ABUNDANCE, DISTRIBUTION, ENERGY FLOW AND NUTRIENT DYNAMICS OF FRESHWATER PALAEMONID SHRIMPS IN LOWLAND COSTA RICA

by

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(Under the Direction of Catherine M. Pringle)

ABSTRACT

Migratory shrimps are an important component of the aquatic fauna in many regions of the new and old world tropics. Freshwater shrimp are speciose (655 species) and are distributed in every biogeographic province in the world. In aquatic systems, many studies of secondary production and consumption patterns along different trophic pathways of stream invertebrates have increased our understanding of how animals contribute to energy flux and nutrient cycling in ecosystem flow. On islands and particularly, in Puerto Rico, many studies have contributed to our knowledge of the natural history and ecological role of migratory neotropical shrimps, whereas in continental regions, studies of freshwater migratory shrimp have just begun. Freshwater migratory shrimp are functionally important components of tropical island stream ecosystems and play a key role in food web stability, organic matter processing, and nutrient cycling. In addition, they are conduits for movement of energy and matter between marine and freshwater systems. The goal of this dissertation is to expand our knowledge of amphidromous shrimps by examining their distribution, abundance, and roles in energy flow and nutrient cycling in a continental region.

INDEX WORDS: shrimp, freshwater, streams, *Macrobrachium olfersi*, *Macrobrachium carcinus*, tropical, secondary production, occupancy, nutrient, abundance, growth, biomass

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DEDICATION

This dissertation is dedicated to my Grandmother, Pat Baldauf.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Rates of energy cycling and biogeochemical processes in aquatic ecosystems can vary greatly over space and time and understanding this variation is important for conserving aquatic ecosystems. Animals have long been thought to play important roles in aquatic ecosystem energy flow and biogeochemical cycling rates (Lindeman 1942, Odum and Smalley 1959, Burton and Likens 1975, Grimm 1988). In aquatic systems, many studies of secondary production and consumption patterns along different trophic pathways of stream invertebrates have increased our understanding of how animals contribute to energy flux and nutrient cycling in ecosystem flow (Benke 2010, Benke and Huryn 2010). Studies have also demonstrated the importance of animal consumers to nutrient and carbon cycling directly via excretion (McNaughton et al. 1997, Elser and Urabe 1999, McIntyre et al. 2007), indirectly through modification of the physical environment (e.g. bioturbation) (Pringle et al. 1999, Vanni 2002) or by creating nurient recyling hotspots (McIntyre et al. 2008). Differences in the level of research on the contribution to ecosystem structure and function of aquatic insects and fish vs. shrimps emphasize the need for more research on freshwater shrimp.

Migratory shrimps are an important component of the aquatic fauna in many regions of the new and old world tropics (Pringle et al. 1993, Crowl et al. 2001). Freshwater shrimp are speciose (655 species) and are distributed in every biogeographic province in the world. Atyidae and Palaemonidae families are the most numerically dominant of the freshwater shrimp species, which comprise 25% of the Caridea sub-order (De Grave et al. 2008). Fifty-three species of

Macrobrachium (Palaemoinidae) occur in the neotropics. The majority of *Macrobrachium spp.* display a form of diadromy know as amphidromy; their larvae require saltwater to develop even though they spend most of their lives in freshwater (Chace and Hobbs 1969). Amphidromy is thought to be far more common in the tropics and on islands than in other regions or landforms (McDowall 2007, 2009).

On islands and particularly, in Puerto Rico, many studies have contributed to our knowledge of the natural history and ecological role of migratory neotropical shrimps, whereas in continental regions, studies of freshwater migratory shrimp have just begun (Snyder et al. 2009). Freshwater migratory shrimp are functionally important components of tropical island stream ecosystems and play a key role in food web stability, organic matter processing, and nutrient cycling (Pringle and Hamazaki 1998, Covich et al. 1999, Pringle et al. 1999, Crowl et al. 2001, Rosemond et al. 2001). In addition, they are conduits for movement of energy and matter between marine and freshwater systems. This lack of knowledge of a common component of tropical freshwater ecosystems is surprising given the world-wide range and potential importance to energy flow and nutrient cycling of aquatic food webs.

The goal of this dissertation is to expand our knowledge of amphidromous shrimps by examining their distribution, abundance, and roles in energy flow and nutrient cycling in a continental region. My dissertation proposes to use field surveys and experiments to: (1) determine if freshwater shrimp populations, across elevational, discharge, and solute-richness gradients, exhibit differences in occupancy; (2) determine the contribution of shrimp to invertebrate biomass, secondary production and energy flow (3) determine if freshwater shrimp populations in relatively pristine upstream forested reaches in the Caribbean lowlands of Costa Rica at La Selva Biological Station have changed in terms of abundance, size and/or species richness from data collected twenty years previous in a historical time period (1988-1989); and (4) use ecological stoichiometry theory to refine the role of shrimps in stream nutrient cycling.

Four main chapters present results of my dissertation research. In Chapter 2, I discuss the distribution and habitat preferences of shrimp with low abundance and detection probability in lowland streams at La Selva Biological Station. I use occupancy modeling and logistic regression models to examine effects of natural landscape-scale variation in solute-rich, regional groundwater inputs on shrimp occupancy as well as other local (substrate and stream size) and landscape (distance to confluence with mainstem river) factors.

In Chapter 3, I estimate density, growth rates, and secondary production for three species within the shrimp genus, *Macrobrachium* and explore biotic and abiotic factors that influence growth rate and secondary production. I then estimate the contribution of shrimp to total invertebrate biomass and secondary production and discuss the findings in the context of energy flow rates in streams.

In Chapter 4, I use rare historical data to examine patterns of relative abundance over time in two streams at La Selva Biological Station. I compare shrimp relative abundance between a historical time period (1988-1989) and a recent time period (2008-2011). In order to compare relative shrimp abundance between the historical and recent time periods, first I evaluate evidence that abundance or body size varies seasonally or inter-annually using data from the recent time period. I also evaluate evidence for seasonal patterns in fecundity by measuring the number of gravid females and the proportion of females in shrimp populations to inform future monitoring efforts. I also evaluate the effectiveness of modified minnow traps in collecting shrimp population data for monitoring.

In Chapter 5, I examine how the predictions of stoichiometric theory apply to an omnivorous consumer in tropical streams across a P-gradient. I evaluate shrimp body stoichiometry and its degree of homeostasis, describe rates of phosphorus (P) excretion by shrimps; and compare the importance of shrimp (with low body %P) as nutrient recyclers, relative to fish (with have higher body %P).

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CHAPTER 2

USING OCCUPANCY MODELING AND LOGISTIC REGRESSION TO ASSESS THE DISTRIBUTION OF SHRIMP SPECIES IN LOWLAND STREAMS, COSTA RICA; DOES REGIONAL GROUNDWATER CREATE FAVORABLE HABITAT? [1]

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[3] M.N. Snyder and C.M. Pringle. To be submitted to *Freshwater Biology*.

Abstract

Aim While freshwater shrimps often have a very low probability of detection because of their low abundance in many continental streams, they are an important biotic component. We used occupancy modeling and logistic regression models to examine both local- and landscape-scale effects on shrimp distribution in the Caribbean lowlands of Costa Rica. Local-scale factors included substrate and stream size while landscape-scale factors included presence or absence of regional groundwater inputs and distance to confluence with the mainstem river. Model results can be used to prioritize areas for conservation before landscape changes fragment rivers and alter shrimp access to high-quality patches.

Location La Selva Biological Station, Sarapiquí, Costa Rica

Methods We sampled three of the most common freshwater shrimp species, *Macrobrachium olfersi, Macrobrachium carcinus,* and *Macrobrachium heterochirus,* across a gradient of conductivity (which is an indicator of regional groundwater input) and stream sizes at La Selva Biological Station (1600 ha). Capture rates were sufficient for one species (*M. olfersi*), to compare the fit of occupancy models representing alternative hypotheses of how local and landscape factors might influence occupancy. Occupancy models did not converge for *M. carcinus* and *M. heterochirus* however, *M. carcinus* had high enough occupancy that logistic regression could be used to model the relationship between occupancy and local and landscape predictors.

Results The best-supported models for *M. olfersi* and *M. carcinus* included conductivity, discharge and substrate parameters. High stream conductivity, which reflects the quantity of regional groundwater input into the stream, and stream size were positively correlated with the probability of *M. carcinus* and *M. olfersi* occupancy. Sandy substrates increased the occupancy of *M. olfersi* and decreased the occupancy of *M. carcinus.*

Main conclusions Our models suggest that shrimp distribution is driven by factors that function at local (substrate, discharge) and landscape (conductivity) scales. Findings suggest that high-conductivity stream reaches characterized by regional groundwater inputs are of high habitat quality for shrimp and are potentially important areas to prioritize for conservation of *M. carcinus* and *M. olfersi* populations.

Introduction

Freshwater shrimps are functionally important components of tropical streams. For example, shrimps have been found to play a key role in food web stability and nutrient cycling in island stream ecosystems, where they comprise the dominant macroconsumer biomass (Pringle et al. 1993, Covich et al. 1999, Crowl et al. 2001). Due to their migratory life history, shrimps are vulnerable to changes occurring in river systems, including damming and land use changes that reduce connectivity and alter water quality (Holmquist et al. 1998, Pringle and Scatena 1999, Dudgeon 2000). In Costa Rica, where this study was conducted, hydropower impoundments, non-point sources of pollution (e.g. pesticide run-off from agriculture) and point sources of pollution (e. g. wastewater effluents) potentially alter habitat quality for freshwater shrimps (Castillo et al. 1997, Pringle and Scatena 1999, Anderson et al. 2006).

Two characteristics of freshwater shrimp populations in lowland Costa Rica suggest that they are potentially vulnerable to alterations in stream habitat quality: (1) low densities; and (2) migratory life history strategy. Amphidromous shrimp species migrate between fresh and salt water (Obregon 1986). Some *Macrobrachium* spp. travel the watershed twice; once as larva drifting passively to the estuarine nursery grounds and again as juveniles migrating upstream to headwater streams, where adults reside (Chace and Hobbs 1969). Even though *Macrobrachium* species may have large geographic ranges, extending from Brazil to Florida and including the Lesser and Greater Antilles (Chace and Hobbs 1969, Bowles et al. 2000), their densities in Costa Rica are low (0.06-0.15 individuals m^{-2}) (Ramírez and Pringle 2004).

Understanding which freshwater habitat conditions promote survival of shrimps will help us identify sites that could be of high priority for their protection or conservation. High quality habitats can potentially create areas where source populations of adult shrimps reside, which could be important for maintaining population levels in lower quality habitat areas. However, the survival, abundance, or even the distribution of low-density species can be difficult to determine. Recently, occupancy modeling has been used to provide knowledge of the distribution, occupancy dynamics, and impact of management on species that are rare or of conservation concern (Albanese et al. 2007, Kroll et al. 2010, Wilson and Roberts 2011). Occupancy models relate both presence/absence and detection/non-detection data, from temporal or spatial replicate samples, to environmental predictor values (MacKenzie et al. 2002, Tyre et al. 2003). Occupancy modeling is a useful technique for studying the distribution and abundance of species with low densities, such as freshwater shrimp in continental systems. While these models of species occupancy do not directly measure population abundance or survival, the underlying principle is that changes in probability of occupancy are correlated with changes in population size and density.

In this study, we use occupancy modeling and logistic regression to determine factors driving shrimp distributions across multiple scales in streams draining lowland Costa Rica. There is very little information on the distribution and habitat requirements of freshwater shrimp species in continental stream systems where they often occur at low abundance relative to other stream taxa (but see Walker and Ferreira 1985, Bowles et al. 2000, Hernández et al. 2007). Amphidromous shrimp can migrate long distances (>100 km) and therefore their distribution and abundance are likely to be determined by a combination of local-, network-, and landscape-scale factors. As observed by Falke et al. (2010), occupancy models have been used in >350 peer-reviewed publications to study a wide variety of organisms, although rarely stream-dwelling invertebrates. This is the first study to our knowledge that has used occupancy modeling to study freshwater shrimp distribution.

The aim of our study was to understand the local- and landscape-level factors that influence the distribution of shrimps in streams draining a lowland tropical rainforest, where local habitat is relatively unaffected by agriculture or other land use alterations. In the landscape (1600 ha) of La Selva Biological Station (LSBS) habitat patches of varying quality are created by inputs of solute-rich, regional groundwater rich in phosphorus (Pringle and Triska 1991). In solute-rich habitat patches, macroinvertebrates have increased growth rates, and there is higher basal resource quality (%P body elemental content) (Ramírez and Pringle 2006, Ramírez et al. 2006, Small and Pringle 2010). Solute-rich stream reaches are buffered from seasonal acidification, exhibit more stable pH values (Small et al. in press) and have higher quantities of calcium (Small et al. in press) than solute-poor stream reaches. In this study, we test the effects of natural landscape-scale variation in solute-rich, regional groundwater inputs on shrimp occupancy. We expect that because of the migratory nature of shrimps, landscape-level factors such as regional groundwater input and distance upstream from the mainstem river will be strong predictors of shrimp occupancy. We predict that shrimp occupancy will be positively influenced by regional groundwater and negatively influenced by the distance upstream from the mainstem river. We also expect that in addition to landscape-level factors, local-level factors such as substrate size and stream size will be strong predictors of shrimp occupancy. We predict that the effect of substrate size on shrimp occupancy will differ by species, but expect that stream size will positively affect shrimp occupancy.

Methods

Study Site

This study was conducted at La Selva Biological Station (LSBS) (10° 26” N 84° 01” W) a forested reserve located on the Caribbean slope of Costa Rica at the intersection of the coastal plain with the Cordillera Central. The geomorphology of the region results in heterogeneous stream chemistry because geothermally modified regional groundwater surfaces at the break in the landform (Pringle 1991). Magmatic outgassing and weathering of volcanic rock creates high levels of solutes (P, Cl, Na, Mg, HCO_3, Ca) in regional groundwater, ranging from 13-29 times more concentrated than solute levels in local groundwater (Genereux and Pringle 1997). Conductivity is positively correlated with the concentration of solutes in the rivers and the percent of water that is supplied by regional groundwater (Pringle and Triska 1991). In this study, we use conductivity as a surrogate for quantity of regional groundwater input and solute concentration.

Average annual temperature at LSBS is 25.8° C, with monthly mean daily temperatures ranging from 24.7 ° C in January to 27.2° C in August (Sanford et al. 1994). The elevation of LSBS ranges from 30-132 m asl.

Fifteen species of amphidromous shrimp occur in Costa Rica, with seven species occurring on the Caribbean side (Obregon 1986). We created models for three of the most frequently encountered shrimp species (*Macrobrachium olfersi, M. carcinus* and *M. heterochirus*) on the Costa Rican Caribbean slope (Obregon 1986). The range of *M. olfersi, M. carcinus* and *M. heterochirus* extends from Florida to Brazil and includes the greater and lesser Antilles (Chace and Hobbs 1969, Bowles et al. 2000). Distributions of these three shrimp species in Costa Rica are not well known, but it is known that they extend from lowland elevations to at least 900 m asl (Obregon 1986).

Sampling

We sampled 360 sites on the Salto, Sura, and Saltito rivers between June and August 2008. The streams sampled ranged from first- to third- order in size, and all drain into the Puerto Viejo River. Modified breeder minnow traps, baited with dried cat food in mesh bags, were used to sample for shrimps (Covich et al. 1996). Traps were placed within 14 stream reaches that were distributed across gradients of longitudinal stream distance and conductivity levels (Figure 2.1). Each trap was considered as a site and was randomly placed greater than four meters apart. Traps were placed during the day, left overnight, and checked each of the three subsequent days. Traps were rebaited each day. The carapace length (CL, mm), sex, and gravid state of each captured shrimp was measured and recorded in the field and individuals were then placed back in the stream. Species identities were assigned according to Obregon (1986).

At each trap site, we categorized the substrate in a three-meter radius around the trap into one of five categories based on prevalence of different size substrates (>66% boulder, <33% sand; 33-66% boulder, >33% sand; >66% sand/gravel, <33% boulder; >66% sand/gravel, <33% silt; >66% clay, <33% sand). Substrate was categorized in this manner because of the high heterogeneity of substrate types over a small area (e.g. a 100-m reach could contain areas that were predominantly boulder substrate and areas of predominantly sand substrates). Leaf litter, and coarse wood could be found at a site in any of the substrate categories. Conductivity, turbidity, discharge, and distance were measured for groups of trap sites. Conductivity (μS cm^{-1}) was measured using a hand held Hanna HI9033 meter. Turbidity (cm) was measured with a turbidity tube (120 cm) manufactured by Carolina Biological Supply (CBS, Burlington, NC, USA) on each day the traps were checked. Distance (m) from the tributary site to the Puerto Viejo River was measured using ArcGIS software (ESRI 2009) and was recorded as "distance to mainstem river." Distance to the mainstem was included as a surrogate for the amount of energy expenditure a shrimp would use during migration. Stream discharge (m^3 s^{-1}) was calculated by combining average water velocity measured with a Marsh-McBirney Model 2000 flow meter with cross-sectional area of the wetted channel. Water velocity and water depth were measured every 0.10 m along the transect, which was also used to measure the cross-sectional area. We used stream discharge as a relative measure of stream size.

Statistical Analysis

Our objective was to compare support for alternative models as predictors of shrimp occupancy (Table 2.1), using capture data at sites varying in substrate characteristics, stream discharge, distance to the mainstem, and conductivity. To do this, we applied occupancy models to our capture data, using maximum likelihood methods in Program Presence (Hines 2009). Occupancy modeling allows one to measure the strength of support for habitat covariates as predictors of proportion of sites occupied (ψ) by a species, while accounting for incomplete probability of detection (*p*) (MacKenzie et al. 2002, MacKenzie et al. 2006). In our study, *p* was the probability of detection given that a shrimp was present at the site of a trap, and was estimated from the capture observations on three sequential trap-nights at each site. We expected detection probability to change as traps remained in place, as well as, in relation to turbidity;

therefore *p* was allowed to vary as a function of trap night and turbidity. This approach assumed that the probability of capture at each site was independent of every other site, that sites were closed to changes in occupancy for the duration of the survey period (three nights), and that there were no false detections of shrimps.

To test hypothesized relations between habitat factors and shrimp occupancy, we initially modeled ψ with no covariates (i.e. null model), followed by a suite of models predicting ψ that contained combinations of the covariates that represented different *a priori* biological hypotheses (Table 2.1). In cases when occupancy models did not converge, we used logistic regression to test hypothesized relations between habitat factors and shrimp occupancy. Logistic regressions were modeled in Program R by using generalized linear models with binomial distributions and logit links (R Development Core Team 2011). In the logistic regression models, a shrimp species was counted as present if it was detected once at a site during any of the three sequential trap-nights and was counted as absent if it was not detected during any of the trap-nights.

Relative model support for occupancy and logistic regression models was assessed using Akaike's Information Criterion with a small-sample size adjustment (AIC_c) (Burnham and Anderson 2002). The best supported models were those with the lowest AIC_c score. We created a confidence set of models by including those models that had delta AIC_c scores less than two, which suggests substantial support of the model (Burnham and Anderson 2002). We used the ratio of model weights to compare the relative likelihood of models compared to the best-supported model (Burnham and Anderson 2002). Model fit was assessed by calculating $\hat{c}$ (a measure of over-dispersion) for the top ranked occupancy model (MacKenzie and Bailey 2004). For the logistic regression models, fit was tested by using a chi-square test to compare the intercept model without covariates to the best-supported model (Hosmer and Lemeshow 2000).

To extrapolate effects of covariates on predicted occupancy, we derived model-averaged covariate effect sizes using the regression coefficients from the confidence set of models (Burnham and Anderson, 2002). We calculated a model-averaged value for the beta values of the predictor variables by adding the products of the beta values multiplied by the model weight and dividing that by the total combined weight of the confidence set of models (Burnham and Anderson 2002).

Results

None of the shrimp species studied had high occupancy rates, which confounded tests of relationships between occupancy and habitat variables for two of the species. Out of 1,308 trap nights 276 *M. olfersi*, 32 *M. heterochirus*, and 113 *M. carcinus* were trapped and measured (Table 2.2). The mean occupancy for *M. heterochirus* was 6% across sites. Occupancy models with covariates for this species did not converge, and captures were too sparse to support predictive logistic regression models. However, *M. heterochirus* was only present at sites with boulder substrate (occupancy rates were 16% at sites with >66% boulder and 3% at sites with 33-66% boulder).

The observed occupancy (the occupancy rate without correcting for detection probability at a site) for *M. carcinus* across sites was 23% and occupancy models with covariates and detection probability did not converge. Therefore, logistic regression models were used to model relationships between *M. carcinus* occupancy and habitat variables.

Of the models selected to be tested *a priori*, two models of *M. carcinus* occupancy had $\Delta AIC_c < 2$ and were included in the confidence set of models (Table 2.3). Covariates that occurred in the top two models were conductivity, substrate, and discharge. The top two models were distinguished as the most likely models by the ΔAIC_c score, which was <1.11. The model with the next smallest AIC_c score was 4.5 times less likely to be the best model (w_i=0.09). The top two models accounted for 0.65 of the total model weight.

In the best-supported models, discharge and conductivity increased the probability of shrimp occupancy (Fig. 2.3). Substrate composed of >66% sand and <33% silt consistently decreased the probability of *M. carcinus* occupancy. The confidence intervals for the other substrates included zero, indicating the possibility of no effect.

We averaged, by model weight (w_i), the parameter values from the best two models to simulate *M. carcinus* occupancy values across a range of conductivity values, substrate types, and discharge using the parameters included in the models (Fig. 2.3). At the mean discharge (i.e. stream size), the probability of occupancy increased as much as 3–fold across the range of observed levels of conductivity (Fig. 2.3A). Occupancy also increased with stream size (indexed by discharge), by approximately 1.4-fold over the range of discharge values given a low-solute condition and the highest occupied substrate (Fig. 2.3B). Parameter estimates for the effect of substrate composition showed lower occupancy in microhabitats dominated by clay, and higher occupancy with a higher percentage of boulders.

M. olfersi was detected at 121 of the 360 sites and the observed occupancy rate was 34%. The occupancy rate corrected by detection probability was 55% (standard error= 6%). The model $\hat{c}$ values were near 1.1 indicating adequate model fit, so we did not adjust the model standard error values (MacKenzie and Bailey 2004).

Of the models selected to be tested *a priori*, three models had delta AIC_c <2 and were included in the confidence set of models (Table 2.4). Covariates that occurred in all of the top three models were substrate, discharge, and conductivity. The top three models were distinguished as the most likely models by having a ΔAIC_c score which was <1.05. The model with the next smallest AIC_c score was 3.1 times less likely to be the best model (w_i=0.13). The top three models accounted for 0.91 of the total model weight. All of the top ranking models included turbidity as a covariate in the probability of detection. Including this covariate, and allowing the probability of detection to vary by trap night, improved model supported compared to models with a constant probability of detection (which had higher AIC_c values). In the confidence set of models, the first trap night had a higher probability of detection than the second or third. On the first trap night, turbidity positively influenced detection probability however, on the second and third night higher turbidity decreased the probability of detection. The naïve estimate of occupancy (34%) would have underestimated occupancy by 21%.

In all three of the best-supported models, discharge and conductivity increased the probability of *M. olfersi* occupancy (Table 2.4). Substrate composed of >66% sand and <33% silt consistently increased the probability of *M. olfersi* occupancy, although the confidence interval included '0' indicating the possibility of no effect.

We averaged, by model weight (w_i), the parameter values from the best three models to simulate occupancy values across a range of conductivity values, substrate types, and discharge using the parameters included in the top model (Ψ(substrate, discharge, conductivity) *p*(day, turbidity)) (Fig. 2.4). At the mean discharge (i.e. stream size), the probability of occupancy increased as much as 7–fold across the range of observed levels of conductivity (Fig. 2.4A). Occupancy also increased with stream size (indexed by discharge), by approximately 3-fold over the range of discharge values, given a low-solute condition and the highest occupied substrate (Fig. 2.4B). Parameter estimates for the effect of substrate composition showed higher occupancy in microhabitats dominated by sand or clay and lacking boulders.

Discussion

Our results suggest that larger streams that receive regional groundwater input may be of conservation value for two of the three shrimp modeled in this study, *M. olfersi* and *M. carcinus*. Our models were unable to determine the effects of regional groundwater and stream size on *M. heterochirus* occupancy rates because it occurred very rarely (6% of sites) and only in boulder-dominated streams. *M. olfersi* and *M. carcinus* occupancy rates were best predicted by discharge, conductivity, and substrate factors. Occupancy rates in larger streams with regional groundwater inputs were predicted to be as much as 7-fold higher for *M. olfersi* and 3-fold higher for *M. carcinus*. To the extent that higher occupancy rates reflect higher density (Bart and Klosiewski 1989, MacKenzie 2005), our results imply that these areas could be important for maintaining *M. carcinus* and *M. olfersi* shrimp populations.

Our study suggests that *M. olfersi* and *M. carcinus* distributions are affected by factors at both the landscape- and local-scale. Multiple biological mechanisms may be responsible for the effects of landscape (conductivity) and local-scale predictor variables (discharge and substrate) in occupancy models for these two species. The positive relationship between stream size and occupancy could be explained by an increase in diversity of habitats or more abundant food (Table 2.1). Shrimps are preyed upon by a variety of predators in these streams including fish, caiman, and otters (Bussing, 1994). Both diversity and abundance of fishes has been shown to increase with stream size (Burcham, 1988). Substrate could affect shrimp populations by providing cover from predation or by influencing the variety of food resources available. While the low number of detections and lack of model convergence makes it difficult to infer what factors control *M. heterochirus* occupancy, our findings that *M. heterochirus* was only found among boulders agreed with the findings of Hunte (1978), who only observed this species in high-gradient, boulder-dominated streams in Jamaica. Our results and the findings of Hunte (1978) suggest the importance of local-level factors for this species.

A variety of mechanisms could be responsible for the positive effects of regional groundwater on *M. olfersi* and *M. carcinus* occupancy. One possible mechanism is that higher quality food resources are supporting higher occupancy in these stream reaches. Shrimps are omnivores that consume both periphyton, macroinvertebrates, detritus, and fish (Chace and Hobbs 1969). Other studies at La Selva have shown that the basal resources (leaf litter, epilithon, fine particulate organic matter) within high-solute stream reaches have up to four times the quantity of phosphorus than those in low-solute streams, which indicates a higher quality food resource (Small and Pringle 2010). Moreover, insect macroinvertebrates using these higher quality basal resources can have two-fold increases in elemental body P-content as well as having higher growth rates (Ramírez et al. 2006, Small and Pringle 2010). Other studies across a variety of aquatic ecosystems have found that temporary increases in food resource quality positively influence consumer density and productivity (e.g. Slavik et al. 2004). The only research that we are aware of that relates solute levels and the distribution of shrimps is that of Thorpe and Lloyd (1999), that found no relationship with shrimp density and solutes in streams converted from forest to agriculture on the island of St. Lucia.

High calcium levels in regional groundwater may also have a positive effect on *M. olfersi* and *M. carcinus* occupancy rates. Low-solute streams at La Selva Biologial Station have calcium levels (0.0-0.7) mg L^{-1} that are below the recommended values (12-24 mg L^{-1}) used in aquaculture production of *Macrobrachium rosenbergii* (Pringle and Triska 1991, New 2002). High-solute streams at La Selva have higher calcium levels (3.0-19.0 mg L^{-1}), which suggests shrimp in high-solute streams could be experiencing less calcium limitation than shrimp in low-solute streams.

Our finding of higher occupancy of *M. carcinus* and *M. olfersi* in stream reaches receiving regional groundwater (with high-solute levels), also suggest that these streams may serve as refugia for shrimp during seasonal pH drops. Streams at LSBS exhibit seasonal trends in pH, however high-solute streams reaches are rich in bicarbonate and are thus buffered against pH drops, exhibiting more stable pH values (Small et al. in press). Long-term averages of pH for solute-poor streams (5.33-5.77) are 1.5 pH units below values (6.2-7.4) which aquaculture studies have shown negative effects on shrimp growth, moulting frequency, and biomass accumulation of *Macrobrachium rosenbergii* (Chen and Chen 2003). The mean long-term pH value (6.03-6.34) for high-solute stream reaches is near the lower limit of acceptable pH values from aquaculture studies.

Although occupancy modeling was designed to account for incomplete detection, it still has limitations. Occupancy modeling allowed us to create models for a shrimp species with low detection probability, *M. olfersi*, but was not powerful enough to make predictive models of the rarer species such as *M. carcinus* and *M. heterochirus*. For these two taxa, the maximum likelihood models did not reach convergence. Thus logistic regression proved to be the most effective modeling tool in our study for *M. carcinus*. Low detection probability, or the low percentage of traps occupied by *M. carcinus* (22%) and *M. heterochirus* (6%), could be responsible for the lack of model convergence. Alternatively, the lack of model convergence could indicate that the populations were influenced by factors that we did not measure.

In summary, occupancy models specified estimates of occupancy for *M. olfersi* and logistic regression models specified estimates of occupancy for *M. carcinus*, providing insights into factors influencing their distribution. In the new and old world tropics, migratory shrimps play an important role in stream food webs and ecosystem function, and are important conduits for movement of energy and matter between marine and freshwater systems (Pringle and Hamazaki 1998, Covich et al. 1999, Rosemond et al. 2001). Understanding what factors affect distribution patterns of migratory shrimp is a first step towards conservation of these species. Specifically, our results show that regional groundwater input positively influences *M. olfersi* and *M. carcinus*. These models could be used to prioritize areas beneficial for these wide-ranging migratory species before landscape changes fragment rivers and alter shrimp access to high quality habitat patches.

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Table 2.1. The scale and biological hypothesis developed *a priori* for the predictor variables used in the occupancy and logistic regression models to predict occupancy of *Macrobrachium carcinus* and *Macrobrachium olfersi* at La Selva Biological Station, Costa Rica.

Predictor variables	Scale	Biological interpretation (hypothesis)
Substrate	microhabitat	The amount of cover from predation and food resources differs by substrate and influences shrimp behavior.
Discharge	microhabitat	Predator abundance increases as discharge (stream size) increases; food resource availability may increase or decrease as discharge increases depending on the food resource; habitat diversity increases.
Conductivity	landscape	Abundance and quality of food resources increase as conductivity increases.
Distance to mainstem	landscape	As the distance to the mainstem increases shrimp predators may decrease, however shrimps expend more energy during migration, and have a lower probability of reproduction success

Table 2.2. Total number and sex (when determinable) of shrimp sampled across all sites on the Sura, Salto, and Saltito Rivers at La Selva Biological Station, Costa Rica.

	M. olfersi	*M. heterochirus*	*M. carcinus*
Total measured	276	32	113
Male	190	19	40
Female	55	9	49
Male:female sex ratio	3.4:1	2.1:1	0.8:1

Table 2.3. The confidence set of models for predicting occupancy of *Macrobrachium carcinus* showing model predictor variables for occupancy and values for Akaike's Information Criterion with the adjustment for small sample size (AIC_c), change in AIC_c (ΔAIC_c), Akaike weights (w_i), and number of parameters (k) in each of the models. Models were included in the confidence set of models if the change in AIC_c was less than two.

Model	AIC_c	ΔAIC_c	w_i	k
Substrate, conductivity	395.1	0.00	0.41	6
Substrate, conductivity, discharge	396.2	1.11	0.24	7

Table 2.4. The confidence set of models for predicting occupancy of *Macrobrachium olfersi* showing model variables for occupancy (ψ) and detection (*p*) and values for Akaike's Information Criterion with the adjustment for small sample size (AIC_c), change in AIC_c (ΔAIC_c), Akaike weights (w_i), and number of parameters (k) in each of the models. Models were included in the confidence set of models if the change in AIC_c was less than two.

Model	AIC_c	ΔAIC_c	W_i	k
ψ(substrate, discharge, conductivity) *p*(trap night, turbidity)	819.6	0.00	0.41	13
ψ(substrate, substrate*conductivity, conductivity, discharge) *p*(trap night, turbidity)	820.6	0.91	0.26	14
ψ(conductivity, substrate, discharge, distance) *p*(trap night, turbidity)	820.7	1.05	0.24	14

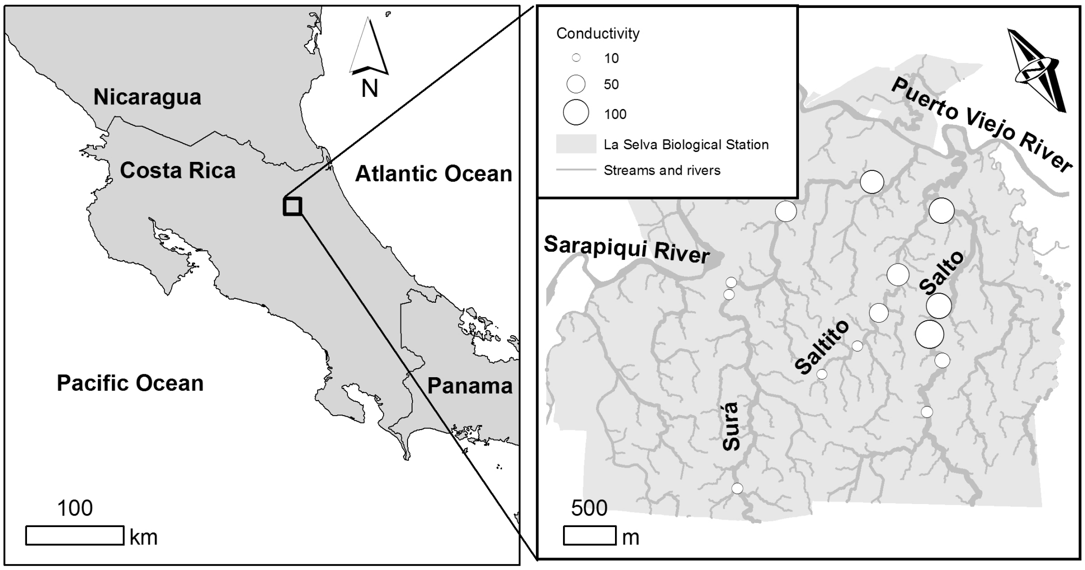


Figure 2.1. Locations of the Sura, Saltito, and Salto streams at La Selva Biological Station, Costa Rica. Shrimp were sampled from June-August 2008 in stream reaches (depicted by circles) varying in solute levels (and conductivity $\mu S\ cm^{-1}$).

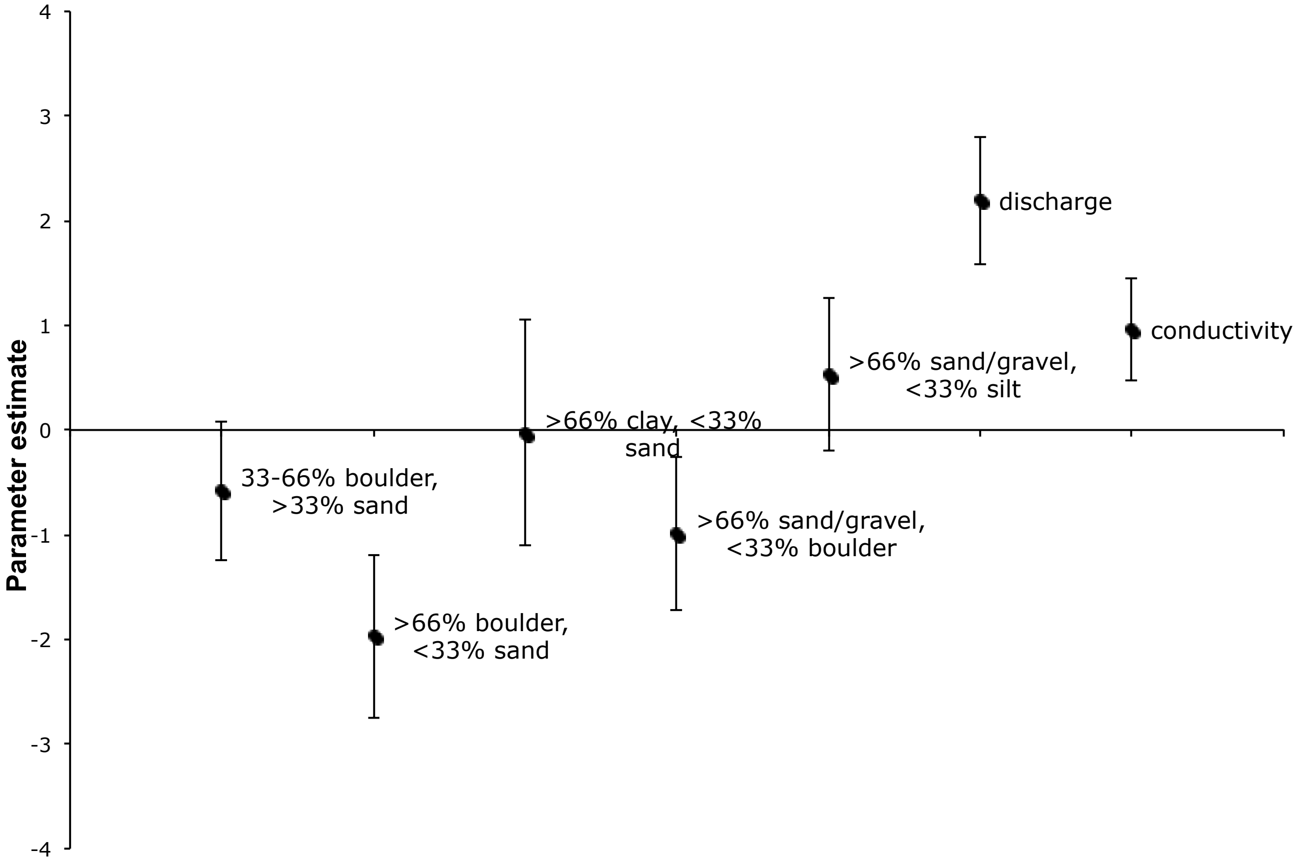
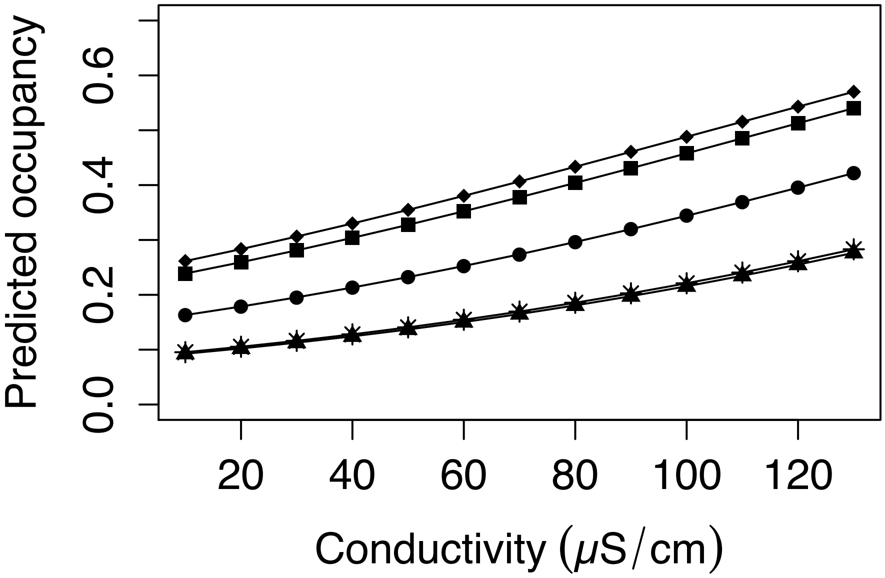


Figure 2.2. The estimated effect size and 95% confidence intervals of the parameters (beta values) for the most well-supported model ψ(substrate, conductivity,discharge)*p*(turbidity) of *Macrobrachium olfersi* occupancy as ranked by AIC_c. Parameter significance is inferred when estimated confidence intervals do not include zero.

(A)


Predicted occupancy
0.0
0.2
0.4
0.6
20
40
60
80
100
120
Conductivity (μS/cm)

(B)

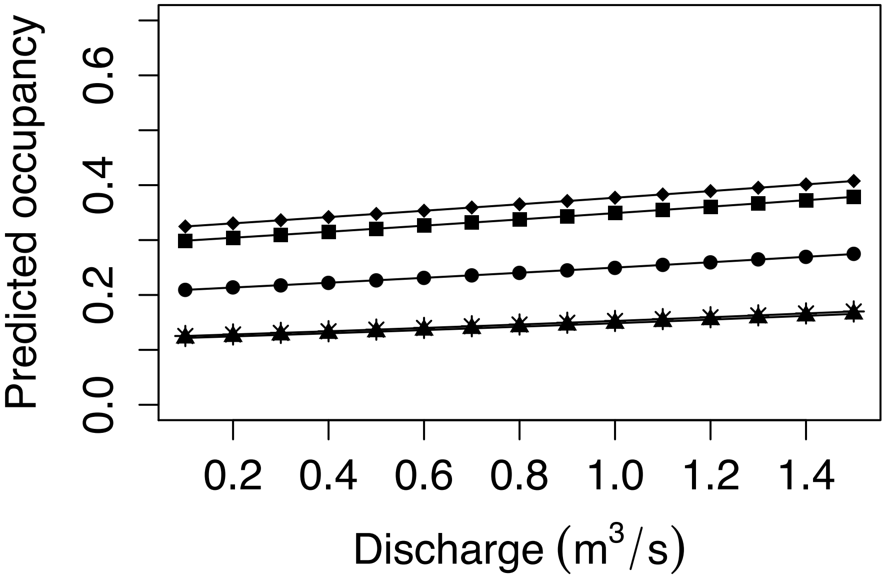
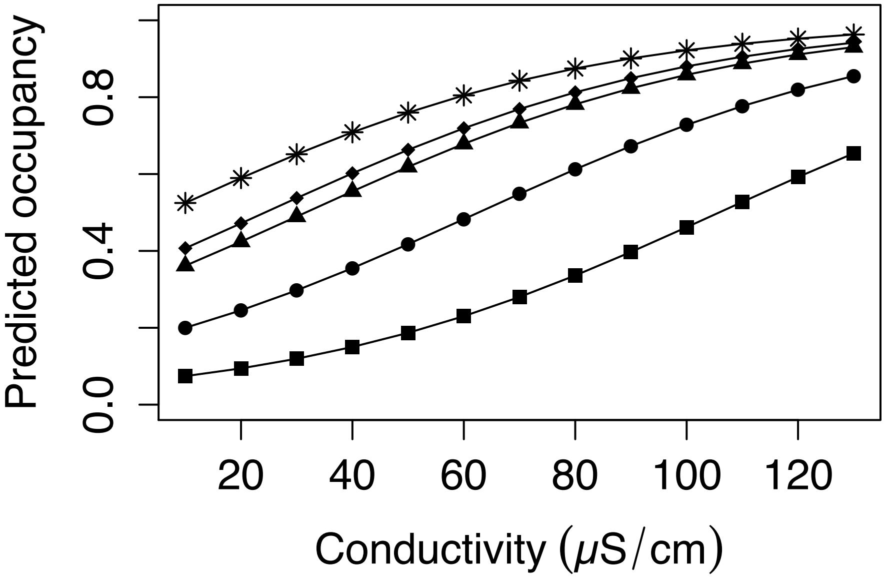

Predicted occupancy
0.0
0.2
0.4
0.6
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Discharge (m³/s)

Figure 2.3. (A) Simulated *Macrobrachium carcinus* occupancy as a function of conductivity (µS cm^{-1}) across five levels of substrate composition. Discharge was held constant at 0.57 m^{3} s^{-1} for this analysis. (B) Simulated macrobrachium carcinus occupancy as a function of discharge (m^{3} s^{-1}). Conductivity was held constant at 0.55 µS/cm for this analysis. Parameters were estimated using logistic regression models and averaged from two top models ranked according to AIC$_{c}$ (Table 4). Each symbol represents a substrate type (squares = >66% boulder, <33% sand; circles= >66% sand/gravel, <33% boulder; triangles = 33-66% boulder, >33% sand; diamonds= >66% clay, <33% sand; asteriks = >66% sand/gravel, <33% silt).

(A)


Predicted occupancy
0.0
0.4
0.8
20
40
60
80
100
120
Conductivity (μS/cm)

(B)

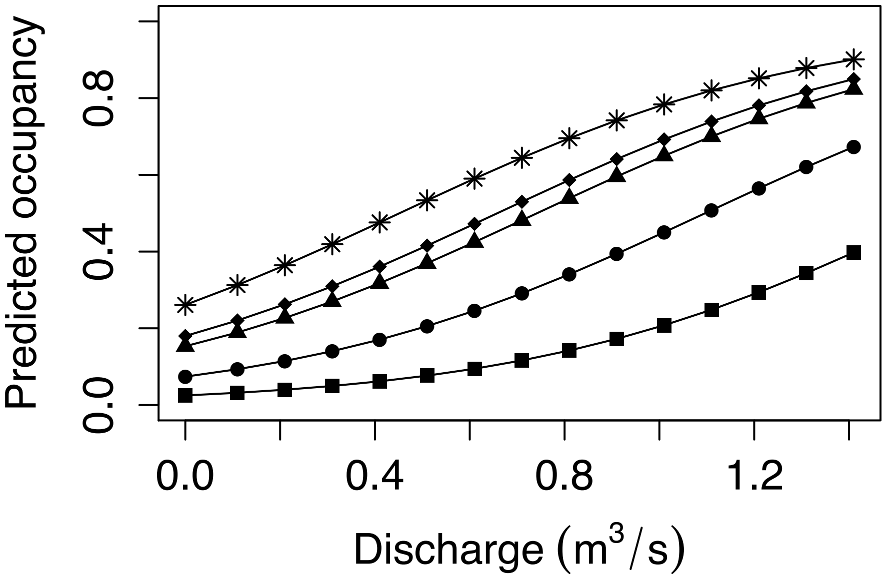

Predicted occupancy
0.0
0.4
0.8
0.0
0.4
0.8
1.2
Discharge (m3/s)

Figure 2.4 (A) Simulated *Macrobrachium olfersi* occupancy as a function of conductivity (μS cm^{-1}) across five levels of substrate composition. Discharge was held constant at 0.57 m^{3} s^{-1} for this analysis. (B) Simulated *Macrobrachium olfersi* occupancy as a function of discharge (m^{3} s^{-1}) across five levels of substrate composition. Conductivity was held constant at 0.16 μS/cm for this analysis. Parameters were estimated using occupancy estimation and averaged from three top models ranked according to AIC$_{c}$ (Table 3). Each symbol represents a substrate type (squares = >66% boulder, <33% sand; circles= >66% sand/gravel, <33% boulder; triangles = 33-66% boulder, >33% sand; diamonds= >66% clay, <33% sand; asteriks = >66% sand/gravel, <33% silt).

CHAPTER 3

LOW DENSITIES OF FRESHWATER SHRIMPS CONTRUBTE SIGNIFICANTLY TO ENERGY FLOW, INVERTEBRATE BIOMASS, AND SECONDARY PRODUCTION IN CONTINENTAL NEOTROPICAL STREAMS, LOWLAND COSTA RICA[3]

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[3] M.N. Snyder and C.M. Pringle. To be submitted to *Freshwater Biology*.

Abstract

1. While freshwater shrimps can comprise an obvious and significant proportion of the overall invertebrate biomass and secondary production on tropical island streams, little is known about their role in tropical continental streams, where they often occur at much lower densities and are largely nocturnally active. In this study, we estimate density, growth rates, and secondary production for three species within the shrimp genus, *Macrobrachium*. Our study site consisted of reaches of five different streams draining La Selva Biological Station (LSBS), a lowland continental site (1600 ha) in Costa Rica, Central America.

2. We determined shrimp growth rates of *M. olfersi*, *M. carcinus* and *M. heterochirus, in situ*, using mark-recapture of 1,171 individually marked shrimp. Generalized linear models were used to investigate environmental factors (discharge, substrate, and solute level) affecting growth rate and secondary production. *Huggins Closed-Capture* models were implemented in Program *Mark* to derive the probability of detection (*p*) and probability of recapture (*c*), which were then used to estimate abundance.

3. Mean instantaneous growth rates for *M. olfersi*, *M. carcinus* and *M. heterochirus* were (mean±SE) 0.002±0.0003 g DM g^{-1} day^{-1} , 0.004±0.0007 g DM g^{-1} day^{-1}, and 0.004±0.002 g DM g^{-1} day^{-1}, respectively. Also, we found that *M. olfersi* growth rate was influenced by sex, original biomass, and density of *M. carcinus.*

4. Our findings suggest that shrimps may account for the majority of invertebrate biomass at our site in Costa Rica, despite their relatively low abundance. When we combined our shrimp biomass and secondary production estimates with aquatic insect invertebrate biomass and secondary production (generated by our previous studies), we found that model-averaged densities of *M. olfersi*, *M. heterochirus,* and *M. carcinus* ranged from 0-2.83 individuals m^{-2}, 0-

0.24 individuals m^{-2}, and 0-0.21 individuals m^{-2}, respectively, representing 94% of the standing invertebrate biomass in the wet season and 87% in the dry season.

5. Lowland rainforest streams draining LSBS yielded a surprisingly high rate of shrimp secondary production. This is the first estimate reported for a non-insect invertebrate for Central American streams, and the highest estimate of secondary production for any *Macrobrachium* spp. that has been published. Total shrimp production at a site ranged from 0.005-10.83 g DM m^{-2} yr^{-1}. Shrimps appear to contribute a significant proportion (33-40%) of stream invertebrate secondary production at our *continental* study site in Costa Rica.

Introduction

While our knowledge of tropical streams has increased dramatically over the past two decades (Dudgeon 2008), knowledge of how different taxa of aquatic biota contribute to energy flow in tropical stream ecosystems is often unavailable. Existing studies may be restricted to one part of the tropics (new world versus old world tropics) or landform (island versus continental), and thus our knowledge is often context-dependent.

For example, in tropical island streams (which are characterized by relatively low abundance and biodiversity of fishes), studies have shown that freshwater shrimps often comprise an obvious and significant proportion of the overall invertebrate biomass (e.g. Cross et al. 2008, Covich et al. 2009) and play an important role in ecosystem structure and function (Pringle 1996, Pringle et al. 1999, Moulton et al. 2004, Moulton et al. 2010). In contrast, little is known about the role of shrimps in continental tropical streams where they typically occur at much lower densities and are largely-nocturnally active (but see Walker and Ferriera 1985, Henderson and Walker 1986, Moulton et al. 2004), which has been attributed to the diversity and density of predatory fishes (Pringle and Hamazaki 1998, Covich et al. 2009, Hein et al. 2011). The genus *Macrobrachium* is globally distributed (Murphy and Austin 2005), a common component of continental tropical freshwater ecosystems (Walker and Ferriera 1985, Henderson and Walker 1986), and even serves as a substantial part of the artisan fishery in many countries (Etim and Sankare 1998, Greathouse et al. 2005). Yet the few studies that have quantified shrimp secondary production have been largely confined to tropical island streams (but see Mantel and Dudgeon 2004, Yam and Dudgeon 2006).

In this study, we quantified biomass, growth, and secondary production of freshwater shrimps within the genus *Macrobrachium*, in continental streams at La Selva Biological Station (LSBS) a relatively undisturbed site on Costa Rica's Caribbean lowlands. Secondary production integrates organism density and other life history attributes (Benke 2010, Benke and Huryn 2010), creating a useful index to understand the importance of this genus in energy flow of a tropical stream ecosystem. To our knowledge no studies of *Macrobrachium* spp. secondary production have been published for continental North or South America.

Our objectives were to (1) estimate the contribution of shrimp to total invertebrate biomass and secondary production by comparing our estimates with previous estimates of insect invertebrate biomass and secondary production in streams at LSBS, and (2) to examine how shrimp growth and secondary production are affected by both abiotic (i.e. stream size, substrate type, and natural landscape-scale variation in solute-rich, regional groundwater inputs) and biotic (intra- and inter-species) effects. We predict shrimps will be a significant component of total standing invertebrate biomass because of their relatively large individual biomass in contrast with insect invertebrates (i.e. compared to previously published studies of insect invertebrate taxa at LSBS). However, because of their larger body size we predicted they will have lower growth rates compared to insect invertebrates and comprise an insignificant portion of total invertebrate secondary production.

Methods

Study Site

Our study site, La Selva Biological Station (LSBS) (10° 26” N 84° 01” W), is a 1600 hectare forested reserve located in the Caribbean lowlands of Costa Rica (30-132 m asl) where the coastal plain transitions into the Cordillera Central. The geomorphology of the region results in heterogeneous stream chemistry because regional solute-rich groundwater surfaces at the break in the landform (Pringle 1991, Pringle and Triska 1991). Regional groundwater has high levels of solutes (P, Cl, Na, Mg, $HCO_{3,}$ Ca), ranging from 13-29 times more concentrated than solute levels in local surface water (Genereux and Pringle 1997). In this study, we use conductivity as a surrogate for solute level because it is positively correlated with the percent of water that is supplied by regional groundwater (Pringle and Triska 1991).

Average annual temperature at LSBS is 25.8° C, with monthly mean daily temperatures ranging from 24.7 ° C in January to 27.2° C in August (Sanford et al. 1994). The long-term annual precipitation average is 3,962 mm. In general, the distribution of precipitation throughout the year at La Selva is characteristic of the Atlantic side of Central America, with January-April receiving the least amount of precipitation, although the monthly precipitation averages are highly variable. Precipitation data used in this study were from the La Selva weather station database (http://www.ots.ac.cr).

Fifteen species of amphidromous shrimps occur in Costa Rica, with seven species occurring on the Caribbean side (Obregon 1986). Here, we focus on three co-occurring omnivorous shrimp, *Macrobrachium carcinus* (Linnaeus 1978)*, Macrobrachium heterochirus* (Wiegmann 1836), and *Macrobrachium olfersi* (Wiegmann 1836). All three species have distributions spanning from Brazil to Mexico (Chace and Hobbs 1969) and are commonly found in our study site (Obregon 1986). *M. carcinus* is much larger and can grow to reach total lengths of >40 cm, while *M. heterochirus* and *M. olfersi* grow to reach total lengths of >10 and >7 cm respectively (Obregon 1986). These shrimps are amphidromous and reproduction is tied to the seasonality of precipitation (Chace and Hobbs 1969, Obregon 1986, Mossolin and Bueno 2002). We created models that examined factors that affect growth rates and secondary production for two of the most frequently-encountered shrimp species (*Macrobrachium olfersi, Macrobrachium carcinus*) and describe growth rate and secondary production for the less-common *M. heterochirus* (Obregon 1986).

Growth

Growth rates of *M. olfersi*, *M. carcinus,* and *M. heterochirus* (>14 mm carapace length) were determined *in situ* using mark-recapture of individually marked shrimp at LSBS. Each shrimp individual was tagged with a Soft Visible Implant Alphanumeric tag (VIalpha, 2.5 mm x 1.0 mm) manufactured by Northwest Marine Technologies (NMT, Tumwater, WA, U.S.A.), and each tag had a unique identifying alphanumeric code. VIalpha tags were implanted in the muscle tissue laterally in the fifth or sixth abdominal segment of shrimps. To prevent mortality, we only tagged shrimp with CL >14 mm (Linnane and Mercer 1998). These tags have high readability (100%) and no significant effect on growth or mortality in crayfish and shrimp (Isely and Stockett 2001, Mantel and Dudgeon 2004).

Shrimp recapture took place monthly within five stream reaches from January to April 2009, in June 2009 and in one month of 2010 (April). All tagged shrimp were sexed and the carapace length (CL) was measured (±0.1 mm) in the field with calipers, and the shrimp were immediately returned to the stream. The biomass of individuals was calculated with length-mass regressions separately for each species. The length (CL in mm) to mass (dry weight in g) was calculated as $DM = a\ CL^b$ where a and b are constants. Instantaneous growth rate was calculated using the equation $Growth= \ln(weight_f / weight_i)/\Delta t$ (Hauer and Lamberti 2006). If an individual shrimp was captured more than once, only the measurement between the initial capture and last recapture were used in the growth calculation.

Generalized linear models were used to investigate which abiotic and biotic factors influence shrimps' growth rates. All statistical models in this study were run using Program R (R Development Core Team 2011). Instantaneous growth rate was log-transformed to assure a linear relationship among growth rates and the predictor variables. The explanatory abiotic factors included in the sixteen *a priori* selected models were conductivity and substrate. Conductivity was measured using a hand held Hanna HI9033 meter. Conductivity (low<50 μS/cm, high 50≥μS/cm) and substrate (boulders present or not) were included in the models as categorical variables. Biotic factors included in the models as explanatory factors were (1) the density of *M. olfersi* at that site, (2) density of *M. carcinus*, (3) density of all shrimp at that site, (4) individual shrimp biomass, and (5) sex.

Models in the confidence set of models were assessed for fit with plots of the residuals and covariates. Model assumption of equal variance was tested with F-tests. Collinearity among continuous predictor values was examined by looking at the VIF values and Pearson correlation coefficients.

Abundance

Mark-recapture of individually marked shrimp was used to determine shrimp abundance at each site (n=8) (Fig. 1) (Table 3.1). Sites were sampled for shrimp with two techniques: modified breeder minnow traps (Gee's, Memphis, Tennessee), baited with dried cat food in mesh bags (Covich et al. 1996), or by two-pass electrofishing. At each site, thirty baited traps were haphazardly placed between three and five meters apart on both sides of a stream reach in the afternoon and checked the subsequent morning. Individual shrimps were marked with VI Alpha tags and measured as described above. Streams sampled ranged from first- to third-order in size and flow to the Puerto Viejo River. Each site was sampled for a capture period of five consecutive days between one and seven times from January to April 2009, in June 2009 and in one month of 2010 (April). Sites were sampled during the typically drier (Jan-April) and wetter parts (May-December) of the year. Sampling in both seasons ensured that seasonal variability in abundance was well represented in annual secondary production estimates. Because the quantity of precipitation can vary widely in both the dry and wet season, we also examined the relationship between shrimp abundance and monthly precipitation with generalized linear mixed models at sites with more than one closed capture period. Site (n=5) was included as a random effect.

Huggins closed capture models were implemented in Program *Mark* to derive the probability of detection (*p*) and probability of recapture (*c*), which were then used to estimate abundance (Yip et al. 1996, White and Burnham 1999). Each monthly capture period of five consecutive days was modeled as an independent closed capture period. Closed capture models assume that the population is closed, that animals retain their marks through the study period, and that the individuals are correctly identified (Otis et al. 1978). *Huggins closed capture* models differ from standard closed capture models in that, instead of using maximum likelihood to estimate abundance (N), probability of capture (*p*), and recapture (*c*) at once, they use maximum likelihood estimation to model *p* and *c* which can then be use to estimate values of abundance (N). *Huggins closed capture* models were fitted to examine two types of changes in the probability of capture, changes that occurred over time, and behavioral responses to capture. Models were run which allowed *p* and *c* to change based on covariates, which included site, sex, shrimp biomass, and precipitation the day of sampling. Covariates were included to increase the precision of *p* and *c* (Otis et al. 1978, Huggins 1989). All of the individual histories from each site that used the same capture method were pooled together to estimate the probability of capture at sites with little information on probability of detection (White 2005).

An information-theoretic approach was used to describe which models of shrimp probability of capture were best-supported (Otis et al. 1978, Anderson et al. 2000, Burnham and Anderson 2002). Akaike's Information Criterion (AIC_c) was used to rank the models, with the best model having the lowest AIC_c score. Then AIC_c weight values were used to select a subset of the best-supported models to include in the confidence set.

Abundance for each site was calculated by first estimating the probability of detection (*p*) for all sites with the same method of capture, and then applying that probability of detection modified by the covariates in the confidence set of models to each individual sampling period at a site. Some sites did not have recaptures, but by applying the pooled estimated probability of detection (*p*) to the number of shrimp captured at a site this allowed us to estimate abundance at those sites as well. The number of unique shrimp encountered at a site on the first day of a closed capture session was divided by the calculated *p* value for that day and time to estimate the abundance. Then the abundance calculations were weighted by the model weight of the two models in the confidence set of models to get the final abundance. By using the *p* value from all the sites together, we were able to distinguish absence of shrimp (true zeroes) from areas with very low probability of recapture. The density of shrimp was calculated by dividing abundance by the trapping area.

Co-linearity of predictor variables was tested using F-tests and VIFs between categorical and non-categorical predictors. Predictors with correlations above 0.60 were not included in the same models. AIC_c scores were used to rank and select the most supported models (Burnham and Anderson 2002).

Production

In situ growth rate estimation and mark/recapture techniques allowed for realistic estimation of production. Secondary production of *M. olfersi and M. carcinus* were calculated separately for each closed capture period (stream reach (n=8), date, size class (5 mm intervals), and sex) by multiplying the estimated instantaneous growth rate (g DM g^{-1} day^{-1}) by the site, date, size class and sex-specific estimated abundance (g m^{-2}). Estimated instantaneous growth rates used in secondary production calculations were calculated by using the model-averaged coefficients from the confidence set of models describing the factors affecting instantaneous growth rates. The average growth rate from all recaptures (n=4) was used to estimate production of *M. heterochirus* because of limited sample size.

Contribution of shrimps to overall invertebrate biomass and secondary production

To estimate the contribution of shrimps to overall standing invertebrate biomass and secondary production we combined our shrimp estimates from LSBS with previously published data of insects from LSBS (Ramírez and Pringle 1998, Ramírez et al. 2006). Ramírez et al. (2006) measured insect biomass at six second and third order stream reaches at LSBS. We compared the mean biomass of shrimp (g AFDM m^{-2}) in this study to the mean insect biomass in the dry and wet season (g AFDM m^{-2}) from Ramirez et al. (2006).

To make inferences about the contribution of shrimp to overall invertebrate secondary production, we compared total shrimp production at two low-solute stream reaches in the Sura and the Sabalo Rivers. Insect secondary production at our Sura2 stream reach was calculated by multiplying the insect biomass from the same site (Ramírez et al. 2006) by the production to biomass ratio measured in the Sabalo River (Ramírez and Pringle 1998) for the most specific taxonomic resolution (family or genus). If P:B ratios were not available for a specific family, we averaged the P:B ratio for all the families in the order and used that value to calculate secondary production. We calculated production using the method described in Benke (1993) for the Plecoptera (*Anacroneuria*) because no information on the P:B ratio of insects from the order Plecoptera were available from the Sabalo. The method utilizes regression coefficients based on the maximum size (total length = 7 mm) found in the Sura and stream temperature (24° C) to estimate production. Previous estimates of insect secondary production used in the comparison for the Sabalo River were directly from Ramírez and Pringle (1998).

Results

Growth

Between January 2009 and April 2010 a total of 1,171 shrimps were captured and tagged within five stream reaches (150-225 m^2). Of the shrimps marked, 62 *M. olfersi* and 17 *M. carcinus* were recaptured >55 days apart and used to estimate instantaneous growth rates. The longest period between final and initial capture was 450 days. Measurements of carapace length (mm) were converted to dry mass (g) for the calculation of instantaneous growth rates. The relationship between carapace length and dry weight for *M. olfersi*, *M. carcinus* and *M. heterochirus* respectively are:

$W=0.0005CL^{2.585}$ ($r^2=.92$, $p<.0001$)

$W=0.0001CL^{3.1159}$ ($r^2=.92$, $p<.001$)

$W=0.16882x-1.9967$ ($r^2=0.80$, $p<.001$)

Instantaneous growth rates of *M. olfersi* ranged from -0.004-0.008 g DM g^{-1} day^{-1}. Negative growth rates were excluded from calculations. The mean instantaneous growth rate (mean±SE) for *M. olfersi* was 0.002± 0.0003 g DM g^{-1} day^{-1}. For *M. carcinus,* 17 shrimp were recaptured >55 days apart. The longest period between final and initial capture was 144 days. Instantaneous growth rate of *M. carcinus* ranged from 0.0005-0.01 g DM g^{-1} day. The mean instantaneous rate of growth for *M. carcinus* (0.004±0.0007 g DM g^{-1} day^{-1}) was greater than *M. olfersi.*

M. heterochirus was only captured in one stream reach (n=4). The longest period between final and initial recapture was 146 days. The mean instantaneous growth rate for *M. heterochirus* was 0.004±0.002 g DM g^{-1} day.

Response of Growth Rate to Physical and Biological Predictors

Before fitting models, potential correlations between covariates were examined. Density of *M. olfersi* and density of *M. carcinus* were both highly correlated with the density of all shrimp (0.70, 0.90) and were therefore not included in models together. *M. olfersi* density, *M. carcinus* density, total density, and original biomass of an individual were significantly correlated as was conductivity with the density of *M. olfersi* and substrate but the correlations were small (correlation coefficients < 0.4, $r^2<0.21$). *M. heterochirus* was only found at one site, so we did have enough samples to measure the effect of environmental parameters on growth rate.

For *M. olfersi*, sixteen models were selected and fitted to test the effect of (1) sex, (2) original biomass, (3) substrate, (4) conductivity, (5) density of *M. olfersi*, (6) density of *M. carcinus*, (7) total density, and a combination of these factors on instantaneous growth rate (Table 3.2). The most supported model (Akaike weight = 0.89, pseudo r^2=0.39) included, sex, original biomass, and density of *M. carcinus*. All models indicated male shrimps grew faster than females. Original biomass was negatively correlated with shrimp growth rate (e.g. as shrimp got larger they grew more slowly) (Table 3.3, Fig.3.2). Density of *M. carcinus* was positively correlated to growth rates of *M. olfersi* (Fig. 3.2). The best model included sex, biomass, and *M. carcinus* density. Instantaneous growth rate that was natural log-transformed (G), fitted with covariates of sex, size and density of *M. carcinus* yielded the following relationship:

For male *M. olfersi*:

G = -6.28366 + 1.49202 + -0.91768 * (biomass) + 0.12093 * (density *M. carcinus*)

For female *M. olfersi*:

G = -6.28366 + -0.91768 * (biomass) + 0.12093 * (density *M. carcinus*)

We modeled growth rate of *M. carcinus* with thirteen *a priori* selected models, which included sex, original biomass, substrate, conductivity level, density of *M. olfersi*, density of *M. carcinus*, total density, and a combination of these covariates. Based on the confidence intervals of the estimated beta values, none of the fitted models were good predictors of the instantaneous growth rate.

Abundance

Twelve *Huggins closed capture* models were fit in Program *Mark* to determine the probability of shrimp capture with modified minnow traps (n=5). Out of the twelve models tested, two were included in the confidence set of best-supported models (Table 3.4). In both models in the confidence set of models, the probability of capture varied over time. In the top model, precipitation and size increased the likelihood of capture, and there was a decreased likelihood of capture for *M. carcinus* (w_i=0.54). The second best-supported model (w_i=0.22) included precipitation and species covariates, but did not include size as a predictor. The third best-supported model was similar in model weight to the second most likely model and differed by one additional covariate (sex), however, we did not include it in the confidence set of models because the confidence intervals for the added covariate indicated that the effect was not significant. The model-averaged probability of capture (mean±SE) for *M. olfersi and M. carcinus* on the first day of capture across all the sites ranged from 0.08-0.17±0.008, and 0.14-0.32±0.008, respectively.

Six *Huggins closed capture* models were fit in *Program Mark* to determine the probability of shrimp capture with electrofishing (n=3). Out of the six models fitted, two were included in the confidence set of models (Table 3.4). The best-supported model was one which included a behavioral effect (i.e. differing probabilities of capture, p, and recapture, c; w_i=0.80). The model confidence set also included the time-varying model (p(t)=c(t), w_i=0.20). The model evaluation was somewhat limited by low recapture rates because when more then one covariate (or a site effect) was included the models became unsolvable. The model-averaged probability of capture for *M. olfersi* and *M. carcinus* on the first day of capture across all the sites was 0.25±0.08.

When we applied the probability of capture, modified by the covariates, to the number of shrimps captured, we found the model-averaged abundance of *M. olfersi* and *M. carcinus* density ranged from 0-2.83 individuals m^{-2}, and 0-0.21 individuals m^{-2}, respectively (Table 3.5, Fig. 3.3). Biomass of *M. olfersi* and *M. carcinus* ranged from 0-5.30 g DM m^{-2}, and 0-9.8 g DM m^{-2}, respectively (Table 3.6, Fig. 3.3). *M. heterochirus* was only encountered at one site (salto5). Densities of this taxon ranged from 0.08-0.24 individuals m^{-2}, and biomass ranged from 0.25-0.75 g DM m^{-2}.

Precipitation in the month before sampling was highly variable and ranged from 21.2 – 595.18 mm. Monthly precipitation was not a significant predictor of abundance at a stream reach for either *M. carcinus* or *M. olfersi* (p=0.96, 0.96).

Production

For *M. olfersi*, mean production ranged from 0.0059-1.65 g DM m^{-2} $year^{-1}$ (Fig. 3.4). P:B ratios were highly variable ranging from 0.24-2.69 (Fig. 3.4). Male *M. olfersi* accounted for ~76% of the total production on average at a site because of their higher density, larger mean biomass, and higher growth rates. *M. carcinus* mean production at a site ranged from 0-8.75 g m^{-2} $year^{-1}$. *M. heterochirus* production at one site was 0.68 g DM m^{-2} $year^{-1}$. Production to biomass ratio (P:B) ratios was 1.59 and was consistent across sites because growth rates were the same at all sites. Total production at a site ranged from 0.005 – 10.83 g m^{-2} $year^{-1}$. *M. carcinus* percentage of shrimp secondary production at a site ranged from 0-89%. The mean percent of shrimp secondary production from *M. carcinus* was 60%.

Production of *M. olfersi* was highly correlated with abundance of *M. carcinus,* and *M. carcinus* abundance explained 53% of the variation (r^2=0.53) (Fig. 3.5). Combining *M. olfersi* and *M. carcinus* abundance explained 88% of the variation. There was a significant interaction between *M. olfersi* and *M. carcinus* abundance. Abundance of *M. olfersi* alone explained 34% of the variation (Fig. 3.5).

Discussion

Our study strongly suggests that shrimps account for the majority of invertebrate standing biomass in streams at LSBS despite their low densities. We found that shrimp comprise 94% and 87% of standing biomass in the wet and dry season, respectively. Estimates are based on comparing the mean shrimp biomass at LSBS (3.12 g AFDM m^{-2}) with previously published estimates of aquatic insect biomass at LSBS in the wet (0.183 g AFDM m^{-2}) and dry (0.450 g AFDM m^{-2}) seasons (Ramírez et al. 2006). Again, despite their relatively low abundance at our continental study site, shrimps comprise a higher percentage of the overall invertebrate biomass than they do on the islands of Palau or on continental Hong Kong (Table 3.7). Our study found the contribution of shrimp to overall invertebrate standing biomass to be higher than in Puerto Rican streams downstream from waterfalls (57%). Interestingly, the overall contribution of shrimps to overall invertebrate standing biomass is almost as high as in headwater Puerto Rican streams (95%) where shrimp are very abundant as a result of reduced predation pressure upstream from waterfalls. Predation pressure is reduced above waterfalls because predaceous fishes cannot pass the waterfalls and are restricted to reaches below waterfalls.

In rivers, where relatively long-lived shrimps (2-7 years) comprise the majority of standing invertebrate biomass, energy flow to higher trophic levels may be slower than in rivers where short-lived insects (2 weeks-2 years) comprise a higher proportion of the biomass. *Macrobrachium* spp. are omnivorous and their diet, which potentially overlaps with insect diets, includes detritus, algae, and insects (Chace and Hobbs 1969). Shrimps are slower-growing and larger-bodied than insects; if they are accumulating resources that would otherwise be available to aquatic insects, these relatively long-lived organisms could be acting as energy sinks slowing energy flow through the food web.

Surprisingly, shrimps appear to contribute a significant percentage (33-40%) of the total stream invertebrate secondary production at LSBS. This proportion was determined by combining our estimates of 0.32 and 6.69 g AFDM m^{-2} $year^{-1}$of shrimp secondary production from the Sabalo and Sura Rivers, respectively, to previous estimates of insect secondary production from the same rivers (0.194 and 13.6 g AFDM m^{-2} y^{-1}) (Ramírez and Pringle 1998, Ramírez et al. 2006). When we compare our estimate of shrimp contribution to total secondary production to estimates from other sites, our estimate was higher than the Hong Kong continental site (14%) (Mantel and Dudgeon 2004), but lower than the Palau (84%) (Bright 1982) (Table 3.7). It is clear that shrimps can be significant components of the energy flow in both *island* as well as *continental* streams, where they are less abundant.

Our estimates of shrimp contribution to overall invertebrate secondary production are from the Sabalo and Sura Rivers, which appear to be representative in terms of physical and biological characteristics of other streams in the Caribbean lowlands of Costa Rica. Caution is called for in using the exact percentage because insect biomass and secondary production data was collected twelve years prior to our study. However, the combined estimates are providing us with a relatively robust and rare snapshot of the role of shrimps in energy transfer in continental neotropical streams. For example, if estimates of insect secondary production were to double, shrimp would still comprise 20% of overall invertebrate secondary production and our conclusion that shrimp are a significant component of overall secondary production would remain unchanged.

We have most likely underestimated true secondary production for two reasons. First, shrimps with carapace length < 15 mm were not included in the estimate. Second, our estimates of secondary production do not include reproductive output. Female shrimp are gravid year round at LSBS, and eggs can be a significant source of input into the system because they compose up to 21% of female body mass (Anger et al. 2002). While the data has some limitations and makes some assumptions, measuring growth rates *in situ* and using maximum likelihood methods allowed us to calculate the most precise estimates of biomass and secondary production possible.

Published estimates of secondary production of *Macrobrachium spp.* vary by an order of magnitude between study sites and do not seem to be predictable based on whether a site is continental or located on an island. Rates of secondary production at LSBS (4.6 g DM m^{-2} y^{-1}) are the second highest estimate of secondary production for any *Macrobrachium spp.* that has been published, with the island of Palau being higher (4.74 g AFDM m^{-2} y^{-1}) (Bright 1982, Cross et al. 2008). Cross et al. (2008), who estimated growth in side-channels, found secondary production values to be an order of magnitude lower for the *Macrobrachium* spp. (0.019 g AFDM m^{-2} y^{-1}), and Mantel and Dudgeon (2004) also published rates (0.484-0.606 g AFDM m^{-2} y^{-1}) for *Macrobrachium hainanse* (Parisi 1919) in Hong Kong far below our estimates.

Insect production to biomass (P:B) ratios are much higher than P:B ratios for shrimps, indicating that they are replacing themselves at a much faster rate than slow-growing, large-bodied shrimps. Comparing the P:B ratio of shrimp (0.3-2.5) to insects (5-200) at LSBS explains how shrimp comprise 87-94% of the standing invertebrate biomass but contribute only 38-40% of stream secondary production. Stream-dwelling insect invertebrates are thought to be under high predation pressure by fishes at LSBS (Pringle and Hamazaki 1998), which could explain the higher P:B ratio of insect invertebrates. If there was a disturbance that decreased shrimp biomass, it would take them a longer period of time to recover to previous levels of production than insects that have a higher P:B ratio (Margalef 1994). In this regard, shrimp populations are potentially less resilient to a disturbance and could support a lower rate of predation than insects that are turning over their biomass at a much faster rate.

The P:B ratio of shrimps in this study had a wide range (0.3-2.5), yet our P:B estimates are similar to values reported by two of the three studies that have estimated P:B of *Macrobrachium spp.* (Table 3.7). Our lowest estimate of P:B is within the range of estimates for *Macrobrachium spp.* (0.01-0.38) measured in artificial stream channels in Puerto Rico (Cross et al. 2008) and our highest estimate is within the range of values found for *Macrobrachium hainense* (1.7-2.3) in Hong Kong (Mantel and Dudgeon 2004). However, on the island of Palau, *Macrobrachium lars* has a slightly higher P:B (2.5-5.0) (Bright 1982) compared to this study. P:B ratios can be used to understand energy flow through food webs but our ability to predict how these ratios might change is limited by the small number of studies estimating P:B ratios for *Macrobrachium spp*.

Studies that estimate insect and shrimp secondary production commonly use growth rates measured at the genus level (Cross et al. 2008, Ramirez and Pringle 1998). However, genus level estimates might not be appropriate for studies of shrimp secondary production. Growth rate data from our study, combined with other studies, suggests that species within the genus *Macrobrachium* have different growth rates. We found the mean instantaneous growth rate of *M. olfersi* (0.00167 g AFDM g^{-1} day^{-1}) was 50% less than *M. carcinus* (0.0034 g AFDM day^{-1}). Because taxon within the *Macrobrachium* genus grow at different rates, using genus level growth rates for *Macrobrachium spp*. may give less accurate estimates of overall secondary production.

Abiotic features (e.g. seasonalilty, shade) vary in their effect on shrimp secondary production. Temperatures vary little in the Caribbean lowlands of Costa Rica (Sanford et al. 1994), so we would not expect that to be the main driver of patterns of shrimp secondary production. However, other studies of shrimp secondary production have found that growth rates of *M. hainanense* were slower during the colder season (Mantel and Dudgeon 2004). Yam and Dudgeon (2006) found both seasonal and site-level differences in both *Caridina cantonensis* and *Caridina serrata.* They found higher production in less shaded streams and higher production in the dry season. They suggest that seasonal differences in production (40% higher in the dry season) were due to shrimp mortality in response to spates.

In summary, this study provides one of the few estimates of overall invertebrate standing biomass and secondary production (including both shrimp and insects) in tropical streams that we have seen in the published literature, and the only estimate from *continental* streams in the neotropics. We found that shrimp had relatively high levels of standing biomass and secondary production when compared to previously published estimates of insect invertebrate biomass and secondary production at LSBS. Individual species and their interactions were a significant factor affecting the secondary production of shrimps at LSBS. Although other studies have examined palaemonid shrimp growth and intra-specific interactions, this is the first *in situ* study to consider the inter-species interactions on shrimp growth and production. Our results suggest that shrimp are potentially very large components of the invertebrate biomass, and consequently significant components of the stream energy flow. These findings point out the need for not only more studies at *continental* sites, but studies across all kinds of landforms and environmental gradients.

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Table 3.1. Physical and chemical description of study sites at La Selva Biological Station, Costa Rica. Monthly means were measured from January - May in 2009.

Site	Discharge ($m^3 s^{-1}$)	Substrate	Conductivity ($\mu S\ cm^{-1}$)	Stream order	Temperature (°C)
Pantano	0.14	sand	68	1	24.9
Piper	0.05	sand	18	1	25.0
Sabalo	1.01	sand	47	4	25.4
Salto5	0.80	boulder	98	3	25.7
Sura2	0.37	sand	17	3	24.8
Sura3	0.37	boulder	17	3	24.8
Sura6	0.81	sand	86	3	25.4
Sura7	0.81	sand	86	3	25.4

Table 3.2. The suite of models fit to evaluate what factors influence *Macrobrachium olfersi* instantaneous growth rate (log g DM g^{-1} day^{-1}) and showing values for number of parameters in the model (k), Akaike's Information Criterion with the adjustment for small sample size (AIC_c), Akaike weights (w_i), change in AIC_c (ΔAIC_c) and calculated pseudo R^2 value. Instantaneous growth rate was measured *in situ* in streams at La Selva Biological Station by mark and recapture of individual shrimp.

Parameters	**k**	**AIC_c**	**ΔAIC*c***	**w_i**	**Pseudo R^2**
Sex, biomass, density *M. carcinus*	5	164.2	5.9	0.89	39
Sex, biomass, conductivity	5	170.1	1.0	0.05	33
Sex, biomass, density all	5	171.1	1.3	0.03	32
Sex, biomass	4	172.4	1.3	0.01	28
Sex, biomass, substrate	5	172.8	0.4	0.01	30
Sex, density *M. carcinus*	4	175.6	2.8	0.00	24
Sex, density_all	4	179.3	3.7	0.00	20
Sex	3	179.8	0.4	0.00	16
Density *M. carcinus*	3	180.4	0.6	0.00	15
Sex, conductivity	4	180.9	0.5	0.00	18
Sex, density_all	4	181.7	0.8	0.00	16
Density all	3	184.3	2.6	0.00	10
Conductivity	3	189.2	4.9	0.00	3
Substrate	3	189.7	0.5	0.00	2
Density *M. olfersi*	3	190.6	0.9	0.00	0
Biomass	3	190.8	0.2	0.00	0

Table 3.3. Mean instantaneous growth rates (g DM g^{-1} day^{-1}) for female and male *Macrobrachium olfersi* of different carapace lengths measured *in situ* using mark-recapture in study stream reaches at La Selva Biological Station. Ranges represent 95% confidence intervals.

Sex	**Carapace length (mm)**	**Growth (g DM g^{-1} day^{-1})**
Female	15-19	0.002 (0.001-0.003)
	20-24	0.002 (0.001-0.003)
	25-29	0.0003
Male	15-19	0.007 (0.006-0.008)
	20-24	0.004 (0.002-0.005)
	25-29	0.003 (0.001-0.004)

Table 3.4. Closed capture Huggins models fit for shrimps (*Macrobrachium olfersi* and *Macrobrachium carcinus*) showing the covariates for each model on the probability of capture (*p*), and probability of recapture (*c*), the number of model parameters (k), Akaike's Information Criterion with the adjustment for small sample size (AIC$_c$), change in AIC$_c$ (ΔAIC$_c$), and Akaike weights (w_i). Model fit was estimated separately for stream reaches sampled with traps and electrofishing.

Model	k	AIC$_c$	ΔAICc	W_i
Trap sampling				
precipitation, species, size [p(t)=c(t)]	9	3812.1	0	0.54
precipitation, species [p(t)=c(t)]	8	3813.9	1.8	0.22
precipitation, species, size,	10	3814.0	2.0	0.20
precipitation, size [p(t)=c(t)]	7	3817.5	5.5	0.04
precipitation [p(t)=c(t)]	6	3826.2	14.1	0.0004
precipitation, size [p(t)=c(t)]	7	3828.2	16.1	0.00017
[p(t)=c(t)]	5	3842.5	30.4	0
[p(.),c(.)]	2	3858.7	46.6	0
size [p(t)=c(t)]	2	3954.5	142.4	0
size, sex [p(t)=c(t)]	3	3956.2	144.6	0
[p(.)=c(.)]	1	3961.0	149.0	0
sex [p(t)=c(t)]	2	3963.0	151.0	0
Electrofishing sampling				
[p(.),c(.)]	2	500.0	0	0.80
[p(t)=c(t)]	5	502.7	2.8	0.20
[p(.)=c(.)]	1	513.4	13.4	0.00096
sex [p(.),c(.)	2	514.4	14.5	0.00058
size [p(.),c(.)]	2	515.3	15.4	0.00037
size, sex [p(.),c(.)]	3	516.4	16.5	0.00021

Table 3.5. Estimated density (indiviuals/m^2) of *Macrobrachium olfersi* and *Macrobrachium carcinus* in eight study reaches at La Selva Biological Station during different sampling periods (Jan.-Apr. 2009, Jun. 2009, and Apr. 2010). Ranges represent 95% confidence intervals.

Site	**Jan-09**	**Feb-09**	**Mar-09**	**Apr-09**	**Jun-09**	**Apr-10**
M. olfersi						
Sura2	0.394 (0.366-0.426)	0.266 (0.241-0.297)	0.252 (0.229-0.279)	0.337 (0.307-0.373)	0.044 (0.042-0.047)	0.000
Sura3	0.019 (0.015-0.029)	0.010 (0.007-0.014)	0.010 (0.007-0.014)	0.010 (0.007-0.014)	.	0.000
Sura7	1.20 (1.13-1.29)	1.06 (0.98-1.16)	2.83 (2.56-3.16)	1.65 (1.50-1.84)	0.698 (0.644-0.736)	1.72 (1.56-1.92)
Sura6	0.558 (0.420-0.833)	0.289 (0.217-0.431)	0.212 (0.159-0.316)	0.346 (0.260-0.517)	0.327 (0.246-0.488)	0.404 (0.304-0.603)
Salto5	0.484 (0.451-0.522)	0.049 (0.046-0.052)	0.447 (0.405-0.98)	0.092 (0.086-0.098)	0.263 (0.239-0.293)	0.089 (0.081-0.10)
Piper	.	.	0.473 (0.449-0.499)	.	.	.
Sabalo	.	.	0.411 (0.309-0.614)	0.283 (0.213-0.422)	.	.
Pantano	.	.	0.304 (0.290-0.319)	.	.	.
M. carcinus						
Sura2	0.039 (0.037-0.040)	0.128 (0.120-0.134)	0.218 (0.205-0.229)	0.219 (0.205-0.230)	0.068 (0.065-0.069)	0.062
Sura3	0	0	0	.	.	.
Sura7	0.067 (0.063-0.068)	0.028 (0.026-0.028)	0.066 (0.068-0.061)	0.033 (0.030-0.034)	0.053 (0.050-0.053)	0.033 (0.031-0.034)
Sura6	0.019 (0.014-0.029)	0	0	0	0	0
Salto5	0.125 (0.118-0.129)	0.039 (0.038-0.040)	0.120 (0.112-0.126)	0.033 (0.034-0.032)	0.186 (0.174-0.195)	0.086 (0.080-0.909)
Piper	.	.	0	.	.	.
Sabalo	.	.	0	0	.	.
Patano	.	.	0	.	.	.

Table 3.6. Estimated biomass (g DM m^{-2}) of *Macrobrachium olfersi* and *Macrobrachium carcinus* in eight study reaches at La Selva Biological Station during different sampling periods (Jan.-Apr. 2009, Jun. 2009, and Apr. 2010). Ranges represent 95% confidence intervals.

Site	**Jan-09**	**Feb-09**	**Mar-09**	**Apr-09**	**Jun-09**	**Apr-10**
M. olfersi						
Sura2	0.737 (0.676-0.798)	0.498 (0.441-0.555)	0.471 (0.421-0.522)	0.630 (0.562-0.699)	0.083 (0.078-0.087)	0.000
Sura3	0.036 (0.018-0.054)	0.018 (0.009-0.027)	0.018 (0.009-0.027)	0.018 (0.009-0.027)	.	0.000
Sura7	2.256 (2.091-2.420)	1.989 (1.803-2.175)	5.302 (4.685-5.919)	3.093 (2.735-3.450)	1.307 (1.236-1.378)	3.22(2.85-3.60)
Sura6	1.045 (0.530-1.561)	0.541 (0.274-0.807)	0.396 (0.201-0.592)	0.649 (0.329-0.969)	0.613 (0.311-0.915)	0.757 (0.384-1.13)
Salto5	0.907 (0.836-0.978)	0.091 (0.086-0.097)	0.836 (0.741-0.932)	0.172 (0.169-0.183)	0.493 (0.437-0.548)	0.167 (0.148-0.186)
Piper	.	.	0.885 (0.836-0.934)	.	.	.
Sabalo	.	.	0.770 (0.390-1.150)	0.529 (0.268-0.791)		.
Pantano	.	.	0.569 (0.540-0.598)	.	.	.
M. carcinus						
Sura2	1.74 (1.65-1.90)	5.74 (5.37-6.04)	9.80 (9.20-10.28)	9.83 (9.22-10.31)	3.04 (2.93-3.12)	2.79 (2.64-2.90)
Sura3	0	0	0	.	.	.
Sura7	3.01 (2.85-3.07)	1.24 (1.16-1.27)	2.96 (2.74-3.06)	1.47 (1.36-1.52)	2.36 (2.25-2.39)	1.48 (1.37-1.53)
Sura6	0.86 (0.65-1.29)	0	0	0	0	0
Salto5	5.59 (5.32-5.79)	1.77 (1.70-1.82)	5.39 (5.04-5.67)	1.48 (1.41-1.53)	8.33 (7.80-8.75)	3.85 (3.60-4.05)
Piper	.	.	0	.	.	.
Sabalo	.	.	0	0	.	.
Pantano	.	.	0	.	.	.

Table 3.7. Values of shrimp and insect biomass and secondary production in tropical rivers are summarized from the scientific literature. Values were included from a site where both insect, gastropod and *Macrobrachium* spp. shrimp invertebrate biomass or secondary production information had been published. Columns titled A = shrimp and B=insect and gastropod.

A			B								
Biomass (g m^{-2})	**Prod (g AFDM m^{-2} yr^{-1})**	**P:B**	**Biomass (g m^{-2})**	**Prod. (g AFDM m^{-2} yr^{-1})**	**P:B**	**Total biomass (g AFDM m^{-2})**	**Total Prod. (g AFDM m^{-2} yr^{-1})**	**% biomass decapod**	**% prod. decapod**	**Location**	**Reference**
3.12	6.69	1.6, 2.6	0.47	13.6	5-70	3.59	20.29	87	33	Sura River, LSBS, Costa Rica	This study, Ramirez and Pringle 1998, Ramirez et al. 2006
0.33	0.131	0.3	0.32	0.194	5-103	3.44	0.325	91	40	Sabalo River, LSBS, Costa Rica	This study, Ramirez and Pringle 1998, Ramirez et al. 2006
0.12	0.02	0.01-0.38	0.21			0.33		57		Bisley, Luquillo, Puerto Rico	Cross et al. 2008, Ramirez and Hernandez-Cruz 2004
4.34	0.89	0.01-0.38	0.09			4.43		95		Prieta, Luquillo, Puerto Rico	Cross et al. 2008, Ramirez and Hernandez-Cruz 2004
1.26	4.74	2.5-5.0	0.2	0.93	2.5-5.0	1.46	5.67	86	84	Palau	Bright 1982
0.545	1.102	1.7-2.3	0.74	6.64	8.3-9.5	1.285	7.742	42	14	TPKS, Hong Kong	Dudgeon 1999b, Mantel and Dudgeon 2004

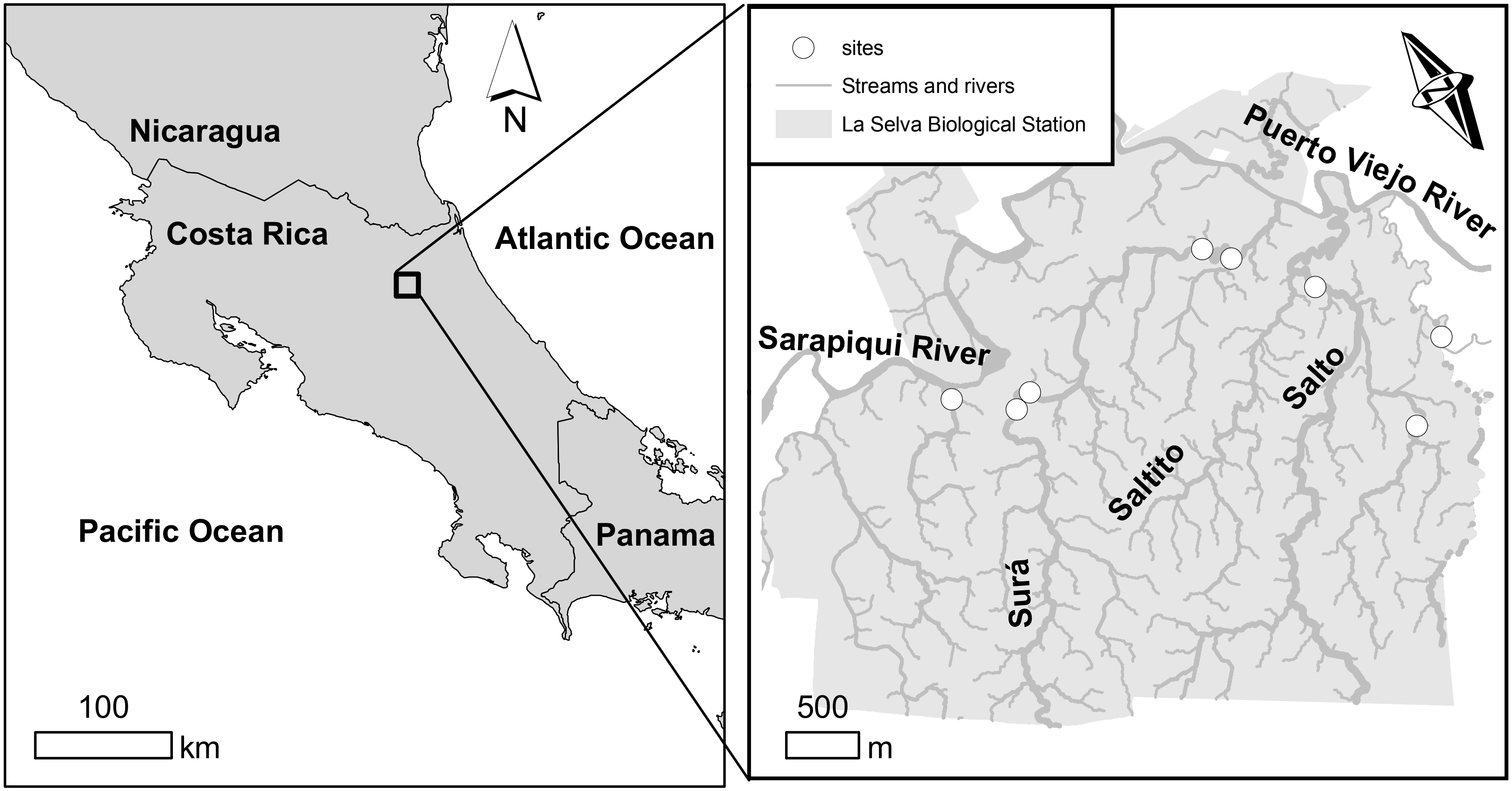


Figure 3.1. Map showing the location of La Selva Biological Station within Costa Rica and the study stream reaches (n=8) in which this study calculated abundance and secondary production during January-April 2009, June 2009 and April 2010.

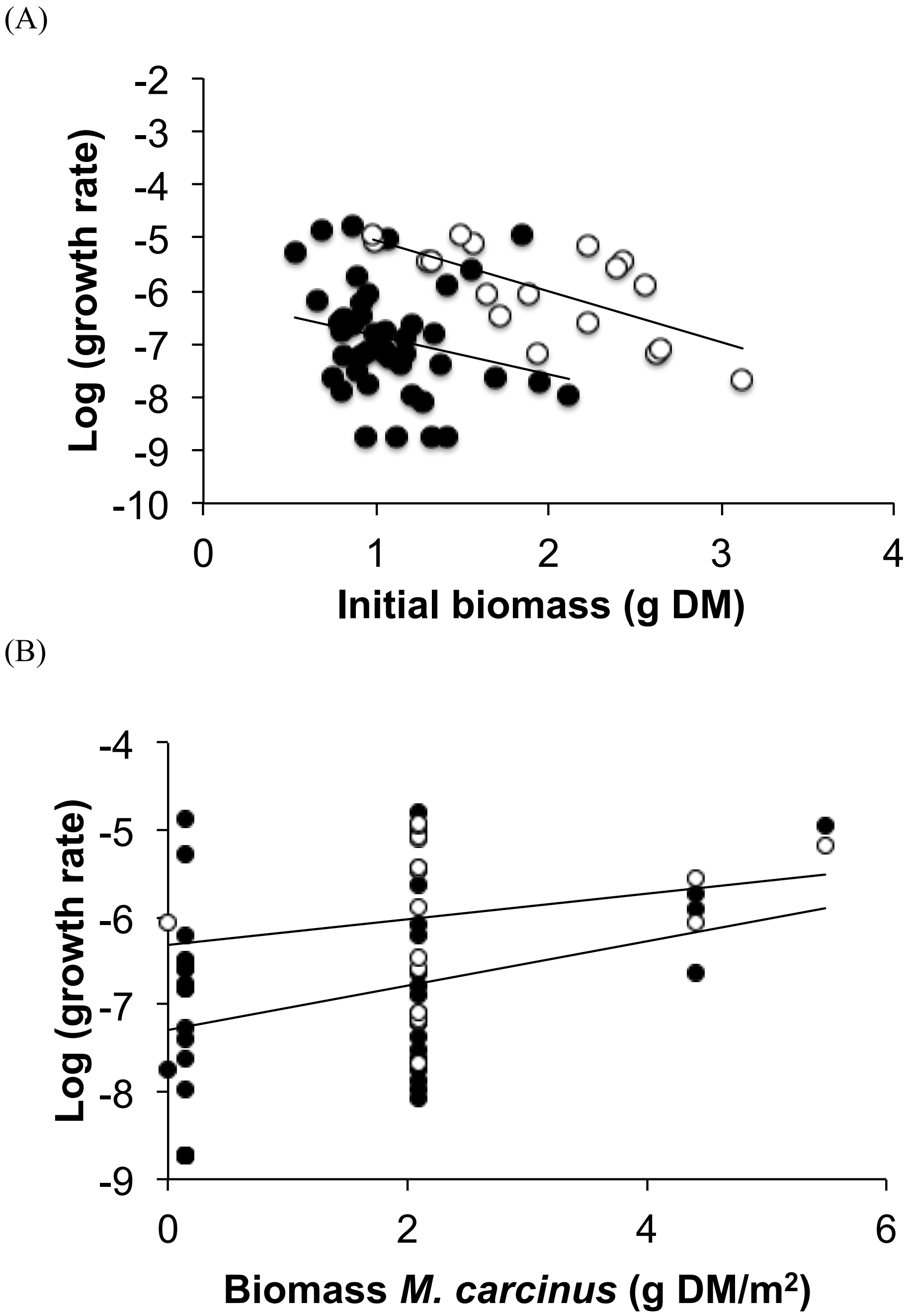
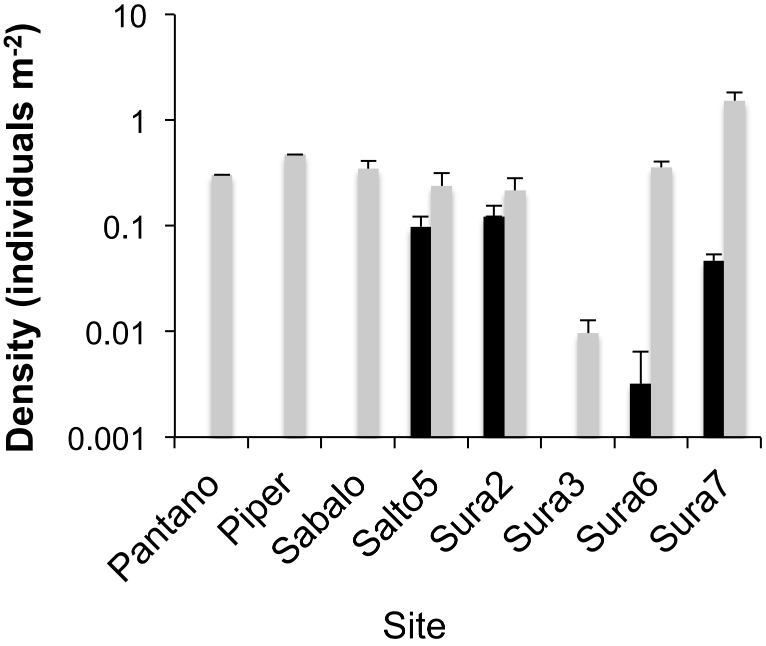


Figure 3.2. In the most supported model of *Macrobrachium* olfersi instantaneous growth rate, (A) original biomass and (B) density of *Macrobrachium carcinus* were included as explanatory variables. *M. olfersi* instantaneous growth rates log(g DM g-1 day-1) are higher for males (open circles) than females (filled circles) and negatively related to biomass of initial capture.

(A)


Density (individuals m⁻²)
10
1
0.1
0.01
0.001
Pantano
Piper
Sabalo
Salto5
Sura2
Sura3
Sura6
Sura7
Site

(B)

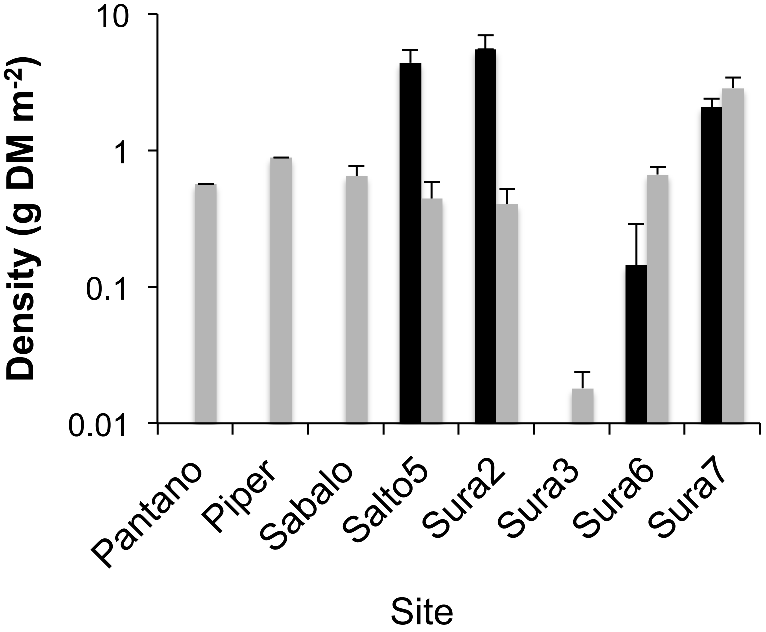
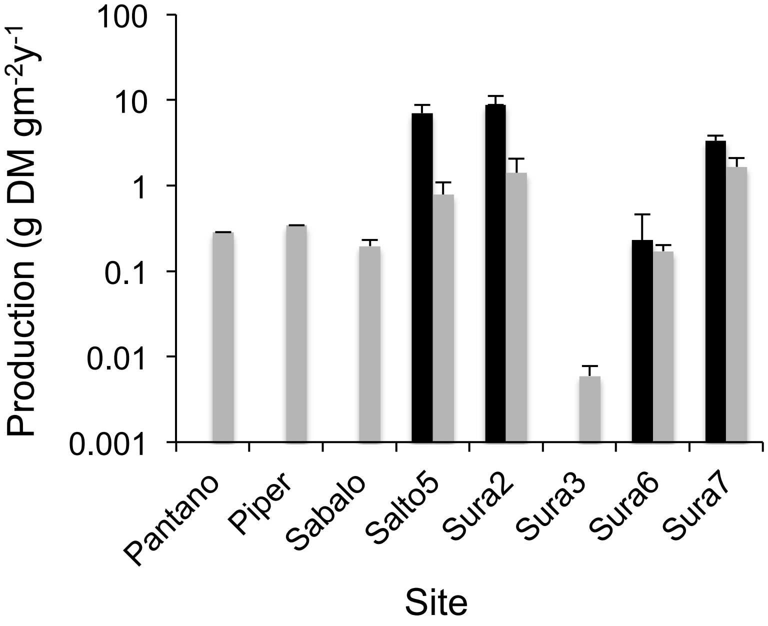

Density (g DM m⁻²)
10
1
0.1
0.01
Pantano
Piper
Sabalo
Salto5
Sura2
Sura3
Sura6
Sura7
Site

Figure 3.3. Estimated (A) mean density (individual m^{-2}) and (B) mean shrimp biomass (g DM m^{-2}) from eight stream sites sampled from January-April and June of 2009 at La Selva Biological Station. Error bars represent standard errors. Grey bars represent *Macrobrachium olfersi* and black bars *Macrobrachium carcinus*.

(A)


Production (g DM gm-2y-1
100
10
1
0.1
0.01
0.001
Pantano
Piper
Sabalo
Salto5
Sura2
Sura3
Sura6
Sura7
Site

(B)

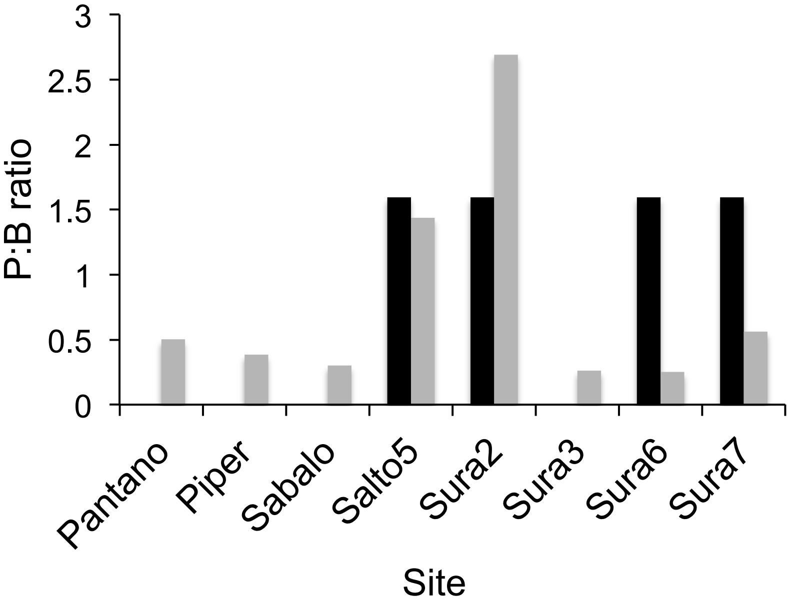
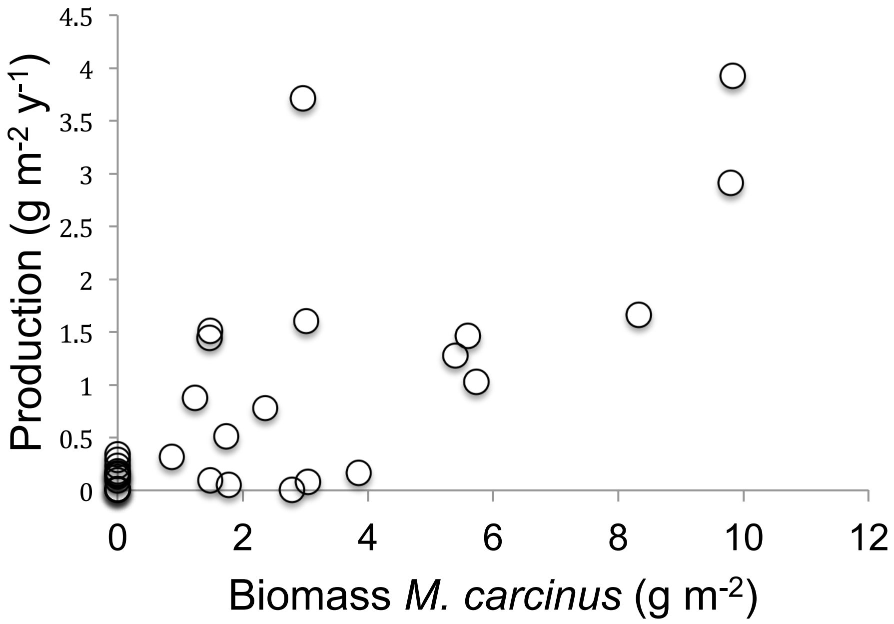

P:B ratio
3
2.5
2
1.5
1
0.5
0
Pantano
Piper
Sabalo
Salto5
Sura2
Sura3
Sura6
Sura7
Site

Figure 3.4. Estimated (A) secondary production (g DM m^{-2} $year^{-1}$) and (B) production to biomass ratio (P:B) from eight stream sites sampled from January-April and June of 2009 at La Selva Biological Station. Error bars represent standard errors. Grey bars represent *Macrobrachium olfersi* and black bars *Macrobrachium carcinus*.

(A)



(B)

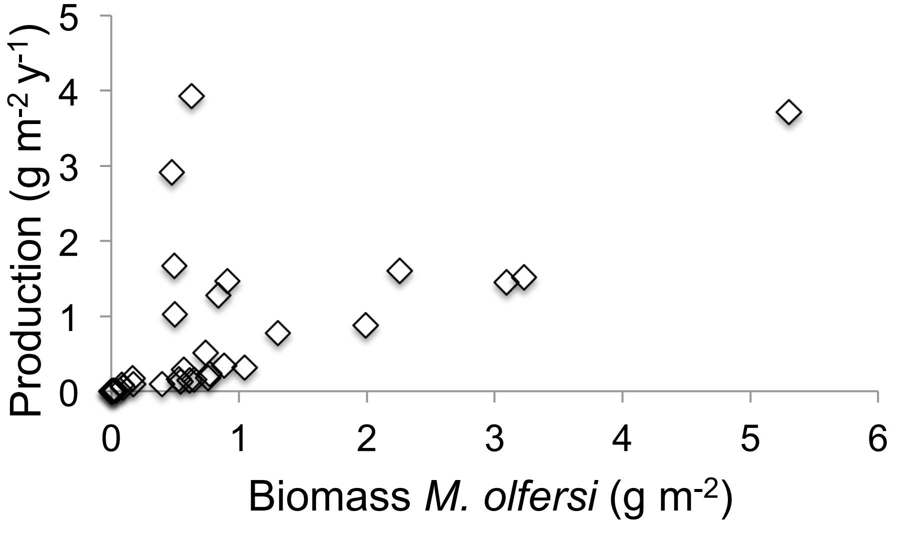


Figure 3.5. Secondary production (g DM m^{-2} year^{-1}) of *Macrobrachium olfersi* in eight stream reaches at La Selva Biological Station compared with (A) standing biomass (g m^{-2}) of *Macrobrachium carcinus* and (B) standing biomass (g m^{-2}) of *Macrobrachium olfersi* during the same site and time period.

CHAPTER 4

SEASONAL AND LONG-TERM (20+ YEARS) DYNAMICS OF *MACROBRACHIUM OLFERSI* POPULATIONS IN LOWLAND NEOTROPICAL STREAMS, COSTA RICA: RESISTANCE AND RESILIENCE OF SHRIMP TO LANDSCAPE SCALE DOWNSTREAM DISTURBANCE[4]

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[4] M.N. Snyder, C.M. Pringle and R.T. Soto-Mayor. To be submitted to *Journal of Tropical Ecology*.

Abstract

Freshwater amphidromous shrimps are important components of tropical aquatic ecosystems, and during their upstream and downstream migration they are vulnerable to factors associated with land use change. At La Selva Biological Station in the Sarapiquí region of Costa Rica, shrimp population data was collected between 1988-1989, before massive land use change occurred downstream that could potentially affect shrimp recruitment upstream. Using generalized linear models and a Bayesian inference framework, the relative abundance of *M. olfersi* between recent (2008-2011) and historical time periods (1988-89) was compared in three stream reaches. Shrimp relative abundance in two stream reaches within the protected area of La Selva was relatively constant yearly and between recent post-disturbance (2008-2011) and historical pre-disturbance (1988-89) time periods. In contrast, a stream reach bordered by pasture accessible to fisherman, showed a 7-fold decrease in relative abundance between recent and historical time periods suggesting site-level disturbance, possibly from shrimp harvesting. The lack of change between historical and contemporary sampling periods within interior forest stream reaches suggests shrimp populations in protected forested reaches, are resistant or resilient to landscape-scale changes in land-use occurring downstream. No seasonal patterns were found in the percentage of gravid females or the percentage of females.

Introduction

Amphidromous shrimps migrate up- and down-stream during their life cycle, as planktonic larvae drifting passively to the estuary and then again as juvenile shrimp emerging from the sea and moving upstream to the freshwater rivers and streams where they live as adults (Chace and Hobbs 1969). The distance migrated can be on the order of hundreds of kilometers

(Rome et al. 2009) and shrimp may be subjected to multiple stressors, depending on the level of alteration of the landscape through which they migrate. Land use change takes many forms (e.g. Pringle and Scatena 1999); and shrimp may be negatively impacted by the increasing conversion of forests and pasture to intensive agriculture (e.g. banana or pineapple plantation) along their migration route.

Factors associated with agricultural expansion, such as increased levels of pesticides in surface waters, can potentially threaten the long-term persistence of migratory shrimp. Aquatic ecosystems in Costa Rica are vulnerable to contamination with pesticides because of the intensity of the application, type of application, toxicity of the compounds used and extensive drainage areas of the cultivation combined with high rainfall (e.g., Castillo et al. 1997). Intensive banana cultivation requires high quantities of agrochemicals to control pests (Castillo et al. 1997) and massive mortality of fish in rivers draining banana plantations in Costa Rica have been reported to occur between five and six times a year (Gatgens 2005). Traditional methods of toxicity testing indicate that the surface waters in the region downstream from banana plantations are of impaired quality potentially leading to acute or chronic toxicity in invertebrate aquatic organisms (Castillo et al. 2006), but little information exists on the impacts of agriculture on shrimp populations.

Although shrimp are important components of tropical freshwater ecosystems, population data are uncommon and historical population data are rarer still (but see Lewis et al. 1966, Walker and Ferreira 1985, Collart and Magalhaes 1994). Like many Neotropical regions inhabited by shrimp, the Sarapiquí region of Costa Rica, downstream from La Selva Biological Station (LSBS), has experienced dramatic land use change and river fragmentation over the past few decades, as a result of massive conversion of secondary forests to banana plantations and pasture (Figure 1). Unlike many other, sites, however, we collected historical shrimp population data at La Selva Biological Station prior to massive landscape conversion downstream, making comparisons between historic and contemporary shrimp populations possible.

In this study, our main objective was to compare shrimp relative abundance between a historical time period (1988-89; pre-disturbance) and a recent time period (2008-11; post-disturbance). We hypothesized that shrimp relative abundance would be lower at three stream sites located within or bordering La Selva Biological Station, potentially as a result of downstream changes in land use affecting recruitment success upstream. A secondary objective was to measure inter- annual variability in shrimp abundance; and intra-annual (seasonal) variability in abundance, body size, and fecundity using data from 2008-11 to inform future monitoring efforts. We hypothesized that shrimp abundance would exhibit little inter-annual variability but would exhibit seasonal variability. We also hypothesized that *Macrobrachium olfersi* would exhibit aseasonal fecundity (constant body size, percentage of females, and percentage of gravid females),

A final objective, was to evaluate the efficacy of using modified minnow traps to collect shrimp population data.

Methods

Study Site

This study was conducted at La Selva Biological Station (LSBS) (10° 26” N 84° 01” W), a 1600 ha forested reserve that is relatively protected from hunting and fishing, located in the Sarapiquí drainage of Costa Rica’s northern Caribbean slope (Fig. 1). The Sarapiquí watershed covers an area of 2,793 km^2 in a landscape that has undergone dramatic land-use changes over the past half century following a pattern typical for the Neotropics. Agricultural production and settlement of the region started in the late 1800s and there have been various waves of settlement and agricultural development since then (Butterfield 1994). The Sarapiquí region had very little intensive banana production until the late 80s and early 90s when an unprecedented increase in conversion of forest and pasture to banana production occurred (Montagnini 1994), increasing the aerial extent from <5% of the Sarapiquí region to >15%. Specifically, from 1991 to 1994 the aerial extent of banana production increased three-fold from 2,673 hectares to 6,506 hectares and the population increased 185% in the Sarapiquí region (Vargas 1995). In the late 90s and early 2000s (post-expansion) the area under intensive agricultural cultivation remained relatively constant, however the production switched from being primarily dominated by banana and other crops (e.g. black pepper, ornamental plants) to a mix of pineapple and banana cultivation. Forest cover in the Sarapiqui region declined from 73% to 34% from 1963 -1996). Since the late 1990s, pastures used for cattle production have decreased and some regrowth of forests has occurred. The most recent land use data indicate that, in 2005, the aerial extent of forest, including forest regrowth, had increased to 56% of the Sarapiquí region while agricultural land use (both intensive agriculture and pasture) was 43% of the aerial extent (Montoya and Malavasi 2008).

Stream reaches sampled in this study were in the Sabalo and Salto rivers (Figure 1, Table 1). The Salto River is a third-order river with a completely forested watershed and its headwaters are encompassed by Braulio Carillo National Park, which is contiguous with LSBS (Fig. 1). The Sabalo River defines part of the border of LSBS and is bordered on the other side by a cattle pasture. The watershed of the Sabalo is a mix of protected forest and agricultural land use, with cattle pastures comprising the majority of the extent of agricultural land.

Fifteen species of amphidromous shrimps occur in Costa Rica, with seven species (*Atya scabra, Atya crassa, Atya innocous*, *Macrobrachium acanthurus, Macrobrachium. heterochirus, Macrobrachium olfersi,* and *Macrobrachium carcinus*) occurring on the Caribbean side (Obregon 1986). Adult and juvenile shrimp occurring at LSBS have migrated between 50-55 km upstream from the ocean. In this study we focus on *Macrobrachium olfersi* because it occurs at high enough densities to allow us to estimate its abundance.

Average annual temperature at LSBS is 25.8° C, with monthly mean daily temperatures ranging from 24.7 ° C in January to 27.2° C in August (Sanford et al. 1994). The elevation of LSBS ranges from 30-132 m asl. The long-term annual precipitation average is 3,962 mm. The distribution of precipitation through out the year at La Selva is characteristic of the Atlantic side of Central America with January-April receiving the least amount of precipitation although monthly precipitation averages are highly variable. Precipitation data used in this study are from the La Selva weather station database (http://www.ots.ac.cr).

Sampling

From December 1988-December 1989 and January 2008-December 2011, relative abundance of shrimp was measured from one to three times per month from three stream reaches: one in the Sabalo River (Sabalo) and two in the Salto River (Salto1 and Salto2). During each time period, three modified minnow traps were placed in pools along each 100-m stream reach, baited with raw chicken and left for two nights before being collected. On the morning of the second day, all shrimps were identified to species, sex was determined and total length (cm) measured. Shrimps were collected in the same stream reaches and following the same methodology during the two time periods.

To investigate seasonality of reproduction, twenty shrimp traps were placed once a month, from January 2008 to December 2011 at the Salto1 and Sabalo stream reaches. The traps were placed every 5-m along both sides of a 100-m stream reach. Baited traps were placed in the afternoon and on the second morning the shrimp were collected, measured (total length), sex identified and reproductive state determined. The traps in the Sabalo and Salto reaches were located in pools, riffles and runs. In the Sabalo reach, the pool habitat was characterized by sand or clay substrate, whereas the riffles and runs contained a mix of cobble and sand substrates. In the Salto reach, the pool habitat was characterized by clay or sand substrate whereas the runs contained a mix of boulders and sand.

Statistical analysis

The relative abundance data (counts of individuals per stream reach) were examined with an auto-correlation function to test for temporal correlations between repeated samples at a stream reach. Auto-correlation analysis uses Pearson correlation coefficients to relate the abundance from t+1 with the abundance at time t, where t can represent various time lags and determine if there is a significant relationship (Zuur et al. 2009).

To examine the effects of season on *M. olfersi* relative abundance we used the number of *M. olfersi* per stream reach as the response variable and season (dry or wet) and precipitation thirty days previous to the sampling date (mm) as explanatory variables. A quasi-generalized linear model (GLM) with a Poisson distribution was used to account for over-dispersion, which is typical of low count data.

An ANCOVA was used to examine the seasonality of *M. olfersi* fecundity. Our response variables included the percentage of gravid females, the proportion of the population that is female, or individual body size; and explanatory variables included precipitation 30 day previous to the sample date and season. Percent of gravid females and percent of females from the population were arcsine transformed to better meet assumptions of normality.

A combination of approaches was used to examine the effect of site and year on *M. olfersi* relative abundance. Generalized linear models were used to determine the most appropriate distribution for modeling counts and a Bayesian approach was used to estimate means and confidence intervals for each explanatory variable on relative abundance. The effect of site and year on relative abundance was estimated separately for the dry and wet season. A Poisson error distribution and dispersion parameter were specified in the generalized linear and Bayesian model structures to better account for zero-inflation in count data and to adjust precision of standard errors. We used a Bayesian approach, implemented with JAGS software in Program R with package rjags, to calculate the means and confidence intervals for each site and time period (Plummer 2011). JAGS is a program for analysis of Bayesian models which using Markov Chain Monte Carlo (MCMC) methods to calculate posterior distributions for model parameters. All models were fit using 5,000 iterations, 2,000 iterations of burn in, and diffuse priors. Using the pwr package in Program R (Champely 2011), we performed a power analysis to examine the effectiveness of traps to detect changes in relative abundance. Model inputs were the mean number of shrimp individuals across all the sites from 2008-2011 and the standard deviation. All other analyses were performed using the R statistical program (R Core Development Team 2011). For the analysis, alpha was set *a priori* to 0.05.

Results

During the historical sampling period (1988-89), three species of shrimps were captured and identified, with *Macrobrachium olfersi* (n=343) being most common and *Macrobrachium* *carcinus* (n=15), and *Macrobrachium heterochirus* (n=4) being rare. Similarly, during the recent time period (2008-2011) five species of shrimps were captured and identified, with *Macrobrachium olfersi* (n=399) being most common and *Macrobrachium heterochirus* (n=2), *Macrobrachium acanthurus* (n=1), *Macrobrachium carcinus* (n=23), and *Atya scabra* (n=1) being rare. Given the disparity in common versus rare species counts during both time periods, we focus the rest of the manuscript on *M. olfersi* because its higher density allowed us to create statistical models to discern true changes in abundance over years and seasons. Auto correlation function analysis showed that the abundance of *M. olfersi* at a stream reach was not significantly related over any of the temporal correlations. Thus each sample of relative abundance at a stream reach through time was considered an independent sample of the population in all other analyses.

Recent population dynamics (2008-2011)

Within stream reaches from 2008-2011, *M. olfersi* populations exhibited little inter-annual change in relative abundance (Table 2, Fig. 2). From 2008-2011, mean abundance was somewhat variable, ranging from 0.42-4.2 shrimps per sampling period across all the stream reaches. However, relative abundance of *M. olfersi* in the Sabalo reach was significantly lower than in the Salto1 or Salto2 reaches. Mean body size was consistent annually and seasonally during the current time period (Figure 3).

Relative shrimp abundance was significantly lower in the wet season (1.56±0.15 SE) compared to the dry season (1.84±0.19 SE; df=303, P = 0.0454; Fig. 4). Cumulative precipitation thirty days prior to sampling had no effect on relative abundance. In the dry and wet seasons, *M. olfersi* exhibited the same patterns in relative abundance across stream reaches and years during the recent time period.

There was no difference in the proportion of females (60%) or the percentage of gravid females (69%) in Salto1 and Sabalo reaches. Neither precipitation nor season was associated with the percentage of gravid females (df = 41, F=0.6953, P=0.8850) (Fig. 5a) or the percentage of female shrimp in a reach (df=3, F=0.8881, P=0.3513) (Fig. 5b).

Comparisons between two time periods

We found shrimp relative abundance in two reaches (Salto1 and Salto2) to be relatively constant among years and between recent (2008-2011) and historical (1988-89) time periods (Fig. 2 and 3). In the Salto2, the mean abundance decreased between time periods in both the wet and dry seasons but the decline was not statistically significant. In comparison, the Sabalo reach showed a significant decrease (7-fold) in relative abundance between current and historical time periods suggesting a site-level change. The Sabalo reach went from having the highest *M. olfersi* relative abundance compared to the other reaches during the historical period to having the lowest relative abundance in the recent period.

The mean and standard deviation (1.89± 1.11) used in the power analysis was derived from all the stream reaches during the recent period. Results indicate that 360 samples are required to observe a decrease in relative abundance of 10%, 60 samples to observe a 25% decline, and 17 samples to observe a 50% decline at our study sites.

Discussion

Our findings suggest that *M. olfersi* populations in protected upstream reaches at LSBS were resistant and/or resilient to major land use change downstream even though the downstream larval drift and upstream migration of juveniles were vulnerable to downstream perturbations. The similarity in relative abundance of *M. olfersi* between pre- and post-disturbance sampling periods was surprising, given the extent of downstream disturbance in the Sarapiquí watershed: While fish and shrimp kills are commonly reported, resulting from pesticide runoff during the first storm event after a dry period (Gatgens 2005,Wheat 1996), *M. olfersi* populations in forested streams at LSBS had the same relative abundance between historical and current sampling periods.

The lack of continuous temporal data from post-banana plantation expansion (1990-2007) makes it difficult to evaluate whether shrimp abundance is relatively resistant or very resilient (or a combination of the two) to this downstream disturbance. If *M. olfersi* was resistant to perturbation, its abundance would change little in response to disturbance and maintain a relatively constant level, whereas a population with high resiliency would decrease but quickly recover to previous levels post-disturbance (Holling 1973).

The resiliency and/or resistance of *M. olfersi* populations inter-annually in recent time periods, and between recent and historical time periods at the two forested sites, could be explained by the nature of their amphidromous migratory life cycle and continuous, year- round breeding. Amphidromous *Macrobrachium olfersi* release much higher quantities of larvae per individual (~10 times more) than non-migratory *Macrobrachium* spp. (Mossolini and Bueno 2002). Shrimp larvae are present year-round in high densities relative to other drifting invertebrates and comprise the majority of drifting invertebrate larvae in streams at LSBS (Ramírez and Pringle 1998, Ramírez and Pringle 2001). We suggest that this large and constant year-round supply of shrimp larvae moving downstream may contribute to a similarly constant upstream migration of juvenile shrimps that could offset potential declines in recruitment resulting from episodic mortality events caused by pesticide runoff. Thus, the life cycle and natural history of amphidromous shrimps may confer some stability to upstream recruitment ,, explaining the similarity in abundance of adult shrimps between current and historical sampling periods in protected upstream forested reaches at LSBS. Moreover, recolonization of downstream stream reaches suffering from massive shrimp and fish kills may also be facilitated by the migratory life cycle of shrimp (McDowall 2007, McDowall 2010), further contributing to population resiliency.

Studies conducted in Puerto Rico similarly suggest that shrimps are highly resilient to disturbances such as whole-reach poisoning events (Greathouse et al. 2005) and stream de-watering above low-head dams (March et al. 2003, Crook et al. 2009). Amphidromous shrimp populations recovered to pre-disturbance densities 12 weeks following a poisoning event where chlorox bleach was used to kill shrimps along an 500-meter reach of a headwater stream (Greathouse et al. 2005). Greathouse et al. (2005) attribute this recovery to nearby sources of organisms for colonization, high mobility of organisms, unimpaired habitat, and location within a national forest. Likewise, March et al. (2003) and Crook et al. (2009) comment on the resiliency of shrimp populations to stream dewatering above low-head dams which can totally disrupt the flow of water to the ocean for up to several months in many rivers draining the Luquillo Mountains of northeastern Puerto Rico. They suggest that as long as some water is released over these low-head dams for some period of the year that shrimps should be able to recruit upstream, however, more research needs to be done on thresholds of connectivity.above low head dams.

We suggest that the major decrease in shrimp abundance (between 1988 and 2011) in the unprotected Sabalo stream reach is due to local factors (e.g. shrimp harvesting) versus regional landscape-scale disturbances downstream. In contrast to the protected status of our two study stream reaches within interior forests of LSBS (which showed no change in shrimp abundance between sampling time periods) the Sabalo study reach is directly adjacent to pasture and therefore accessible to local fisherman. Local people have been observed in the Sabalo collecting shrimp (Sanchez pers comm.), which are typically used as bait for fishing. Thus, our working hypothesis is that direct harvesting of adult shrimp is responsible for declines in shrimp abundance in our Sabalo study reach.. An alternative hypothesis is that, pollution from agricultural run-off is responsible, although this is unlikely given that the majority of the Sabalo watershed is forested and the remaining agricultural extent (3%) is primarily cattle pasture.

The seven-fold decrease in shrimp abundance that we observed in the Sabalo between the 1988-89 and 2009-11 sampling periods could have significant direct impacts on stream foodweb dynamics. For example, lower shrimp abundance could adversely impact animals in higher trophic levels such as otters that rely on shrimp for food (Spinola-Parallada and Vaughan-Dickhaut 1995). Omnivorous shrimps have also been shown to decrease the abundance of insects such as larval Chironomidae and decrease amounts of depositional inorganic matter (Pringle and Hamazaki 1998). In streams with lower shrimp abundance, transport of inorganic sediments could be less tightly linked to shrimp biota and controlled more by abiotic factors associated with precipitation and changes in river discharge.

In this study, the percentage of gravid females, percent females, and body size showed little variability year round suggesting that reproduction is aseasonal and could be monitored during either the wet or dry season. Our findings are in accordance with Ramírez and Pringle (2001) who found no seasonal patterns in shrimp larval drift in streams at LSBS, however this is in contrast to studies in other tropical regions, where gravid shrimp were found only during the wet season when transport of larva to the sea would be facilitated by high discharge events (Walker and Ferreira 1985, Palacios et al. 2008). While La Selva has a dry and wet season they are not very distinct and often the mean monthly precipitation during the dry season is no different than during the wet season (Sanford et al. 1994). The lack of distinct seasonal patterns in precipitation in the Carribbean lowlands of LSBS could explain why our results were in contrast to Palacios et al. (2008) who monitored shrimp in a region (Sinaolo, Mexico) with a distinct dry season. Fecundity is an important demographic parameter to monitor because reproduction has a high energetic cost for shrimp and the percentage of gravid females has been shown to be sensitive to habitat alteration (Covich et al. 2003, Leight et al. 2005). Our results, showing aseasonal shrimp fecundity, will be useful in informing population monitoring of shrimps in the Sarapiqui region of Costa Rica.

In the Sabalo study reach, a large change in shrimp relative abundance occurred between time periods and we were able to detect it with a small number of samples. However, smaller changes in shrimp populations could be occurring that are below our level of detection. Based on the results of the power analysis, to detect a 25% change in *M. olfersi* relative abundance, two times as many samples as those we collected would be required. Our use of three traps per study reach was a tractable way to monitor shrimp populations but was not powerful enough to detect smaller (i.e., < 25%) changes in abundance. The wet season, on average, had smaller confidence intervals around *M. olfersi* mean relative abundance than the dry season (1.95 and 3.43, respectively), suggesting that monitoring shrimp in the wet season would have a higher power to detect changes in the population. Future monitoring might consider sampling more intensely within a stream reach (i.e. a higher density of traps) during the wet season.

In conclusion, our data provide a rare opportunity to examine migratory shrimp population dynamics over both small and large time scales. Our findings strongly suggest that shrimp populations in “protected” stream reaches were resilient or resistent to regional landscape-scale perturbations occurring downstream from our study reaches. We suggest that the large number and constant year-round supply of shrimp larvae moving downstream may contribute to the apparent stability of adult shrimp populations in protected interior forested reaches at LSBS. While our sampling methodology was unable to detect changes in shrimp abundance <25%, we did find a seven-fold decline in relative abundance of *M. olfersi* in our unprotected study stream reach. While we suggest that the mechanism of the decline is due to local shrimp harvesting, this hypothesis needs to be further investigated.

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Table 4.1. Annual means (2008-2011) of physical site descriptors of stream reaches sampled in this study at La Selva Biological Station, Costa Rica. The Sabalo stream reach is located in the Sabalo River and the Salto1 and Salto2 reaches are both located in the Salto River.

Site	River	Wetted width (m)	Temperature (°C)	Conductivity (µS/cm)	Discharge (m^3/s)	pH
Salto1	Salto	8.0	25.3	98	0.79	6.1
Salto2	Salto	6.5	25.3	98	0.84	6.4
Sabalo	Sabalo	12.0	25.5	47	1.01	6.0

Table 4.2. *M. olfersi* relative mean abundance (individuals/site) and 95% confidence intervals (95% CI) at La Selva Biological Station stream reaches in the dry and wet seasons. Means and confidence intervals were estimated with three chains and 5000 MCMC simulation in a Bayesian model with a Poisson distribution corrected for over-dispersion.

		Dry		Wet	
Site	Period	Mean	95% CI	Mean	95% CI
Salto1	1988-89	4.30	(1.64-9.14)	3.19	(1.73-5.37)
	2008	3.42	(1.34-7.64)	1.72	(0.82-3.11)
	2009	1.34	(0.57-2.61)	1.02	(0.54-1.70)
	2010	1.44	(0.61-2.80)	0.91	(0.42-1.68)
	2011	1.09	(0.43-2.20)	1.27	(0.68-2.09)
Sabalo	1988-89	6.66	(3.94-10.01)	3.85	(2.54-5.41)
	2008	0.91	(0.20-2.46)	0.76	(0.28-1.58)
	2009	0.86	(0.34-1.77)	0.54	(0.22-1.04)
	2010	0.76	(0.27-1.60)	0.77	(0.36-1.38)
	2011	0.42	(0.10-1.00)	0.18	(0.04-0.46)
Salto2	1988-89	3.36	(1.67-5.69)	2.97	(1.72-4.67)
	2008	4.21	(1.73-8.66)	3.60	(1.92-6.05)
	2009	2.27	(1.07-4.13)	1.89	(1.09-2.97)
	2010	2.39	(1.23-4.17)	1.31	(0.67-2.25)
	2011	2.51	(1.05-4.88)	2.69	(1.64-4.10)

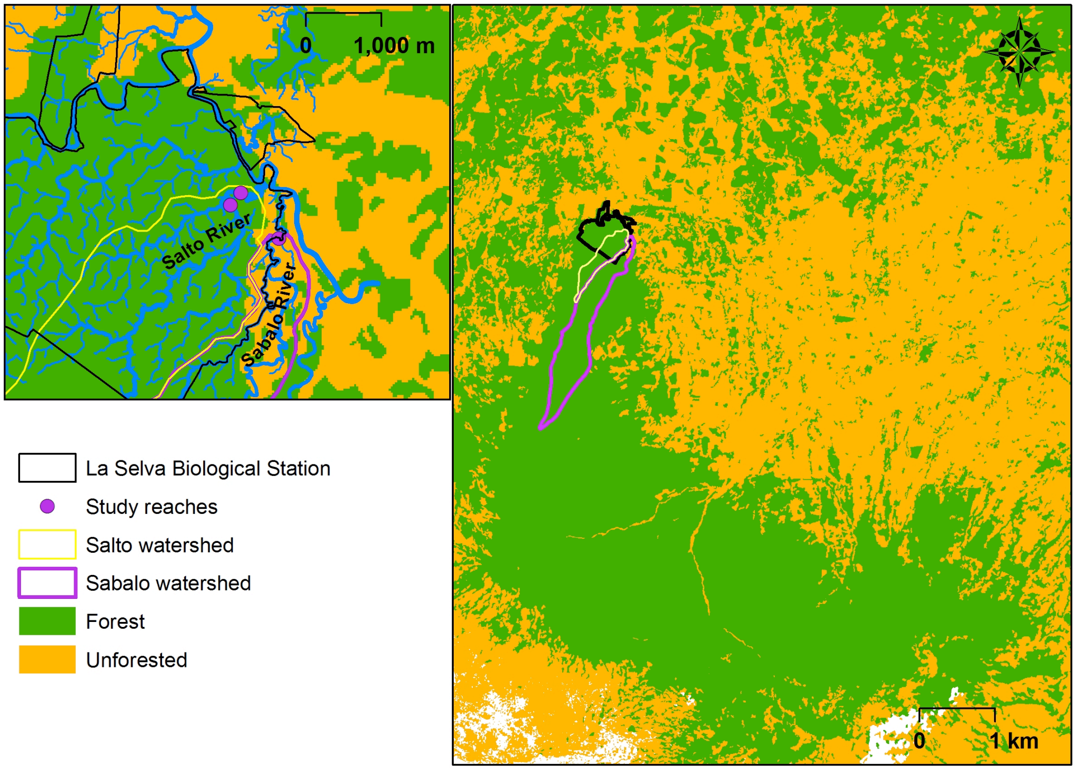


Figure 4.1. The map depicts watersheds of the sampling stream reaches and land use at La Selva Biological Station and in the Canton of Sarapiquí. Land use depicted is a 2005 land cover classification derived from Landsat ETM+ satellite imagery with a 30-m pixel size (Montoya and Malavasi 2008).

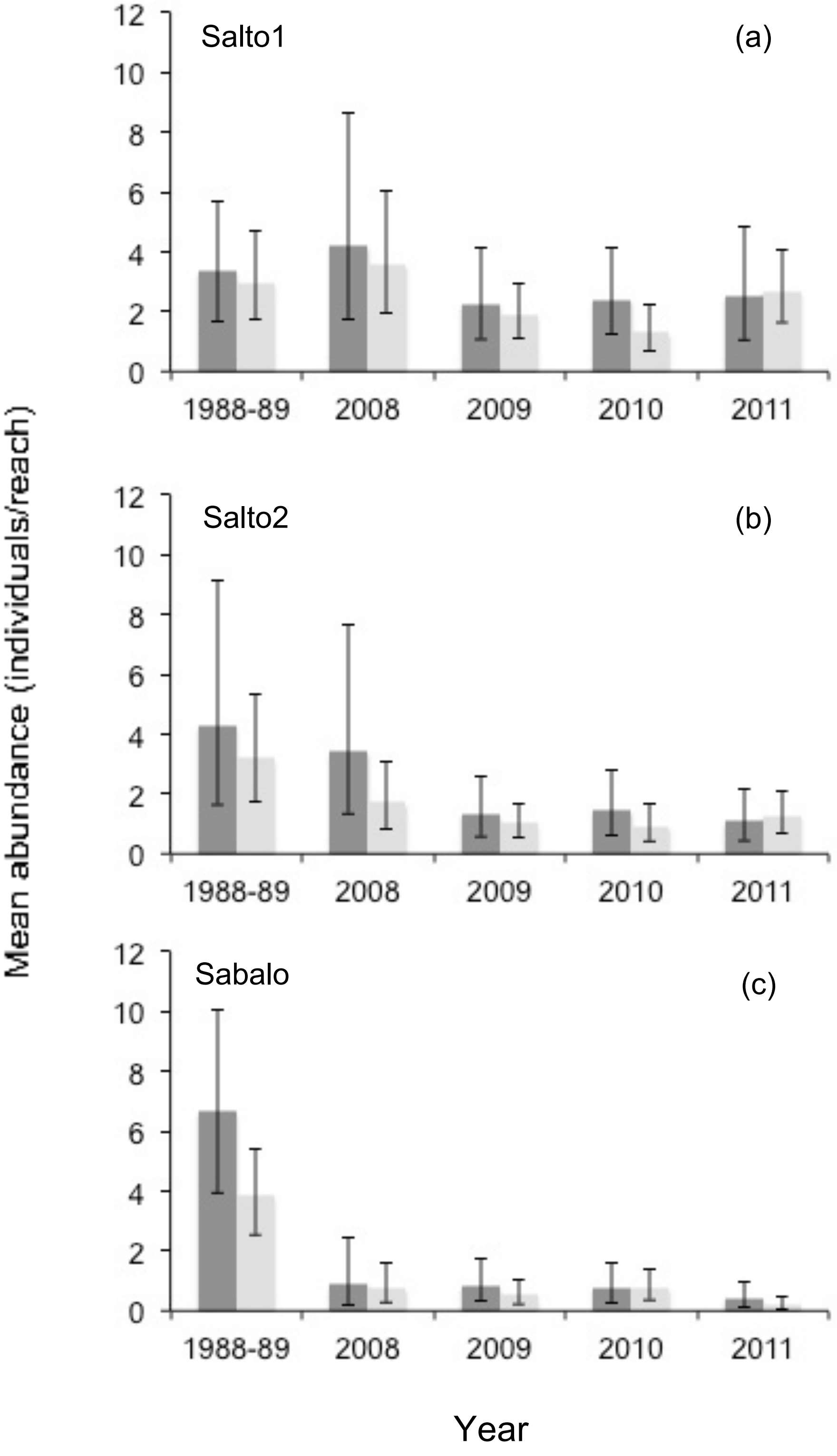

Salto1
(a)
Salto2
(b)
Sabalo
(c)
12
10
8
6
4
2
0
1988-89
2008
2009
2010
2011
Mean abundance (individuals/reach)
Year

Figure 4.2. Relative abundance (individuals/site) of *M.olfersi* during the historical (1988-89) and recent time period (2008-2011) in three stream reaches, Salto1, Salto2, and Sabalo (a,b, and c respectively), at La Selva Biological Station during the wet (light grey bars) and dry (dark grey bars) seasons. Means and confidence intervals were estimated in a Bayesian framework using 5000 MCMC iterations and three chains. A Poisson error distribution and dispersion parameter was specified to account for zero-inflation in the count data and to adjust precision of standard errors.

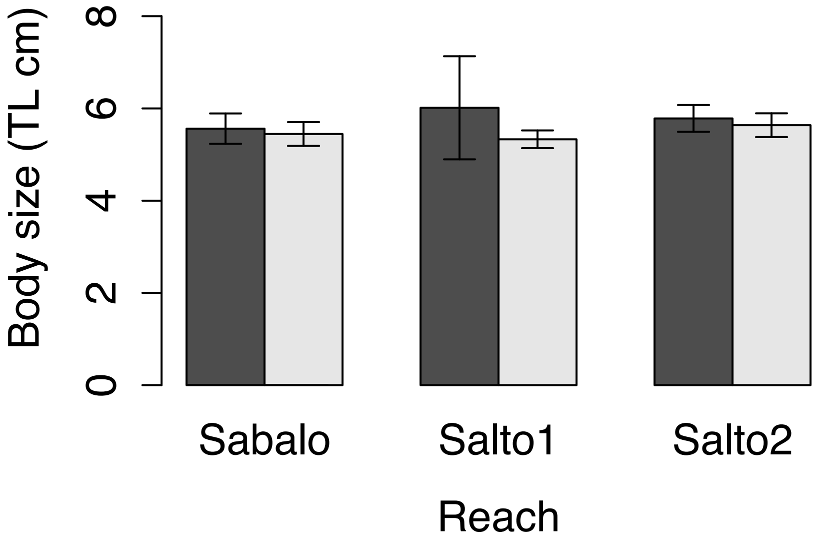


Figure 4.3. *M. olfersi* body size (total length cm) in three stream reaches (Salto1, Salto2, and Sabalo) of the Salto and Sabalo rivers at La Selva Biological Station during the dry (dark bars) and wet (light bars) season. Error bars represent estimated 95% confidence intervals.

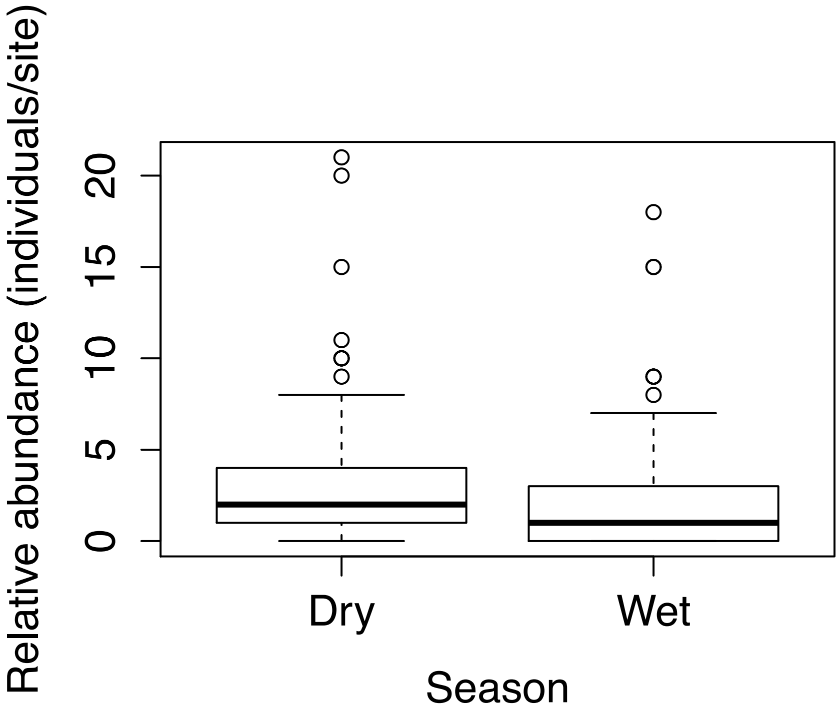
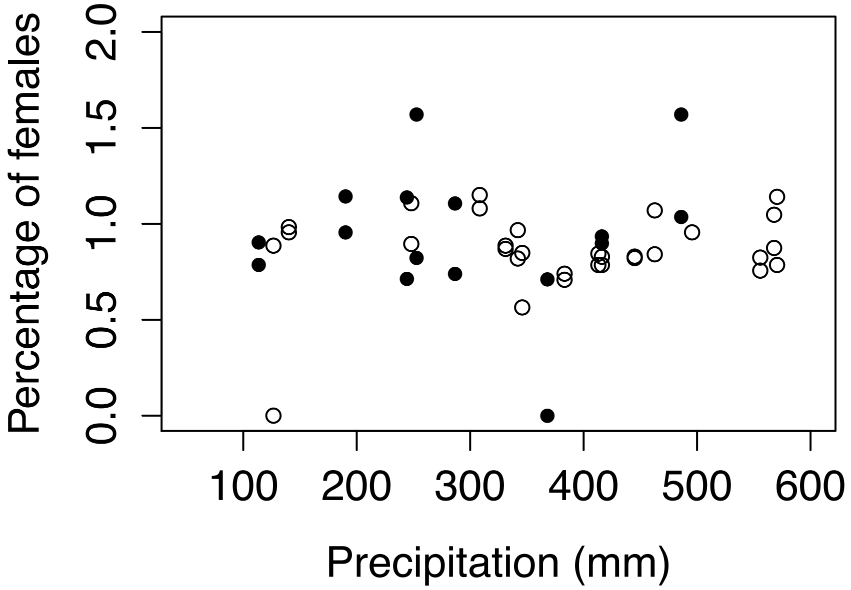


Figure 4.4. *M. olfersi* relative abundance (individuals/site) during the dry and wet season in three stream reaches in the Salto and Sabalo River at La Selva Biological Station from 2008-2011. Dry season had significantly higher relative abundance than the wet season ($p<0.0378$).

(a)


Percentage of females
0.0
0.5
1.0
1.5
2.0
100
200
300
400
500
600
Precipitation (mm)

(b)

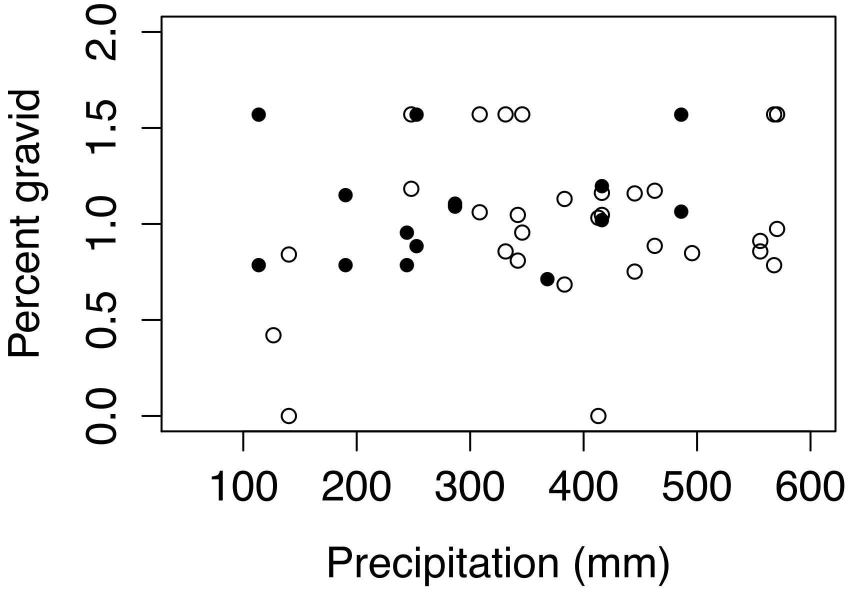

Percent gravid
0.0
0.5
1.0
1.5
2.0
100
200
300
400
500
600
Precipitation (mm)

Figure 4.5. The arcsin transformed percentage of females in the population (A) and arcsin transformed percentage of gravid females (B) of *M. olfersi* from the Sabalo and Salto Rivers during two years (2009-2011) at La Selva Biological Station. Precipitation (mm) is precipitation accumulated 30 days previous to sampling date. Open circles represent the wet season. Closed circles represent the dry season.

CHAPTER 5

STOICHIOMETRY OF OMNIVOROUS SHRIMP CONSUMERS AND NUTRIENT RECYCLING RATES ACROSS A PHOSPHORUS GRADIENT IN LOWLAND TROPICAL STREAMS[6]

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[6] Snyder, M.N., Small G.E. and C.M. Pringle. To be submitted to *Journal of Freshwater Science*

Abstract

Animals have long been recognized as important recyclers of nutrients within aquatic ecosystems. The mass balance approach of ecological stoichiometry suggests that animal growth and the recycling of carbon and nutrients are strongly predicted by the elemental imbalance between food resources and consumer elemental composition. In this study, across a P-gradient in lowland tropical streams at La Selva Biological Station, we describe: (1) shrimp body stoichiometry and its degree of homeostasis; (2) rates of phosphorus (P) excretion by shrimps; and (3) the importance of shrimp (with low body %P) as nutrient recyclers, relative to fish (with higher body %P). We discuss our findings in relation to the predictions of ecological stoichiometric theory based on consumers and food resource stoichiometry.

Our study found that shrimp body nutrient content does not change based on stream P-levels, despite large differences in P-content of their likely food resources, suggesting that shrimps maintain strict homeostasis across this P-gradient. We also found that shrimp P-recycling rates were not tightly coupled with stream SRP levels. Stable isotope results indicate that shrimp show different diet choices over the P-gradient, which may partially compensate for differences in food P-concentrations, but P- recycling rates could not be explained by diet choice alone indicating that some other physiological or behavioral mechanism is involved. Compared to fish species in these same study sites, shrimp species recycle P at lower rates, despite their lower body P-demand. Our results indicate that diet and behavioral traits are important for large-bodied shrimp in mediating the relationship between consumer and food resource stoichiometry, and nutrient recycling rates.

Introduction

In aquatic systems, animals can play important roles in nutrient and carbon cycling directly through translocation of nutrients between ecosytems (e.g. migrating salmon) (Mitchell and Lamberti 2005), via excretion (McNaughton et al. 1997, Elser and Urabe 1999, McIntyre et al. 2007), or indirectly through modification of the physical environment (e.g. bioturbation) (Pringle et al. 1999, Vanni 2002). Excretion by consumers can directly influence nutrient cycling, by changing the quantities of nutrients available to autotrophs (Flecker et al. 2002, Vanni 2002) and facilitating their downstream transport by mobilization, (Small et al. 2011). Nutrients from food resources ingested and assimilated by consumers are excreted after they have used what is necessary for metabolic maintenance and growth (Vanni 2002).

The effect of anadromous fish migrations on stream nutrient cycling has been well-studied (Mitchel and Lamberti 2005, Schuldt and Hershey 1995, Wipfli et al. 1999) but little is known about how invertebrates and more specifically migratory shrimp affect stream nutrient and carbon cycling. Many freshwater shrimp of the *Macrobrachium spp*. display a different form of migration, known as amphidromy, where the shrimp assimilates most of its biomass and lives the majority of its life in the freshwater but transitions from larvae to juvenile in saltwater. Amphidromous shrimp migrate upstream once but unlike semelparous salmon this does not mark the end of their lives. While both salmon and shrimp are migratory the mechanism by which shrimp affect nutrient and carbon cycling is more likely via excretion than translocation of nutrients from the marine system.

Ecological stoichiometry provides us with a theoretical framework in which we can link individual consumers with the ecosystem level process of nutrient cycling (Frost et al. 2002, Sterner and Elser 2002). The mass balance approach of ecological stoichiometry suggests that animal growth and the recycling of carbon and nutrients are strongly predicted by the elemental imbalance between food resources and consumer elemental composition (Sterner and Elser 2002). Growth rates of animals can be diminished by insufficient supply of one or more mineral nutrients (Urabe et al. 1997, Elser et al. 2000). Stoichiometrically-explicit models of consumer growth have assumed limitation by one element at a time, with a threshold elemental ratio (TER) at which growth limitation switches from one dietary element to another (e.g. growth limited by phosphorus to growth limited by carbon) (Frost et al. 2006). Growth of herbivores and detritivores, who are feeding on relatively low-quality (high carbon:nutrient) food, are generally considered to be limited in aquatic systems by nitrogen or phosphorus (Elser and Urabe 1999, Elser et al. 2000). However, consumers can also be limited by carbon or energy (Schindler and Eby 1997, Elser and Urabe 1999, Tiunov and Scheu 2004).

Consumers have long been recognized as important recyclers of nutrients within ecosystems (Sterner 1986, Grimm 1988). Stoichiometric theory posits that body size, body nutrient content, plasticity of body nutrient content, diet, gross growth efficiency, and elemental imbalance between consumers and food resources determine rates of nutrient recycling (Sterner and Elser 2002). Factors determining the importance of consumers as nutrient recyclers remain uncertain, but studies have found that in streams consumer excretion can become important to overall stream nutrient demand when either a consumer species represents a unique pathway for a high-nutrient food subsidy (i.e. terrestrial insects) (Carpenter et al. 1992, Small et al. 2011) or when their diet is high in nutrients relative to the TER (Schindler and Eby 1997, Vanni et al. 2002). A recent study on nutrient recycling by fish at La Selva Biological Station (LSBS) found that body nutrient content and diet interact to mediate excretion rates in omnivorous fish (Small et al. 2011). One fish (*Astyanax aeneus*) with a low body %P was found to be a keystone recycler of P, contributing ~90% of stream P-demand in a low-P stream (Small et al. 2011). In addition to fish, shrimps are also a common component of the macroconsumer fauna in these streams and have been shown to significantly contribute to nutrient flux in streams in Puerto Rico (Benstead et al. 2010). While shrimps are at relatively low densities (0.2-3 m^{-2}) in our study streams at LSBS, shrimps' body P content is lower then that of fish, due to the absence of bones, suggesting that they require less P for growth and thus may be expected to recycle P at a higher rate.

In this study, we contribute to stoichiometric theory by examining how the strength of homeostasis, body nutrient content, and species identity mediate rates of P-recycling for a large invertebrate consumer across streams ranging in P. We explore factors contributing to phosphorus excretion rates of two common shrimp species in lowland tropical streams. Specifically our objectives are to: (1) determine the strength of homeostasis in two shrimp species (*Macrobrachium carcinus* and *Macrobrachium olfersi*) across a P gradient; (2) determine if stream P-levels influence rates of shrimp P excretion; and (3) compare rates of shrimp P excretion with previously published estimates of fish consumers in these streams.

Methods

Study site

This study was conducted at La Selva Biological Station (LSBS) (10° 26" N 84° 01" W), a 1600 hectare forested reserve located on the Caribbean slope of Costa Rica at the intersection of the coastal plain with the Cordillera Central. Daily and seasonal stream temperature have low variability (24-26 °C). The long-term annual precipitation average is 3,962 mm of precipitation (Sanford et al. 1994). The geomorphology of the region results in heterogeneous stream chemistry because regional solute-rich groundwater surfaces where the foothills transition to the lowlands (~60 m elevation) (Pringle 1991). Regional groundwater has high levels of solutes (P, Cl, Na, Mg, HCO_3, Ca), ranging from 13-29 times more concentrated than solute levels in local surface water (Genereux and Pringle 1997). The stream sites used in this study (1^{st}-4th order) are a subset of sites that are part of a long-term study that has continuously monitored them monthly since 1997 (Pringle and Triska 1991, Triska et al. 2006, Small et al. 2011) (Table 5.1).

This study builds upon previous work done on ecological stoichiometry and nutrient cycling in tropical forested streams with elevated phosphorus levels from naturally solute-rich seeps at La Selva Biological Station (LSBS) (Small and Pringle 2010, Small et al. 2011). Aquatically derived food resources (detrital biofilm, epilithon, and aquatic insects) have higher phosphorus (P) content in high-solute stream reaches along the phosphorus gradient. Comparing the low-P Sura-60 and the high-P Sura-30, the P content increases 1.6-fold for periphyton, 1.5-fold for aquatic insects, and 2.4-fold for coarse particulate organic matter (Small and Pringle 2010). Aquatic insects from four orders and five functional groups were found to deviate from strict homeostasis and demonstrate a reduced imbalance with their food resources (Small and Pringle 2010).

Fifteen species of amphidromous shrimps occur in Costa Rica, with seven species occurring on the Caribbean side (Obregon 1986). *Macrobrachium* spp. are considered omnivorous and consume a combination of algae, detritus, insects, and other shrimps (Kilham and Pringle 2000, March and Pringle 2003). We examine abiotic and biotic factors that affect body nutrient content and phosphorus excretion rate for two of the most frequently encountered shrimp species (*Macrobrachium olfersi, Macrobrachium carcinus*) (Snyder Chapter 1) and we describe body nutrient content for the rarer taxon (*Macrobrachium heterochirus*).

Body nutrient and stable isotope analysis

We analyzed the elemental composition (C, N, and P) and natural stable isotope abundance ($\delta^{15}N$ and $\delta^{13}C$) of shrimp as well as their potential food resources (coarse particulate organic matter, periphyton, aquatic insects). Shrimps were collected in streams that had a wide range of soluble reactive phosphorus (SRP) concentration (2-96 μg/l). For body nutrient composition shrimps (*M. olfersi* and *M. carcinus*) were sampled from ten reaches with varying SRP along two streams (Salto and Sura) at La Selva Biological Station from June-August 2008. Stream reaches were selected with a stratified random design, either high or low phosphorus and near or far from the mainstem river. Shrimp were collected using modified minnow traps baited with cat food. The digestive tract was extracted and excluded from chemical analysis. For stable isotope analysis, periphyton and insect invertebrates were collected from seven study streams in February and July 2006. Epilithon was collected with loeb samplers and from unglazed ceramic tiles after 4 weeks of incubation in a stream.

Samples were kept frozen until they could be freeze-dried or oven-dried at 50 ºC for 48 hours and then homogenized. For C and N analysis of shrimp, samples were analyzed on a Carlo Erba NA 1500 CHN analyzer (Carlo Erba, Milan). For P analysis first samples were acid-digested (Aqua Regia double acid; Jones et al. 1991), and then analyzed spectrophotometrically using the ascorbic acid method. Ground pine needles (US National Institute of Standards and Technology, 1575a) and poplar leaves (Analytical Chemistry Laboratory, University of Georgia) were used as external standards for P and N analyses.

Samples for stable isotope analysis were oven-dried for 24 hours at 80 ° C or freeze-dried for 48 hours before being ground and weighed into tin combustion capsules. For natural abundance of $\delta^{15}N$ and $\delta^{13}C$, stable isotopes samples were analyzed with a mass spectrometer.

All samples were analyzed at the University of Georgia's Odum School of Ecology Analytical Chemistry Laboratory.

Excretion

We quantified P excretion rates for *M. olfersi* and *M. carcinus* across our four study streams during April and May of 2010. Shrimps were collected using modified minnow traps. The traps were baited, but the shrimps were prevented from consuming the bait by a physical barrier. Shrimps were immediately placed into individual plastic bags (1 individual/bag) containing between 180-250 mL of water for small individuals (<18 mm CL) and 500-1000 mL of water for larger individuals (>18 mm CL). Water for all excretion trials was collected from a low phosphorus site (Sura 60) and filtered to remove suspended particles using Whatman Grade number 1 filter paper (11 µm pore size). Water samples were collected from each incubation chamber immediately after the shrimp had been placed and again after a one-hour incubation, and filtered through a 0.45-µm Millipore filter (Millipore, Billerica, Massachusetts, USA) to remove feces and other particulate matter (e.g. eggs). Stress can impact excretion rates (Whiles et al. 2009) and any shrimp showing signs of stress were not included in the analysis. A one-hour incubation period was selected based on a study published by Whiles et al. (2009), which determined the time based on trade-off between stress and starvation. Water samples were immediately frozen and transported on ice to the University of Georgia's Analytical Chemistry Laboratory for analysis. Total P was measured using the absorbic acid method (APHA 1998). We measured excretion rates for 116 individual shrimp. Shrimp carapace lengths were measured in the field and each individual was sexed. Not all species were present in all four streams. Per capita P excretion rates were calculated as the increase in total phosphorus relative to the sample taken at the beginning of the 1-hour incubation.

For each stream reach, nutrient concentrations were measured from filtered water samples (0.45-µm Millipore filters) for soluble reactive phosphorus (ascorbic acid method), NO_3–N (cadmium reduction method), and NH_4–N (phenate method; APHA 1998).

Statistical Analysis

To examine if body nutrient content and elemental ratios (%P, %N, %C, C:N, C:P, and N:P) show ontogenetic effects, we used linear regression with data from all our sites pooled together. To examine if body elemental content was affected by species identity, we performed Analysis of Variance (ANOVA). Percentage values were scaled by dividing by 100 and arcsin transformed. A linear mixed model was used to test the effect of site phosphorus level on shrimp body element ratios to test how homeostatic shrimp nutrient content is relative to shrimp food resources. Stream reach phosphorus level was considered as a factor in the models, either high (>40 µg/L) or low (<40 µg/L). *M. olfersi* and *M. carcinus* were tested separately and site was included as a random effect.

We used $\delta^{15}N$ stable isotope data to inform our models about relative trophic level. To test for changes in shrimp diet (relative consumption rates of higher order trophic levels), we examined the effect of stream SRP on shrimp $\delta^{15}N$ signature. To compare shrimp $\delta^{15}N$ values to potential food resources, we used an estimated fractionation rate of 3.4 (Post 2002).

We examined what factors influenced the phosphorus excretion rates with linear regression models. Different alternative hypotheses were tested to examine the factors that control phosphorus excretion based on model building and ranking. Models were ranked with Akaike's Information Criteria corrected for small sample size (AIC_c); additionally, a psuedo R^2 value was calculated to assess model fit. We used linear models to rank the relative support for different hypothesises as to what factors control shrimp total phosphorus excretion. Log of phosphorus excretion was the response variable. Models built tested if the effect of species identity, body size (dry mass), site SRP level (high or low), and sex were important predictors of P-excretion rate. Twenty-eight of the 104 individual shrimp measured for total phosphorus excretion had final values of total phosphorus equal to or less then the quantity of total phosphorus in the chamber at the start time (i.e. P-excretion was not detected). Statistical models included a minimum detection value of 0.1 μg/L for those samples with rates <0 before log transformation to ensure a positive excretion rate. We used 0.1 μg/L as the minimum detection value because it was close to the detection limit of a sample.

Comparison of shrimp excretion to fish recycling in the low-P focal stream

To calculate abundance of shrimps at Sura-60, we first calculated the probability of capture and recapture based on eight different stream reaches that varied in abiotic and biotic variables. To determine shrimp abundance at each site (n=5), mark-recapture of individually marked shrimp was used (Table 5.1). Sites were sampled for shrimp with modified breeder minnow traps (Gee’s, Memphis, Tennessee), baited with dried cat food in mesh bags (Covich et al. 1996). At each site, 30 baited traps were haphazardly placed between 3-5 m apart on both sides of a stream reach in the afternoon and checked the subsequent morning. Individual shrimps were marked with VI Alpha tags and measured as described above. Streams sampled ranged from first- to third- order in size and flow to the Puerto Viejo River. Each site was sampled for a capture period of five consecutive days between one and seven times from January 2009-July 2009 and in April 2010. Sites were sampled during the typically drier (January-April) and wetter (May-December) parts of the year.

Huggins closed capture models were implemented in Program *Mark* to derive the probability of detection (*p*) and probability of recapture (*c*), which were then used to estimate abundance (Yip et al. 1996, White and Burnham 1999). Closed capture models assume that the population is closed, that animals retain their marks through the study period, and that the individuals are correctly identified (Otis et al. 1978). *Huggins closed capture* models differ from standard closed capture models in that, instead of using maximum likelihood to estimate abundance (N), probability of capture (p), and recapture (c) at once; they use maximum likelihood estimation to model p and c which can then be used to estimate values of abundance (N). *Huggins closed capture* models were fitted to examine two types of changes in the probability of capture; changes that occurred over time and behavioral responses to capture. Models were run which allowed p and c to change based on covariates, which included site, sex, shrimp biomass, and precipitation the day of sampling. Covariates were included to increase the precision of p and c (Otis et al. 1978, Huggins 1989). To estimate the probability of capture at sites with little information on probability of detection, all of the individual histories from each site that used the same capture method were pooled together (White 2005).

An information-theoretic approach was used to describe which models of shrimp probability of capture were the most well-supported (Otis et al. 1978, Anderson et al. 2000, Burnham and Anderson 2002). AIC_c was used to rank the models, with the best model having the lowest AIC_c score. Then AIC_c weight values were used to select a subset of the best-supported models to include in the confidence set.

Shrimp abundance at Sura-60 was calculated by first estimating the probability of detection (p) for all sites, and then applying that probability of detection, modified by the covariates in the confidence set of models, to each individual sampling period at Sura-60. There were no recaptures during some sample periods, but by applying the pooled estimated probability of detection (p) to the number of shrimp captured at a site this allowed us to estimate abundance at those sites as well. The number of individual shrimp encountered at a site on the first day of a closed capture session was divided by the calculated *p*-value for that day and time to estimate the abundance. Then the abundance calculations were weighted by the model weight of the two models in the confidence set of models to get the final abundance. By using the *p* value from all the sites together we were able to distinguish absence of shrimp (true zeroes) from areas with very low probability of recapture. Shrimp density was calculated by dividing abundance by the trapping area. For more details on the models and methods see Chapter 2. Shrimp abundance (individuals/m^2) was converted to shrimp biomass using length to mass regressions (Chapter 2).

The mean P-excretion rate (µg TP/ L/DM/hr) was multiplied by the density of shrimp (g/m^2) to get an estimate of TP excreted (µg/TP/m^2/hr) for the stream reach. This estimate of TP excreted was compared with previously published estimates of areal uptake rate for the low-P Sura-60 stream reach.

Results

Shrimp body nutrient content and natural stable isotope abundance

Species identity was significantly related to two descriptors of shrimp body nutrient content. *M. olfersi* has a significantly higher %N ($F_{1,57}$=10.64, p=0.002), %C ($F_{1,57}$=16.03, p=0.0002), and higher %P ($F_{1,57}$=5.98, p=0.02) when compared with *M. carcinus* (Table 5.1). Species identity was not significantly related to the other measures of body stoichiometry (p>0.05). *M. olfersi* (84.4) and *M. carcinus* (86.4) had similar mean C:P ratios. *M. heterochirus* (105.6) had a C:P ratio of 105.6 but this ratio was based on a limited sample size (n=2) (Table 5.1). One measure of body elemental content changed ontogenetically with shrimp body size; as *M. olfersi* total length increased the C:N of body elemental content increased ($F_{1,28}$=12.61, p=0.002) (Fig. 5.1). None of the measures of body nutrient content changed as *M. carcinus* increased in size (Fig 5.1). Shrimp consumers were found to be in strict homeostasis; no significant relationships were found between stream SRP level and shrimp body nutrient composition in *M. carcinus* or *M. olfersi* (p>0.05).

Neither species identity or body size was related with $\delta^{15}N$ values for shrimp biomass (p>0.05). The $\delta^{15}N$ values for *M. carcinus* and *M. olfersi* biomass, pooled across all sites, were 7.83 (±0.14 SE) and 7.84 (±0.10 SE), respectively. The mean $\delta^{13}C$ for *M. carcinus* and *M. olfersi* was -25.92 (±0.29 SE) (Fig. 5.2). However, shrimps $\delta^{15}N$ abundance was significantly lower (-0.61) in high-P stream reaches ($F_{1,57}$=15.62, p=0.0002) (Fig. 5.2). Neither species identity or body size was related with $\delta^{15}N$ abundance.

The mean $\delta^{15}N$ for periphyton, aquatic insects, and CPOM from all the sites was 5.43±0.28, 4.37±0.23, and 3.13±0.13 respectively. Aquatic insect mean $\delta^{15}N$ showed a large variation across the most common families collected in our grab samples, with Chironomidae having the lowest abundance (3.42) and Perlidae having the highest (7.00). After accounting for fractionation of $\delta^{15}N$, shrimps are classified as omnivores and across these sites are consuming a combination of the food resources that we sampled (periphyton, aquatic insects, and CPOM) (Fig. 5.3). No significant relationship was found between stream phosphorus level and the $\delta^{15}N$ abundance of shrimp food resources (CPOM, periphyton, and aquatic insect food resources) (p>0.05).

Effects of stream SRP on P excretion rate

Shrimp excretion rates ranged from 0.42-1.18 μg/L/hr TP for *M. olfersi* across our study sites (Table 5.3). *M. carcinus* P-excretion rates exhibited a larger range across our sites (0.09-1.92 μg/L/hr). We tested eight different linear models to assess support for hypothesis selected *a* *priori* about which factors would best predict shrimp total phosphorus excretion. Two of the explanatory factors, species identity and body mass, were highly correlated (R^2=0.76, p<.00005) so we only included body mass in our models. The most well-supported model (pseudo R^2=0.17, w_i=0.96) includes both body size and site as important predictor factors of P-excretion rate and is 96 times more likely than the next most well-supported model (Table 5.2). According to the most well-supported model, body size is positively correlated with P-excretion rate. Stream SRP level was not included in the most well-supported models, which indicates little evidence that stream SRP influences phosphorus excretion rates.

Discussion

In this study, we did not find differences in body elemental composition in a large-bodied invertebrate consumer in stream reaches across a P-gradient. The body nutrient content of shrimps differed from other smaller bodied macroinvertebrates, which showed two-fold increases in body %P in the high-solute Arboleda-30 relative to the low-solute Sura-60 (Small and Pringle 2010). Strict homeostasis by shrimps could explain these results, but it is also possible that the mobile, omnivorous shrimps compensate for lower P food resources in the low-P streams by consuming more food, consuming food items higher in P, and/or foraging across a larger area that includes high-P reaches.

While we have no direct evidence that shrimp are changing their diet in different sites, shrimps' $\delta^{15}N$ abundance increased as stream SRP decreased, suggesting that shrimps are feeding at a higher trophic level (i.e. a larger fraction of their diet consists of aquatic insects which are high in P relative to periphyton and detritus). Such a diet shift may partially offset the effects of lower P content in each of their food resources in low P streams, such that their overall diet is similar in P content. Our results are consistent with other studies of decapod crustaceans in nutrient enriched systems which demonstrated strict homeostasis of body nutrient content (Evans-White and Lamberti 2006), but are in contrast to studies of Atyid shrimps which showed differences in body N:P and C:P across a nutrient loading gradient in urban streams (Tsoi et al. 2011).

Our analysis indicates that the P-excretion rate of shrimps was not dependent on stream P-levels; this result is in contrast to previously published studies on cichlid fish excretion at these sites (Small et al. 2011). Shrimp excretion rates in this study were more similar to fish of the Poecilidae and Atherinidae families, which had lower body %P than the cichlids and fairly consistent P-excretion rates across sites. Ecological stoichiometry predicts that, as the imbalance between food and consumer (C:P) gets smaller, the limiting element (P) will be released more quickly back into the ecosystem. Threshold elemental ratios take into account the gross growth efficiency of an organism (essentially the elemental cost of maintenance), and therefore these ratios are a more precise way to determine when an organism is P-limited than by simply comparing the ratios of consumer C:nutrient and food C:nutrient. For example, if the $TER_{C:P}$ for an organism is greater than food resource C:P, the organism should be excreting less P than if the $TER_{C:P}$ is less than the food resource C:P. To test how we would expect shrimp excretion rates to change over the P-gradient in our study streams given body and food resource stoichiometry, we use an estimated $TER_{C:P}$ of 201 for shrimp based on estimates of other crustaceans by Frost et al. (2006) and C:P ratios of epilithon from the previously published estimates of Small and Pringle (2010). We develop predictions based on two different feeding regimes (100% epilithon and 100% aquatic insect) because our $\delta^{15}N$ abundance suggested that shrimp were consuming some proportion of them both. If we assume that shrimp are eating 100% epilithon or 100% insects at our high-P site, their $TER_{C:P}$ is greater than the epilithon C:P (90), and the insects (156), so with either diet we would expect to see a high rate of P-excretion. At the low-P site, the epilithon has a C:P of 230 and the insects 181 so we would expect P limitation and lower excretion rates if they are consuming 100% epilithon. Based on $TER_{C:P}$ to maintain consistent excretion rates in the low-P site they would need to consume at least 60% aquatic insects.

Our results are not in accordance with predictions based on $TER_{C:P}$. There are a number of possible reasons shrimp P-excretion rates would differ from predictions based on $TER_{C:P.}$ Shrimp could be growing at different rates, diet could vary by location, they could be food limited, they could have a different limiting nutrient, and/or they could be consuming more in low-P sites and have higher assimilation rates of P. The increase of $\delta^{15}N$ abundance in low-P sites suggests that shrimps are consuming different relative proportions of CPOM, insects and periphyton in high- and low- phosphorus reaches. Other studies have found food choice to be an important determinant of nutrient recycling rates. For example, in a study of gizzard shad, Pilati and Vanni (2007) found that nutrient excretion rates more closely related to diet choices then to body stoichiometry. However, while $\delta^{15}N$ stable isotope abundance increased from high- to low-P streams, shrimp P excretion rates of shrimps exhibited no clear pattern across P-levels. Growth rates of *M. olfersi* and *M. carcinus* were not correlated with stream SRP (Chapter 2). This suggests that P-excretion rates are not predictable by body stoichiometry, growth rate, or diet alone and that individual behavior or physiology could be important predictors of P-recycling rates.

When we compare mass-specific P-excretion rates of shrimp with previously published rates of fish (Small et al. 2011), at sites across the P gradient, surprisingly, shrimps are excreting P at a lower rate than the majority of the fish species (90% *M. olfersi* and 79% *M. carcinus*).

When we compare mean P excretion rate per individual fish in the Sura-60 a low-solute stream reach, both *M. carcinus* and *M. olfersi* are excreting at a higher rate than two of the five fish species (*Astatheros alfari* and *Archocentrus septemfasciatus*) (Table 5.5). By calculating shrimp density for an entire low-solute stream reach (Sura-60) we are able to compare excretion rates of *M. olfersi* and *M. carcinus* on a per unit area basis (m^2). In the Sura-60, shrimps excretion rates for *M. olfersi* were similar to the two fish taxa with the lowest excretion rates and *M. carcinus* was excreting at a higher rate than two (*Archocentrus septemfasciatus* and *Astatheros alfari*) of the five fish taxa. *Archocentrus septemfasciatus* and *Astatheros alfari* are omnivorous cichlids with higher body %P than shrimps, which indicates that shrimps are excreting at levels lower then would be predicted by their body %P. Shrimp excretion at Sura-60 (0.2 μg $TP/m^2/h$) seems to be a relatively small flux when compared with the total fish contribution (45.2 μg $SRP/m^2/h$) to P-demand through excretion which is not what we predicted based on the body %P and density of shrimp.

This study has provided us with the opportunity to compare P-excretion rates across different faunal taxa with different body P-content in the same streams. Compared to fish, shrimps have lower body P-content so stoichiometric theory predicts that they would recycle P at a higher rate, assuming similar diets and growth rates, however, in both high- and low-P streams, shrimps are recycling P at lower rates than fish. We do not have growth data for fish, but the quantity of P excreted suggests that shrimps have higher gross growth efficiency (i.e. a larger fraction of P ingested goes to production of new biomass) than fish and therefore excrete less P. Our results suggest that other factors (i.e. diet, growth rate) are as important as body nutrient ratios in predicting P-excretion rates.

In summary, the shrimps *M. carcinus* and *M. olfersi* are large-bodied consumers that, unlike other stream invertebrates, show no variation in body nutrient content over a stream P-gradient. Despite feeding on food resources that are elevated in P-content in high-P streams, shrimps did not have higher P- excretion rates, likely due to dietary shifts. Therefore, our results highlight the importance of diet choice and behavioral traits in mediating the relationship between consumer and food resource stoichiometry, and nutrient recycling rates.

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Table 5.1. Physical and chemical properties of the four stream reaches used in this study to examine phosphorus excretion rates by shrimp at La Selva Biological Station, Costa Rica. Values were means of monthly samples collected from January – April 2009. SRP is soluble reactive phosphorus.

Site	Discharge (m^3/s)	Conductance (µS/cm)	pH	Temperature C	NO_3-N (µg/L)	NH_4-N (µg/L)	SRP (µg/L)
Salto	0.17	98	6.0	25.1	227	19	92.2
Sabalo	0.28	47	6.0	25.5	137	29	8.0
Sura-60	0.19	20	5.6	24.8	199	21	3.0
Sura-30	0.61	157	6.1	25.4	163	18	83.0

Table 5.2. Shrimp body nutrient content (percentage of dry mass) and C:N, C:P, and N:P ratios for the three shrimp species examined in this study. The shrimp were sampled from eight stream reaches across a phosphorus gradient on the Sura and Salto Rivers at La Selva Biological Station. Mean values are reported with the range in parenthesis.

Species	%C	%N	%P	C:N	C:P	N:P
M. carcinus	35.6	9.16	1.18	4.55	86.38	19.14
(n=29)	27.9-39.6	6.50-10.76	0.79-4.10	4.27-5.02	17.56-127.01	3.51-28.96
M. heterochirus	37.7	9.71	1.01	4.53	105.56	23.48
(n=2)	36.9-38.4	9.31-10.11	0.72-1.31	4.43-4.62	72.58-138.56	15.71-31.25
M. olfersi	38.6	10.03	1.21	4.49	84.38	18.83
(n=30)	31.8-44.9	8.47-12.73	0.70-1.98	4.11-4.97	52.51-137.66	10.92-31.09

Table 5.3. Mass-specific P excretion rates (mean with SE in parenthesis) for three shrimp species in four streams ranging in dissolved P levels from 3-92 μg SRP/L. P excretion rates are in μg total phosphorus [g dry mass]$^{-1}$ h^{-1}. Minimum detection limit values are included in these means.

Species	**Salto** (92 μg/L SRP)	**Sura-30** (83 μg/L SRP)	**Sabalo** (8 μg/L SRP)	**Sura-60** (3 μg/L SRP)
M. carcinus	0.09 (0.03)	1.92 (0.86)		0.13 (0.04)
M. olfersi	0.42 (0.21)	1.18 (0.20)	1.06 (0.22)	0.44 (0.09)
M. heterochirus	2.27 (0.82)		2.34 (0.62)	

Table 5.4. Results of the linear regression models used to predict phosphorus excretion rates [g dry mass]$^{-1}$ h^{-1} showing values for number of parameters (k), Akaike's Information Criterion with the adjustment for small sample size (AIC_c), change in AIC_c (ΔAIC_c), Akaike weights (w_i), and log likelidhood (LL) for each of the models. The pseudo R^2 for the most well-supported model is 0.17. Both *Macrobrachium carcinus* and *Macrobrachium olfersi* were included in these models. Mass refers to the weight (g DM) of the individual shrimp. Site refers to which of the four study stream reaches. Stream P is soluble reactive phosphorus in µg/L. Sex refers to if an individual shrimp was male or female. Null is the model run without any parameters.

Parameters	**k**	**AIC_c**	**ΔAIC_c**	**w_i**	**LL**
Mass, site	6	156.17	0	0.96	-71.58
Site	5	164.68	8.51	0.01	-76.98
Stream P, site	5	164.68	8.51	0.01	-76.98
Null	2	167.99	11.81	0	-81.92
Mass	3	168.32	12.15	0	-81.02
Stream P	3	169.53	13.35	0	-81.62
Sex	4	172.05	15.87	0	-81.79

Table 5.5. Estimated contribution to stream phosphorus recycling for the two common shrimp species and the six most abundant fish species in a stream reach (Sura-60) with low SRP (soluble reactive phosphorus) at La Selva Biological Station. Fish phosphorus excretion rates are from Small et al. (2011).

Shrimp species	Density (individuals/m ^{2})	Mean size (g dry mass)	Density (g DM/m$^{2)}$	Mean P excretion rate (µg TP/ shrimp / hr)	TP excreted(µg TP m^{-2} h^{-1})
M. olfersi	0.26	1.5	0.39	0.44	0.11
M. carcinus	0.12	25.7	3.08	1.50	0.18

Fish species	Density (individuals/m ^{2})	Mean size (g wet mass)	Density (g wet mass/m^2)	Mean P excretion rate (µg SRP fish^{-1} hr^{-1})	TP excreted (µg SRP m^{-2} h^{-1})
Astyanax aeneus	0.5	10.6	5.5	81.0	40.5
Astatheros alfari	0.5	36.6	17.1	0.3	0.1
Archocentrus septemfasciatus	0.6	5.9	3.7	0.2	0.1
Priapicthys annectens	2.5	1.8	4.3	2.2	1.1
Alfaro cultratus	0.4	0.9	0.3	8.6	3.4

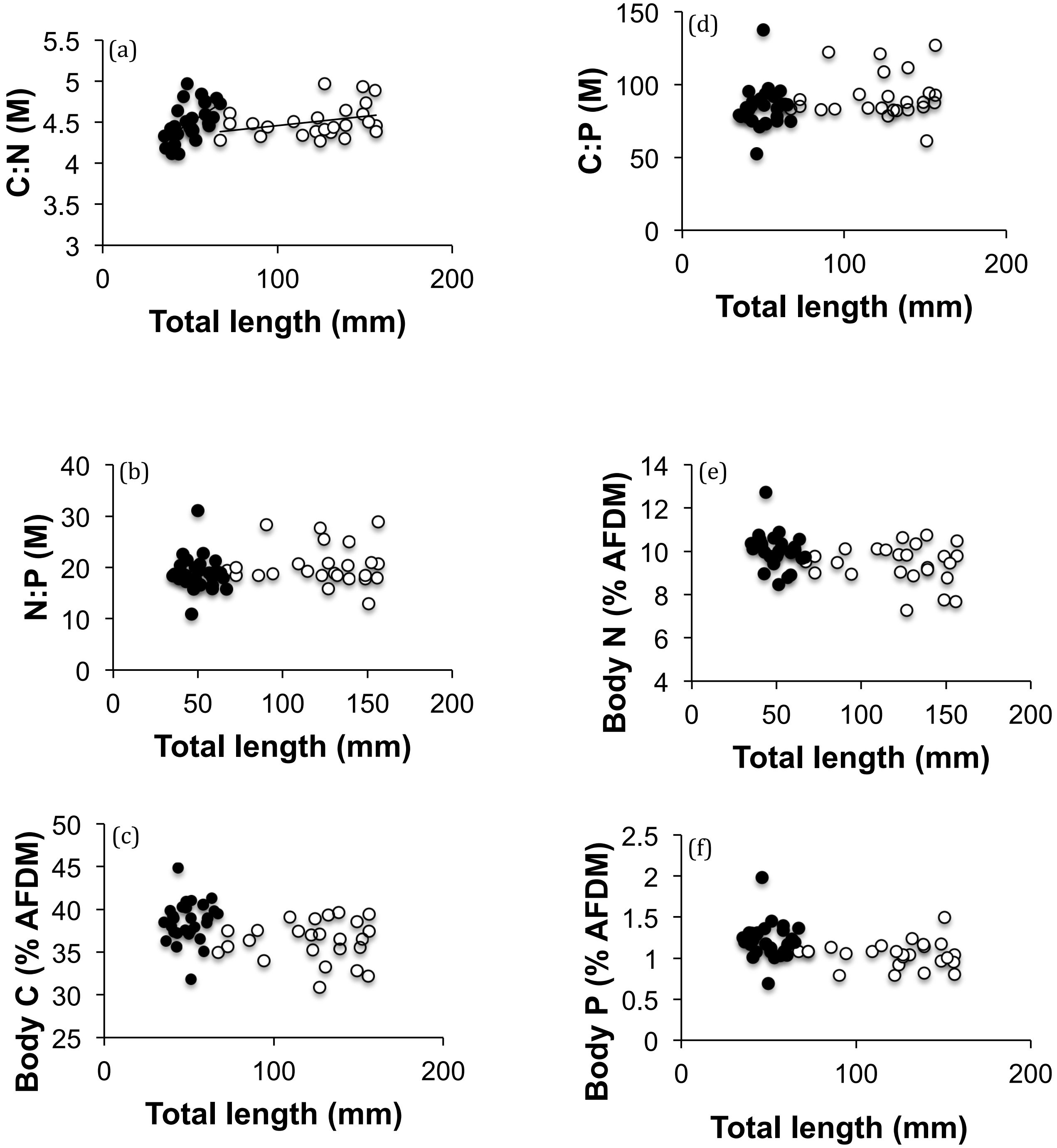
(a)
C:N (M)
5.5
5
4.5
4
3.5
3
0
100
200
Total length (mm)
(d)
C:P (M)
150
100
50
0
0
100
200
Total length (mm)
(b)
N:P (M)
40
30
20
10
0
0
50
100
150
200
Total length (mm)
(e)
Body N (% AFDM)
14
12
10
8
6
4
0
50
100
150
200
Total length (mm)
(c)
Body C (% AFDM)
50
45
40
35
30
25
0
100
200
Total length (mm)
(f)
Body P (% AFDM)
2.5
2
1.5
1
0.5
0
0
100
200
Total length (mm)

Figure 5.1. Relationship between body size (total length mm) and body elemental stoichiometry (%C, %N, %P and C:N, C:P, and N:P ratios) for two shrimps species from eight stream reaches in the Salto and Sura Rivers at La Selva Biological Station. Open circles represent *Macrobrachium olfersi* and closed circles represent *Macrobrachium carcinus.* Only one of the relationships was significant, between body size and body elemental stoichiometry. *M. olfersi* C:N significantly increased with body size ($F_{1,28}$=12.61, p=0.002). Body stoichiometry was different between species; *M. olfersi* has a significantly higher %N ($F_{1,57}$=10.64, p=0.002), %C ($F_{1,57}$=16.03, p=0.0002), and higher %P ($F_{1,57}$=5.98, p=0.02) when compared with *M. carcinus*.

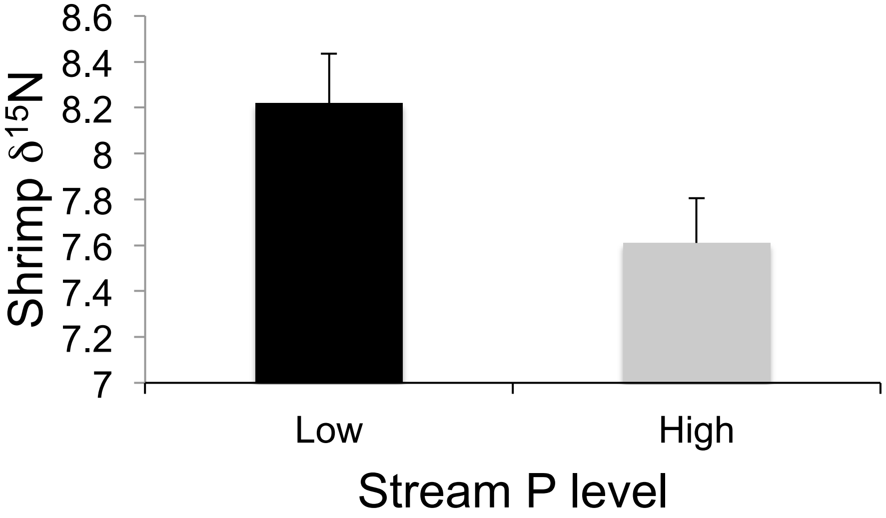

Figure 5.2. Shrimp in low-P (soluble reactive phosphorus) stream reaches have significantly higher body $\delta^{15}N$ signatures ($F_{1,57}$=15.62, p=0.0002). *Macrobrachium carcinus and Macrobrachium* olfersi from high-P (n=4) and low-P (n=4) stream reaches in the Sura and Salto Rivers at La Selva Biological Station were included in this analysis. Error bars represent 95% confidence intervals.

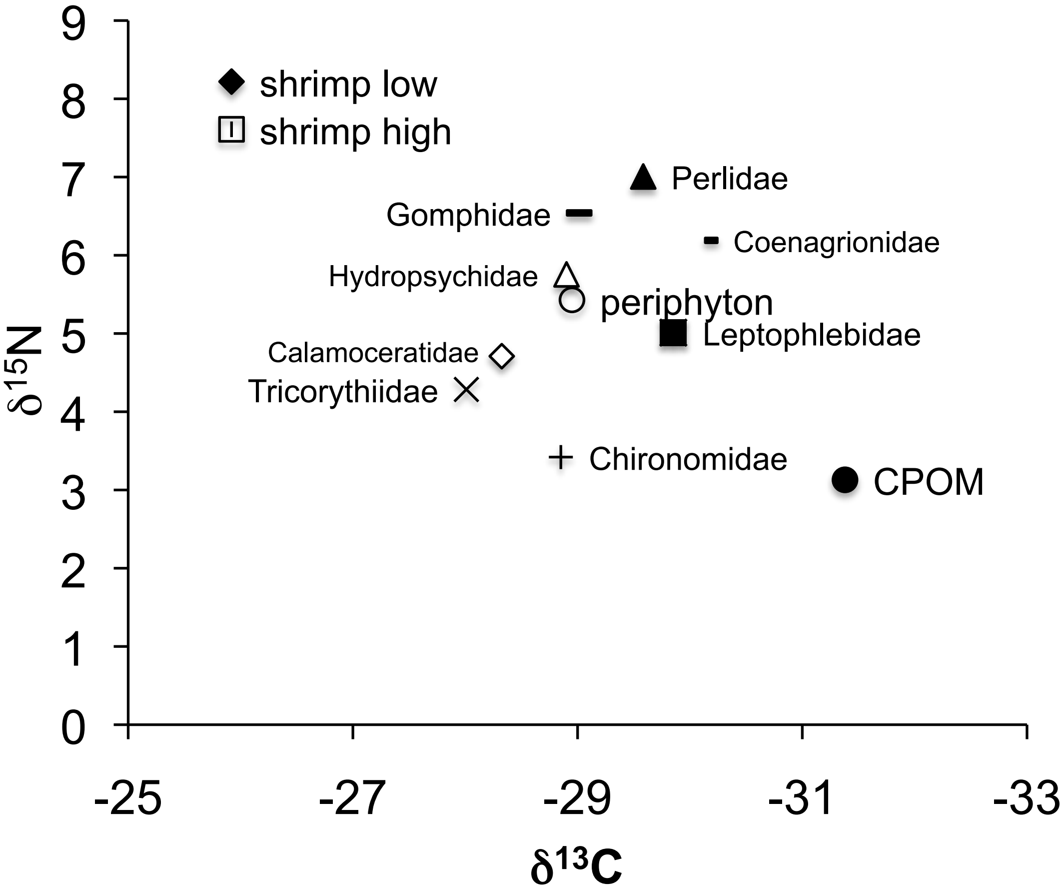

Figure 5.3. Biplot of δ^{15}N-δ^{13}C abundance for shrimp and their potential food resources in streams at La Selva Biological Station. Shrimp mean δ^{15}N abundance is shown separately for the low- and high-P (filled square) sites because the values were significantly different. Values for δ^{15}N-δ^{13}C abundance for aquatic insects, coarse particulate organic matter (CPOM), and periphyton are pooled over low- and high-P sites because they showed no significant difference.

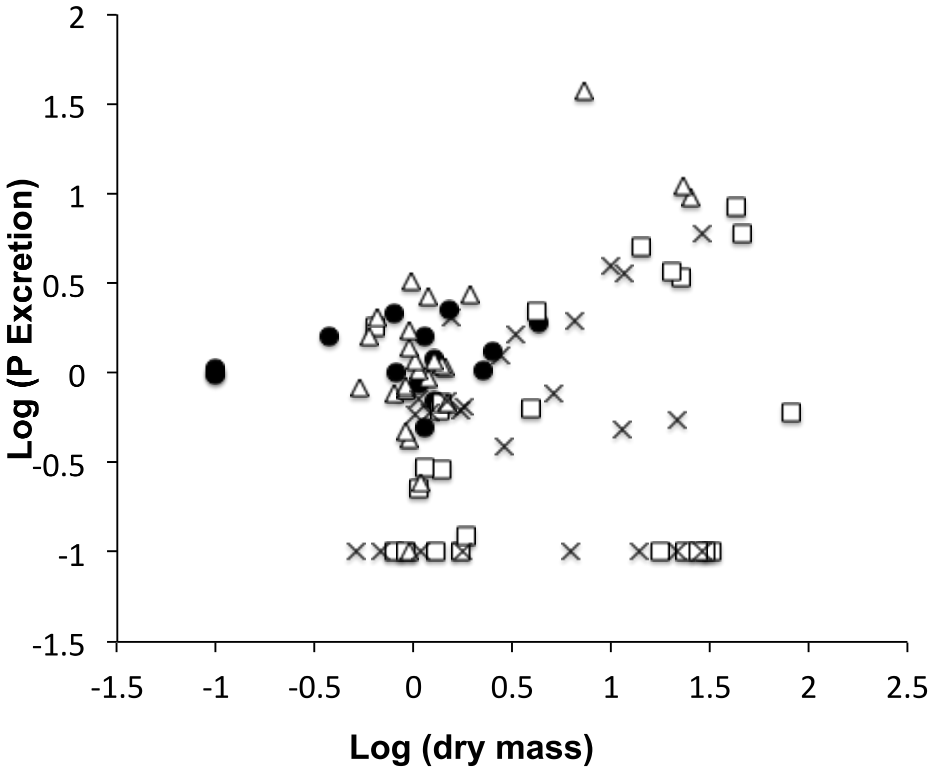

Figure 5.4. Log transformed phosphorus (P) excretion vs. log transformed dry mass (g) of individual shrimp of two species (*Macrobrachium carcinus* and *Macrobrachium olfersi*) in four study streams with different levels of soluble reactive phosphorus (SRP) at La Selva Biological Station. Excretion rate was measured as (μg TP shrimp^{-1} h^{-1}) and biomass was measured in grams. The symbols refer to sites: circles=Sabalo, squares=Salto5, triangles=Sura30, crosses=Sura60.

Sura60.

CHAPTER 6

CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

The findings of this dissertation provide valuable information regarding the abundance, distribution, energy flow and nutrient cycling dynamics of freshwater shrimps. In Chapter 2, I demonstrated that occupancy of two of the most abundant shrimp fauna at LSBS (*Macrobrachium olfersi* and *Macrobrachium carcinus*) in lowland Caribbean streams at La Selva Biological Station are affected by factors at both the landscape- and local-scales. The third most abundant shrimp, *M. heterochirus* was only found in high-gradient boulder dominated streams. Substrate also influenced *M. carcinus* and *M. olfersi* occupancy. *M. carcinus* showed a preference for boulder substrate and *M. olfersi* occupancy increased in areas with primarily sandy substrate. *M. olfersi* and *M. carcinus* occupancy increased with regional groundwater input and stream size. The occupancy rates in larger streams with regional groundwater inputs are predicted to be as much as 7-fold higher for *M. olfersi* and 3-fold higher for *M. carcinus*. We proposed three hypotheses for how regional groundwater could increase occupancy rates (1) increased quality or quantity of food (2) refugia from pH drops or (3) release from calcium limitation.

Chapter 3 results revealed that shrimps (*M. olfersi* and *M. carcinus*) comprise a significant component of total invertebrate standing biomass and secondary production. In contrast to my hypothesis, shrimps were a significant component of energy flow in the food web because they comprised a large part of secondary production (33-40%) in lowland Caribbean streams of Costa Rica. Because of the omnivorous life history of shrimp (Chapter 4), their diet (detritus, periphyton, insects) overlaps with insect diets in these streams and the first-third order detritus-based streams are probably using food resources that would otherwise be used by rapidly growing, small-bodied aquatic insects. I found that shrimps are most likely slowing down the rate of energy flow because they are competing with aquatic insects for food resources.

In Chapter 4, I showed that in forested streams within La Selva Biological Station, the relative abundance of *M. olfersi* was similar during the recent (2008-2011) and historical (1988-89) (pre-banana expansion) time periods. However, in a more easily accessible stream bordering a pasture, *M. olfersi* populations declined 7-fold between the historic and recent time periods. We suggested that shrimp harvest for consumption is responsible for the decline. While our monitoring methodology was not very powerful, it could show large changes in relative abundance of *M. olfersi*. Additionally, I found evidence to support the hypothesis that shrimp fecundity is not seasonal because the percentages of females and females with eggs showed no relationship with season or precipitation.

In Chapter 5, I found that shrimps (*M. carcinus* and *M. olfersi*) showed no variation in body nutrient content over a stream P-gradient unlike other smaller-bodied insect invertebrates. Despite feeding on food resources that are elevated in P-content in high-P streams, shrimps P-excretion did not show a response to a decrease in the imbalance between their bodies and food stoichiometry likely due to dietary shifts. Shrimp body $\delta^{15}N$ abundance suggested a diet shift, which may partially offset the effects of lower P content in each of their food resources in low P streams, such that their overall diet is similar in P content. When I compared mass-specific P-excretion rates of shrimp with previously published rates of fish at sites across the P gradient, surprisingly, shrimps were excreting P at a lower rate than the majority of studied fish species.

In summary, I have described the role that shrimps play in nutrient recycling and energy flow in lowland Caribbean streams in Costa Rica. I have demonstrated that shrimps can be a significant part of the invertebrate biomass and energy flow in continental regions. This study provides one of the few estimates in the published literature for overall invertebrate standing biomass and secondary production (including both shrimp and insects) in tropical streams; further, this dissertation provides the only proposed estimate from *continental* streams in the neotropics. This study has also provided information on the occupancy, habitat preferences, and temporal population dynamics of *Macrobrachium spp.* in continental streams.

Shrimps are under increasing threat from anthropogenic changes to aquatic ecosystems in the tropics, and understanding what factors affect distribution of migratory shrimp is a necessary first step towards shrimp conservation. In Costa Rica, fragmentation of rivers stems from multiple sources including hydropower impoundments, non-point sources of pollution (e.g. pesticide run-off from agriculture) and point sources of pollution like wastewater effluents. Much of Costa Rica is under intensive agricultural production that uses high quantities of pesticides (Castillo et al. 1997). Costa Rica imports 16.0 kg/hectare of pesticides for use each year (Castillo et al. 1997). Studies examining the response of native stream fauna to pesticide loading in streams draining agricultural areas are few, however Castillo et al. (2006) showed that streams directly downstream from banana plantations are at risk for chronic toxicity because of high levels of nematicides.

Rivers in Costa Rica and Central America in general are under increasing pressure to produce power via hydropower and there is a considerable push for increased hydropower. Since 1990, over 30 small to medium sized hydropower plants have been built in Costa Rica (Anderson 2002, Anderson et al. 2006), and more than 400 dams have been constructed or proposed for

Central America (Anderson et al. 2006). In South America, at least 150 dams have been built or planned for the Amazon Basin alone (www.dams-info.org); the effects of these dams will include losses in longitudinal riverine connectivity of many neotropical rivers.

In light of the increasing fragmentation and anthropogenic disturbance of rivers, it is important that we understand consumer resource dynamics within complex food webs if we are to predict how changes in ecosystem structure (e.g. extirpation of a species) will affect ecosystem function. Freshwater shrimp omnivory is an important aspect of shrimp life history that not only influenced how they changed the rate of energy flow through the food web (Chapter 2), but also partially determined their P recycling rate across a stream P gradient (Chapter 4). This study highlights the importance of omnivory in mediating how organisms affect energy flow and nutrient recycling in tropical streams, however, omnivory potentially makes understanding the consequences of consumer extirpation more complex.

Much of the research on shrimp natural history and abundance is in protected forest reserves on islands and therefore we have little idea of the effect of other land use types and harvesting on shrimp populations. Research across the landscape in non-protected sites could help elucidate possible mechanisms responsible for population declines and would also allow us to create spatially explicit species distribution models across land use types. Future research is needed to better understand the effects of shrimp harvesting on shrimp populations and stream ecosystem function. What are the long-term effects of intensive banana and pineapple agriculture on shrimp populations in streams draining agricultural fields? Studies in Puerto Rico have shown that it took shrimp populations three months to fully recover from a shrimp poisoning event, but multiple perturbations might change recovery rates (Greathouse et al. 2005). What are the long-and short-term consequences of shrimp harvesting? What are the consequences for the food webs in streams where shrimp populations have been harvested in continental streams?

My dissertation findings highlight the need for studies across all kinds of landforms and environmental gradients. Would river fragmentation and degradation downstream from shrimps living in short, steep tropical island streams have more or less of an impact on shrimp populations than in mainland streams where shrimp populations occur much farther from the ocean? Are shrimp populations on mainland streams less connected to populations in adjacent watersheds than shrimp populations in island streams? Are tropical island stream shrimp populations quicker to recover from local level disturbance than shrimp populations on mainlands? Future research efforts could include research as to how shrimp population dynamics would change depending on landform-type.

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