from stream
78	B	07/13/05	1	104.8	IV	1	--	--	1	--	1	1	592	In a pool walking near bank

Appendix B.

"Corrected" values were calculated by adding either the C-13 correction factor (+ 0.35 ± 0.1 ‰ $\delta^{13}C$) or the N-15 (+ 0.12 ‰ $\delta^{15}N$ ± 0.12). -- = no correction factor needed. The correction factor was only applied to isotope values in which the whole organism was used.

ID	Stream	N-15 (‰)	Corrected	C-13 (‰)	Corrected
1	A	5.73	5.86	-26.62	-26.27
2	A	5.69	5.81	-26.59	-26.23
3	A	3.88	4.00	-25.91	-25.56
4	A	3.94	4.06	-25.36	-25.01
5	A	3.39	---	-25.66	---
6	A	4.30	---	-24.66	---
7	A	3.02	---	-25.84	---
8	A	5.19	---	-23.05	---
9	A	6.00	---	-24.52	---
10	A	3.60	---	-26.13	---
11	A	5.10	---	-25.61	---
12	A	4.84	---	-24.98	---
13	A	5.76	---	-24.66	---
14	A	5.12	---	-24.05	---
15	A	5.75	---	-24.74	---
16	A	5.54	---	-24.85	---
17	A	4.34	---	-24.40	---
18	A	5.38	---	-24.60	---
19	A	5.80	---	-25.40	---
20	A	6.10	---	-24.06	---
21	A	6.83	---	-24.35	---
22	A	5.90	---	-24.85	---
23	A	5.73	---	-23.34	---
24	A	5.64	---	-24.30	---
25	A	5.27	---	-24.62	---
26	A	4.44	---	-24.11	---
27	A	5.46	---	-24.11	---
28	A	4.99	---	-24.08	---
29	A	5.06	---	-23.73	---
30	A	5.42	---	-23.89	---
31	A	5.37	---	-24.05	---
32	A	4.81	---	-23.96	---
33	B	2.23	2.35	-25.34	-24.98
34	B	1.37	1.49	-25.73	-25.38
35	B	3.48	3.61	-26.74	-26.39
36	B	3.11	3.23	-27.13	-26.78
37	B	2.89	3.01	-27.55	-27.19
38	B	3.31	3.43	-26.96	-26.60
39	B	4.24	4.36	-26.00	-25.65
40	B	3.29	3.41	-25.33	-24.98
41	B	3.80	3.92	-26.82	-26.47
42	B	3.83	3.95	-26.19	-25.84
43	B	2.65	2.77	-25.54	-25.18
44	B	1.74	1.86	-25.31	-24.96
45	B	3.29	3.41	-25.77	-25.41
46	B	1.58	1.70	-25.44	-25.08

47	B	1.97	2.09	-24.36	-24.01
48	B	4.28	4.41	-24.91	-24.56
49	B	2.19	2.32	-24.61	-24.25
50	B	3.28	3.40	-25.76	-25.41
51	B	0.92	1.04	-25.22	-24.86
52	B	2.38	---	-24.72	---
53	B	1.41	---	-26.10	---
54	B	3.21	---	-24.87	---
55	B	4.66	---	-24.76	---
56	B	2.66	---	-25.19	---
57	B	4.17	---	-25.34	---
58	B	4.46	---	-24.49	---
59	B	2.24	---	-24.90	---
60	B	2.22	---	-23.10	---
61	B	4.77	---	-24.60	---
62	B	4.28	---	-24.99	---
63	B	3.23	---	-24.15	---
64	B	2.63	---	-25.11	---
65	B	3.62	---	-24.97	---
66	B	5.02	---	-24.93	---
67	B	4.61	---	-25.49	---
68	B	3.63	---	-24.61	---
69	B	5.50	---	-24.65	---
70	B	3.73	---	-24.42	---
71	B	4.92	---	-24.08	---
72	B	4.81	---	-24.03	---
73	B	5.96	---	-24.19	---
74	B	5.21	---	-24.31	---
75	B	5.47	---	-23.76	---
76	B	6.26	---	-24.11	---
77	B	5.08	---	-23.94	---
78	B	5.65	---	-24.05	---