Use of stable isotope ratios to characterize potential shifts in the isotopic niches of grazing insects following an amphibian decline in a Neotropical stream

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Abstract: Neotropical streams are losing dominant consumer groups as a result of disease-driven amphibian declines. The herbivorous tadpoles of *Lithobates warszewitschii* were once abundant in the Rio Maria in the Eastern Cordillera Central of Panama, where they consumed algae and organic matter. The decline of this once abundant grazer has the potential to affect the resources consumed by insect grazers in this system. Stable isotopes were used to characterize changes in the resource use before and after amphibian declines of four abundant insect grazer taxa: *Stenonema* spp., *Thraulodes* spp., *Psephenus* spp. and *Petrophila* spp. We collected 11 isotope samples of *L. warszewitschii* and 27 isotope samples of these insect taxa in 2006, and then 24 more isotope samples of the same insect taxa in 2008, 20 mo. after a disease-driven amphibian extirpation. We also tested for potential functional redundancy of insects with tadpoles by comparing the post-decline isotopic niche of each insect taxon to the isotopic niche of *L. warszewitschii*. The isotopic niche of *Psephenus* spp., *Petrophila* spp. and *Stenonema* spp. shifted from 2006 to 2008, but none of the insect taxa in 2008 occupied the same isotopic niche as tadpoles. Our study builds on previous evidence that the ecological roles of tadpoles were not replaced through functional redundancy after amphibian declines.

Key Words: amphibian declines, chytrid, emerging infectious disease, functional redundancy, isotopic niches, species loss, stable isotopes, tropical streams

INTRODUCTION

Ecosystems worldwide are facing unprecedented biodiversity declines (Pereira *et al.* 2010). Efforts to understand biodiversity losses generally focus on ecosystem descriptors such as changes in species abundances and community composition. Characterizing changes in energy flow or resource use is more difficult and consequently is usually neglected. The ratio of the stable isotopes of nitrogen (^{15}N) and carbon (^{13}C) can be useful tools for integrating temporal data to characterize the trophic level (δ^{15}N) and energy source (δ^{13}C) of an individual or a population (Newsome *et al.* 2007). The variation in δ^{15}N and δ^{13}C of individuals in a population can be used to characterize the δ space (or isotopic niche) of the whole population (Bearhop *et al.* 2004, Bolnick *et al.* 2003). Layman *et al.* (2007) proposed using the convex hull and the mean distance to centroid to describe the size and location of the isotopic niche of a population. The convex hull encompasses the data points of the population and gives an indication of the isotopic niche width of that population while the mean distance to centroid gives information on how similar two populations are in isotopic space. Recently, methods to statistically test for differences in the location in isotopic space of a single population over time, or between two populations, were developed (Jackson *et al.* 2011, Turner *et al.* 2010).

Stable isotopes have been used to examine community responses to species invasion (Nilsson *et al.* 2012, Vander

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Zanden & Casselman 1999), but not the opposite, species extirpations. Catastrophic amphibian declines occurring in Central America can potentially affect stream ecosystems (Whiles *et al.* 2006). Tadpoles consume algae and detritus and may thus compete with grazing insects (Kupferburg 1997), but they can also facilitate access to these resources for some grazing insects through bioturbation (Ranvestel *et al.* 2004). Therefore, the extirpation of grazing tadpoles could potentially affect access to the resources consumed by grazing insects, with the consequences manifested through changes in the carbon and nitrogen isotopic ratios in the grazing insects.

As part of the Tropical Amphibian Declines in Streams (TADS) project, we used invertebrate samples collected from a Panamanian stream before and after tadpole declines to provide a snapshot of the potential consequences of tadpole declines on the isotopic niches and energy sources of four grazing insect taxa. We also examined the potential for these grazing insects to replace the ecological role of the dominant grazing tadpole *Lithobates warszewitschii.* We measured biomass and examined the diet (using stable isotopes ratios and gut content analysis) of the four grazing insect taxa and *L. warszewitschii* and measured the C and N stable isotope ratios of the epilithon. Post-decline isotopic niches of the grazing insects were compared with the pre-decline isotopic niche of tadpoles to determine if grazing insects occupied a similar isotopic niche to tadpoles after the decline. We predicted that grazing insects would change diets to include more diatoms in the absence of tadpoles, and that at least one grazing insect taxon in 2008 would occupy the same isotopic niche as tadpoles from 2006.

MATERIALS AND METHODS

Study site

Río Maria is a headwater stream near El Valle de Anton in the eastern Cordillera Central of Panama. Río Maria is a high-gradient stream at ~900 m asl with an average wetted width of 3.5 m during the dry season (January to mid-May). Stream flow did not significantly differ between the 2006 and 2008 dry seasons (mean $\pm$ 1 SD = 22.4 $\pm$ 2.9 L s^{-1} in 2006 and 22.9 $\pm$ 2.4 L s^{-1} in 2008). The site remained undisturbed between sampling events, but within 6 mo of our 2008 sampling event a small dam was built and a construction project in the middle of the study reach prevented a second post-decline sampling event.

The tadpoles of more than 20 species were present in the pre-decline amphibian assemblage, including grazing *Hyloscirtus* spp. and *Lithobates warszewitschii.* Amphibian declines occurred at Río Maria during the 2006 wet-season (June–December) and amphibians had declined to

2% of their pre-decline biomass by February 2008 (Whiles *et al.* 2012). The remaining consumer community includes an insectivorous fish (*Brachyraphis roswithae* Meyer & Etzel), the freshwater crab *Pseudothelphusa tristani* Rathburn and ~40 aquatic insect taxa that were common in the headwater streams pre- and post-amphibian decline.

Sampling of insect biomass and resources

Insects were sampled pre-decline (February and March 2006) and post-decline (February and March 2008) along the same 100-m reach. Samples were collected during the dry season because insect biomass and abundance in Neotropical streams are highest during the dry season (Colón-Gaud *et al.* 2010a). Three stove-pipe benthic cores (314 cm^2 sampling area) were collected from depositional habitats (pools) and four Surber samples (930 cm^2 sampling area, 250-μm mesh) were collected from erosional habitats (riffles and runs). All samples were elutriated through a 250-μm sieve and placed in a bag with ~8% formalin. In the laboratory, all insects from coarse fractions (insects with a body length > 1 mm) were removed and identified to genus. Fine fractions (insects with a body length between < 1 mm > 250 μm) were also examined under a dissecting microscope and were occasionally subsampled (from 1/2 to 1/32) with a Folsom plankton splitter.

Taxa were classified into functional feeding groups (FFG) based on Merritt *et al.* (2008) and previous natural abundance stable isotope data (Verburg *et al.* 2007). Individual insects were measured (total body length) and biomass was estimated using published ash-free dry mass (AFDM) length-mass regressions (Benke *et al.* 1999) or regressions developed from our own specimens using methods of Benke *et al.* (1999). The AFDM was then summed on each sampling date to obtain taxon-specific biomass estimates, which were then habitat weighted according to the proportion of each habitat during base flow conditions (64% riffle/run, 36% pool). Biomass estimates from 2006 (pre-decline) and 2008 (post-decline) of grazing insect taxa were analysed using a non-parametric bootstrap. Test statistics were the differences between 2006 and 2008 means. The means were compared to permuted means of the pooled 2006 and 2008 data which were resampled 10 000 times. P values were the percentage of permuted differences that lay outside the test statistic with P < 0.05 considered significant. Four grazing insect taxa, *Stenonema* spp. (Ephemeroptera: Heptageniidae), *Thraulodes* spp. (Ephemeroptera: Leptophlebiidae), *Psephenus* spp. (Cole-optera: Psephenidae) and *Petrophila* spp. (Lepidoptera: Crambidae), were selected for stable isotope and gut content analysis because they constituted > 80% of the

grazer biomass. Immature stages of stream insects are not well described to the species level, and thus we used generic level in our study and indicated spp. because we did not know for sure how many species of each genus were present in our study site. Based on morphological characteristics, it appeared that *Stenonema*, *Psephenus* and *Petrophila*, were represented by one species each and there were no more than three species of *Thraulodes* present.

Epilithon was sampled for biomass and stable isotopes in February–March 2006 (pre-decline) and February– March 2008 (post-decline). A modified Loeb sampler was used to collect a sample from a known area in five to seven riffles and five to seven pools. The samples were filtered onto a glass fibre filter (GFF; particle retention size = 0.7 μm) and dried at 50–60 °C for 24 h to obtain dry mass (DM). Samples were habitat weighted according to the proportion of each habitat during base flow conditions (64% riffle/run, 36% pool). A non-parametric bootstrap using the methods to compare insect biomass was used to compare DM means for 2006 and 2008.

Sampling for analysis of natural isotope abundance

The taxa selected for the stable isotope analyses were the four most abundant grazing insect taxa in Río Maria and included *Stenonema* spp., *Thraulodes* spp., *Psephenus* spp. and *Petrophila* spp., which collectively accounted for 83% of the grazer/scraper biomass in 2006. A minimum of eight individuals of each insect taxon from each sampling date were collected for stable isotope analyses. Individuals were pooled to make sufficient biomass for an isotope sample. For example, if eight individuals were collected, there may only be enough mass for four isotope samples. Because these taxa are < 7 mm, several individuals (two to five) were pooled into a single isotope sample, but a total of 9–22 individuals were sampled per taxon. *Lithobates warszewitschii* tadpoles were also collected for stable isotope analysis in 2006. *Lithobates warszewitschii* was the most abundant grazing tadpole in Río Maria. *Hyloscirtus* spp. were also present but constituted < 5% of the grazing tadpole biomass. Tadpoles of other species were not sampled because they occupy separate guilds (e.g. they live in leaf packs or are filter feeders) and we were interested in examining the consequences of the extirpation of a grazing tadpole on grazing insects. Sampling for pre-decline insects and tadpoles for isotope analyses occurred in February 2006. Tadpoles and insects were randomly sampled from the stream by net or hand-picking (picking individuals from rock with forceps) and placed on ice. A Loeb sampler was used to collect epilithon samples which were then filtered onto glass fibre filters (GFF; particle retention size = 0.7 μm). Epilithon samples were not separated into algal and non-algal components because the largest diatom, *Terpsinoe*

musica Ehrenberg, would not separate from the non-algal component. Samples were kept on ice or frozen until they could be dried at 50 °C. After drying, samples were ground to a fine powder, weighed, and packed into tin capsules for δ^{15}N and δ^{13}C analysis at the University of Georgia's Odum School of Ecology Analytical Chemistry Laboratory. Post-decline samples for isotope analysis were collected in February 2008, 20 mo after the amphibian decline began, which ensured that the insects collected for analysis had consumed resources only available well after the amphibians had declined.

Location and size of isotopic niches

Turner *et al.* (2010) used nested linear models to test for changes in the centroid location of a population over time, which could potentially indicate changes in resource use (Zeug *et al.* 2009). The centroid location for each insect grazer was tested for changes from the pre-decline date (2006) to the post-decline date (2008). The Euclidian distance between two centroids was computed and the centroids were considered to occupy different locations if the distance between the two centroids was significantly greater than zero (Turner *et al.* 2010). A parametric Hotelling's T^2 (a multivariate equivalent to the univariate t-test) test statistic was used to compare population mean vectors (Turner *et al.* 2010).

The position of the convex hull in isotopic space was identified using a statistical approach that uses a multivariate ellipse-based metric to generate a standard ellipse area (SEA). The SEA is the bivariate equivalent to the standard deviations in a univariate analysis, reducing the weight of outliers in the population, and allowing for comparisons of populations with different sample sizes (Jackson *et al.* 2011). For small populations (e.g. $N = 3$), a corrected (SEA$_C$) version of the SEA is an appropriate method for generating the isotopic niche area. SEA$_C$s were calculated for each taxon pre- and post-amphibian declines using the methods described in Jackson *et al.* (2011) and the R package SIAR (Parnell *et al.* 2010). An unbalanced one-way ANOVA for each taxon was used to test for changes in energy sources (δ^{13}C) between the 2006 and the 2008 dates.

Analysis of gut contents

Ten individuals (five individuals from 2006 and five individuals from 2008) of each of the four grazer-insect taxa (*Petrophila* spp., *Psephenus* spp., *Stenonema* spp. and *Thraulodes* spp.) and five individuals of *L. warszewitschii* from 2006 were used for gut content analysis. Gut contents were prepared using the methods of Parker & Huryn (2006) with specimens that were collected

Table 1. Densities of the four focal grazing insect taxa from 2006 (pre-decline) and 2008 (post-decline) and the tadpole *Lithobates warszewitschii* from the Rio Maria in the eastern Cordillera Central of Panama. Length is the body length of individuals used for gut content analysis. The data are reported as mean $\pm$ SE.

Taxa	Biomass (mg DM m^{-2})			Length (mm)		
	2006	2008	P	2006	2008	P
Lithobates warszewitschii	362 ± 210	0		5.52 ± 0.15		
Stenonema spp.	24.0 ± 11.9	11.7 ± 9.95	0.381	6.04 ± 0.86	6.13 ± 1.50	0.95
Thraulodes spp.	66.7 ± 16.7	37.0 ± 7.94	0.006	4.02 ± 0.36	3.48 ± 0.33	0.31
Psephenus spp.	9.18 ± 3.42	3.34 ± 1.65	0.003	2.58 ± 0.24	2.27 ± 0.40	0.53
Petrophila spp.	5.28 ± 1.54	16.9 ± 11.0	0.90	5.96 ± 0.65	7.22 ± 0.83	0.27

to estimate total biomass. Guts were removed under a dissecting microscope, placed in a 30-ml syringe and sonicated for 30 s. For *L. warszewitschii* a foregut segment of 8–10 mm was used. Material was then filtered onto a 13 mm, 0.45 μm pore, nitrocellulose fibre filter (Millipore HAPW01300), placed on a microscope slide, and dried at 50 °C for 30 min. A drop of Type B immersion oil was used to clear the filter and filters were sealed to the slides with a coverslip and nail polish. Ten fields of view were digitized randomly with a digital camera using brightfield optics at 400× (Olympus BH-2). Pictures were quantified using ImagePro (Media Cybernetics, Inc., Silver Spring, MD, USA) and particles in each photograph were categorized as animal or plant fragments, filamentous algae, diatoms, fungi, amorphous detritus or non-algal biofilm. Non-algal biofilm is a mixture of autotrophic and heterotrophic micro-organisms in a glycoprotein matrix that is attached to stream substrata. We assumed non-algal biofilm was bacterial and did not make a distinction between the glycoprotein-matrix produced by bacteria from that produced by cyanobacteria. Gut content data from 2006 and 2008 for each insect grazer were transformed to proportions of total contents and a paired one-tailed t-test was used to assess potential changes in the proportion of each food item consumed between years.

RESULTS

Insect biomass and their resources

Insect densities as measured by biomass did not change from 2006 to 2008 (Table 1) ($F_{3,54} = 1.625$, $P = 0.19$) while epilithon biomass increased from 39.5 ± 6.49 g DM m^{-2} in 2006 to 93.9 ± 31.8 g DM m^{-2} in 2008 ($P < 0.05$).

Isotopic analyses of grazing insects

The standard ellipse areas of the grazing insects from 2006 did not overlap with the ellipse area of tadpoles (Figure 1). The total area of the standard ellipses in isotopic space that represents the isotopic niche of the
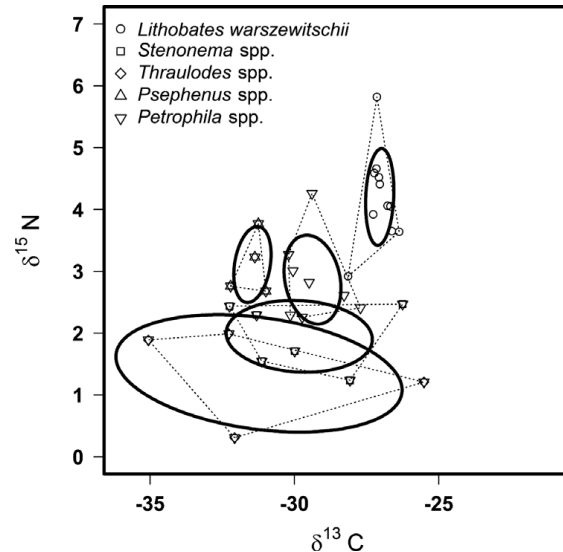

Figure 1. Biplot of δ^{15}N-δ^{13}C of the insects *Petrophila* spp., *Psephenus* spp., *Stenonema* spp. and *Thraulodes* spp. and the tadpole *Lithobates warszewitschii* from 2006 from the Rio Maria in the eastern Cordillera Central of Panama. Each dot represents an isotope sample that could be composed of one to three individuals. The dotted lines represents the convex hulls as described in Layman *et al.* (2007) and the solid line represents the standard ellipses, the bivariate equivalent of the univariate standard deviations (Jackson *et al.* 2011). Note that there are no insect grazer taxa that overlap the isotopic niche of *L. warszewitschii* tadpoles.

grazing-insect populations did not significantly change on the post-decline date (Figure 2). The isotopic niche of *Psephenus* spp., *Petrophila* spp. and *Stenonema* spp. shifted on the post-decline date, but *Thraulodes* spp. did not shift (Figure 2). A shift in the mean centroid location for three grazing-insect taxa occurred post decline (Table 2) while the mean centroid location of the Loeb samples did not significantly change from 2006 to 2008 (distance = 0.57, Hotteling's T^2 = 4.4, P = 0.14). However, no insects sampled in 2008 occupied the tadpole isotopic niche. The centroid location of *Stenonema* spp. in 2008 was the most similar to the tadpole *Lithobates warszewitschii*, but the mean centroid distance between *L. warszewitschii* and post-decline *Stenonema* spp. were significantly different (distance = 1.83, Hotteling T^2 = 21.4, P < 0.01). The δ^{13}C of only one taxon, *Petrophila* spp. ($F_{1,11} = 38.2$,

Table 2. Shifts in the centroid locations of epilithon and the four insect taxa from 2006 (pre-decline) to 2008 (post-decline) from the Rio Maria in the eastern Cordillera Central of Panama. The mean distance is the distance between the centroid of the isotopic niche from the first to the second sampling date for each taxon. No. samples is the number of isotope samples used in the analyses. Numbers in parentheses are the number of individuals in the stable isotope samples. Therefore, there could be one to three individuals in each isotope sample.

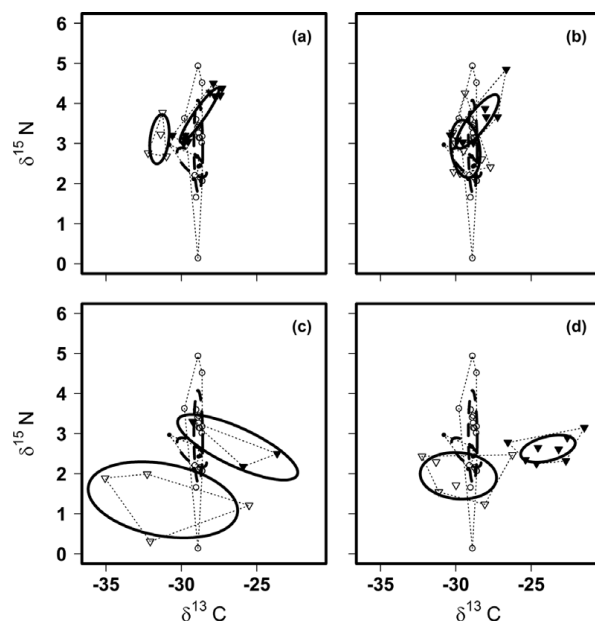| Taxa	No. samples		Mean distance	Hotteling's T^2	P
	2006	2008			
Epilithon	17	5	0.57	4.41	0.14
Stenonema spp.	9 (12)	4 (8)	2.72	29.28	< 0.001
Thraulodes spp.	8 (22)	8 (19)	1.05	4.93	0.13
Psephenus spp.	4 (11)	3 (9)	5.13	15.33	0.03
Petrophila spp.	6 (18)	9 (13)	6.36	41.21	< 0.001

Figure 2. The δ^{15}N–δ^{13}C biplot for *Stenonema* spp. (a), *Thraulodes* spp. (b), *Psephenus* spp. (c) and *Petrophila* spp. (d) from the Rio Maria in the eastern Cordillera Central of Panama. Circles are individual Loeb samples and inverted triangles are individual insect sample while open points are pre-decline samples and filled points are post-decline samples. The finely dotted lines represent the convex hulls as described in Layman *et al.* (2007). The black solid lines are the standard ellipses, the bivariate equivalent of the univariate standard deviations (Jackson *et al.* 2011), for the insect sampled. Dashed black lines are the standard ellipses for the Loeb samples. *Stenonema* spp., *Psephenus* spp. and *Petrophila* spp. occupy a different isotopic niche in 2008 compared with 2006.

Figure 3. Stable carbon (δ^{13}C) isotopes of the four most abundant grazing insect taxa sampled from 2006 (pre-amphibian decline, open bars) and 2008 (post-amphibian decline, hashed bars) from the Rio Maria in the eastern Cordillera Central of Panama. Carbon isotopic ratios represent the carbon sources of an organism. Data are mean and standard deviation. Sample sizes are in Table 2. *** P < 0.001.

P < 0.001, Figure 3) became significantly more enriched from the first sampling date to the second.

Gut content analysis

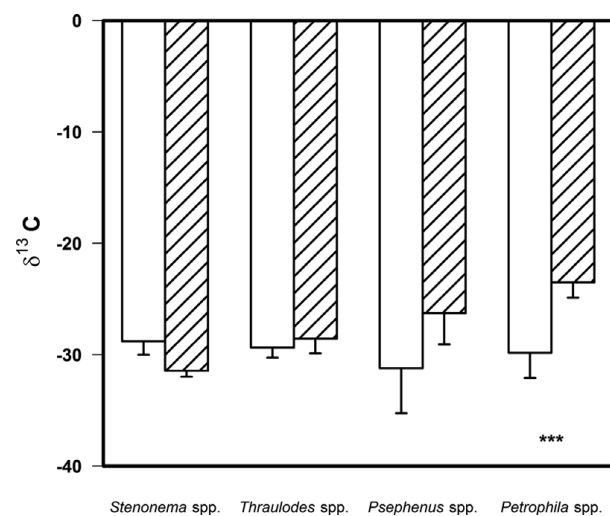*Lithobates warszewitschii* primarily consumed non-algal biofilm, but 1.5% of its diet was animal material. The relative proportions of food items in the guts of grazing insects changed from 2006 to 2008. The proportion of non-algal biofilm increased from 2006 to 2008 in the guts of *Psephenus* spp. (n = 5, $t = -3.1$, *df* = 7.6, P = 0.007) and *Petrophila* spp. (n = 5, $t = -2.08$, *df* = 6.3, P = 0.04), and the proportion of diatoms decreased in the guts of *Psephenus* spp. (n = 5, $t = 4.03$, *df* = 7.5, P = 0.002) and *Petrophila* spp. (n = 5, $t = 2.03$, *df* = 6.2, P = 0.043) (Figure 4). However, the diet of *Stenonema* spp. and *Thraulodes* spp. did not significantly change (Figure 4).

DISCUSSION

Our data suggest that grazing insects were not functionally redundant with respect to tadpoles because
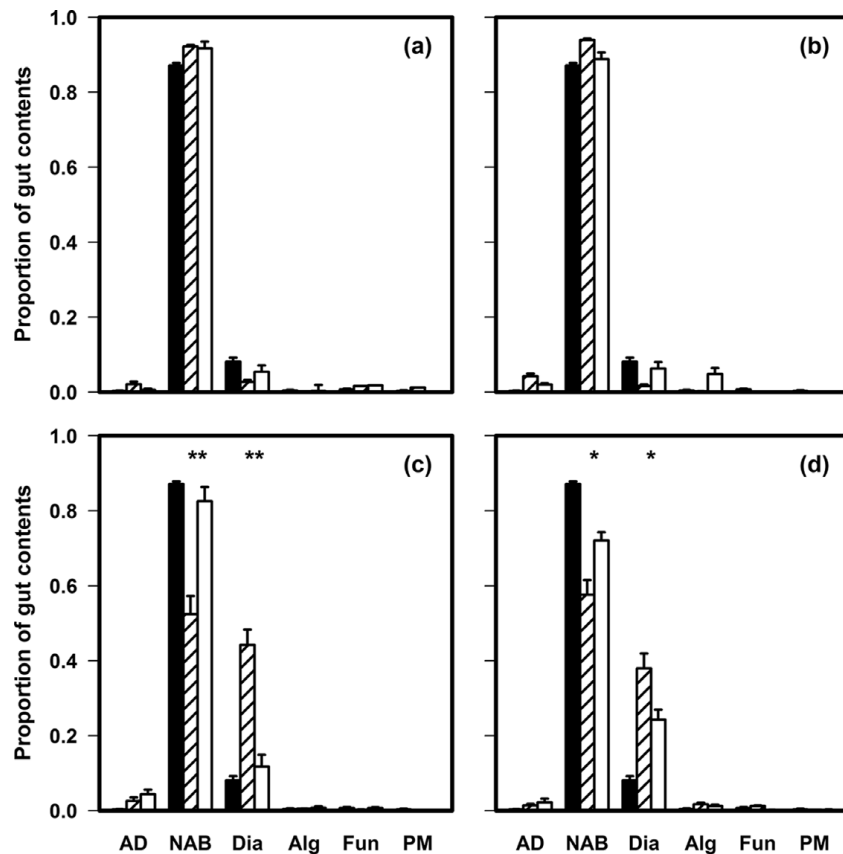
Figure 4. Gut content analysis of the grazing insects *Stenonema* spp. (a), *Thraulodes* spp. (b), *Psephenus* spp. (c) and *Petrophila* spp. (d) pre- and post-amphibian declines from the Rio Maria in the eastern Cordillera Central of Panama. *Lithobates warszewitschii* (tadpoles) was the only taxon with animal material in its guts (not included in graph: 1.5% ± 0.6%). Solid bars are *L. warszewitschii*, hashed bars are pre-decline, and open bars are post-decline. Data are means with standard errors. AD, amorphous detritus; NAB, non-algal biofilm; Dia, diatoms; Alg, filamentous algae; Fun, fungi; PM, plant material; asterisks denote a significant difference between pre- and post-decline diets of the insect and are not a comparison between the insect and *Lithobates warszewitschii.* * $P < 0.05$, ** $P < 0.01$.

of increased epilithon, coupled with the lack of response in grazing-insect populations to tadpole declines. Additionally, the observed change in the isotopic niches of three grazing-insect taxa from 2006 to 2008 suggests that tadpoles may have influenced the grazing insect community by reducing sediment accrual on stream substrata through bioturbation (Ranvestel *et al.* 2004). However, the populations of the insect grazers did not increase as the biomass did not change from 2006 to 2008. Together, these results suggest that the response of grazing insect to amphibian declines could be more subtle than can be detected through changes in relatively coarse measurements such as total biomass.

The diets of grazing-insect taxa do not appear to be functionally redundant with grazing tadpoles as none of the four insect taxa occupied the same isotopic niche in 2008 as tadpoles in 2006. The animal material present in the guts of *L. warszewitschii* would enrich the ^{15}N of tadpoles compared with the ^{15}N of grazing insects which

could limit the potential of the insects to occupy the same isotopic niche as tadpoles. However, the isotopic niche of all four insects trended towards the isotopic niche of *L. warszewitschii,* and the diets of *Psephenus* spp. and *Petrophila* spp. became more similar to the diets of *L. warszewitschii,* e.g. more non-algal biofilm and fewer diatoms. However, the biomass of *L. warszewitschii* in 2006 was more than five times higher than the grazing insect biomass in 2008, suggesting that the diet shifts of grazing insects alone do not compensate for the loss of tadpoles.

The shift in the isotopic niche of *Psephenus* spp., *Petrophila* spp. and *Stenonema* spp. was unlikely to be caused by changes in isotopic signatures of their resources because the isotopic niche of the epilithon did not change significantly between sampling dates. Gut content analyses of these three taxa revealed that they primarily consumed different proportions of the compartments in the epilithon (e.g. non-algal biofilm, diatoms and

filamentous algae) but had little vascular plant material in their guts. The increased percentage of non-algal biofilm in *Psephenus* spp. and *Petrophila* spp. in 2008 may have driven the slight ^{15}N enrichment of their isotopic niches. The slight ^{15}N enrichment could have been the result of an increase in heterotrophic bacteria in their diets. The bacteria decomposing the senescent material would have been δ^{15}N enriched relative to the other epilithon, and the δ^{15}N could consequently have increased the δ^{15}N signature of the grazing insects.

Tadpoles may have also facilitated access to diatoms for small-bodied grazing invertebrates through bioturbation. The grazing insects in this study are small, with late instars 6–7 mm in size and sediment accrual may affect their movement and foraging. Tadpoles can reduce sediment accrual on stream substrata through bioturbation (Connelly *et al.* 2008, Flecker *et al.* 1999), and small-scale manipulation experiments in a similar Panamanian stream showed that baetid mayflies were more abundant on artificial substrates to which tadpoles had access compared with tadpole exclusions (Ranvestel *et al.* 2004). Tadpoles may also have facilitated diatom access by influencing the diatom community structure. When grazing tadpoles were present, the diatom community consisted of adnate diatom taxa, and then shifted to larger-bodied and more erect diatom taxa following amphibian declines (Connelly *et al.* 2008). The shift in diatom taxa may have reduced the abundance of edible diatoms available to the smaller-bodied grazers, particularly *Psephenus* spp. Further studies are needed to determine if grazing insects consume similar diatom taxa as tadpoles and if the diatom taxa consumed by grazing insects differ in the presence and absence of tadpoles.

The lack of population increases by grazing insects is consistent with previous studies in the region. In a similar stream in Panama, the Río Guabal, algal standing stocks increased following amphibian declines (Connelly *et al.* 2008), but the biomass of only one insect taxon (*Farrodes*) out of the 12 examined (including *Stenonema* spp., *Thraulodes* spp., *Psephenus* spp. and *Petrophila* spp.), increased immediately following amphibian declines (Colón-Gaud *et al.* 2010a). The lack of change in biomass of grazing insects in our study stream lends further support to the lack of functional redundancy by grazing insects with respect to tadpoles. Our results also suggest that changes in trophic pathways may occur in the absence of changes in population biomass. Notably, *Petrophila* shifted energy sources from a more algae-based diet to a diet with more non-algal biofilm.

The observed changes in the isotopic niches and diets were unlikely related to annual variation alone. Seasonal and annual changes in insect biomass are well documented in temperate streams, but tropical streams may experience less year-to-year variation because of constant temperature and light availability (Boyero *et al.* 2009). Furthermore, highland Neotropical streams have unique assemblages compared with lowland Neotropical streams because tadpoles are the most abundant consumers while fish diversity and abundance is low. Long-term data on insect populations are not common from highland Neotropical regions (Boyero *et al.* 2009), but a 2-y study in two mountain streams, the Quebrada Chorro and the Tube stream, in western Panama (~500 km west of our focal study stream) showed no changes in the biomass or abundance of insects in the grazer, shredder, gatherer and predator functional feeding groups from the first to the second study years (Colón-Gaud *et al.* 2010b). Additionally, algal-biofilm standing stocks measured as AFDM also did not significantly change from the first to the second year (Colón-Gaud *et al.* 2010b). Furthermore, in a similar Panamanian stream, Colón-Gaud *et al.* (2010a) showed that biomass of 10 of 12 insect taxa in the scraper functional feeding group did not vary between dry seasons, even with the loss of amphibians. The limited long-term insect population data available from highland Neotropical headwater streams suggests limited inter-annual variability, and, when coupled with our own biomass data, suggest that the stable isotope patterns are not driven by annual variation.

The results of our study are significant from a methodological perspective because detecting changes in centroid locations is a relatively untested analytical method. Turner *et al.* (2010) raised the issue of the number of samples required to detect changes in the centroid location of a population and questioned whether statistically significant changes in centroid location are biologically meaningful (Turner *et al.* 2010). Our study showed significant changes in centroid location occurred in three taxa, with fewer than ten isotope samples for each taxon. The changes in centroid location for two taxa, *Psephenus* spp. and *Petrophila* spp., were also coupled with changes in gut contents, suggesting that the shifts in centroid locations were biologically meaningful. In contrast, the shift in the centroid location for *Stenonema* spp. from 2006 to 2008 was not coupled with changes in gut contents. The results of our study suggest that changes in centroid location should be interpreted with caution and should be coupled with other analyses.

While our study is limited to a single stream and two points in time, our findings are from a field-based survey, rather than mesocosms or small-scale manipulations, and therefore reflect changes in a natural community. Our study also represents a quantitative examination of a stream system at the reach scale in a biologically imperiled region, providing insight into how remaining consumers may respond to the loss of a dominant vertebrate

consumer. The ongoing biodiversity crisis necessitates the need for field-based studies that examine changes in biomass and trophic pathways of an assemblage, even when only limited data are available, to fully assess the consequences of biodiversity declines.

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