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PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

Changes in digestive traits and body nutritional composition accommodate a trophic niche shift in Trinidadian guppies

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Abstract A trophic niche shift can occur as an adaptive response to environmental change such as altered resource quality, abundance or composition. Alterations in digestive traits such as gut morphology and physiology may enable these niche shifts and affect the persistence of populations and species. Relatively few studies, however, have assessed how niche shifts influence suites of digestive traits through phenotypic plasticity and evolutionary mechanisms, and how these trait changes can subsequently alter the nutrition, fitness and life history of organisms. We investigated how population divergence and plasticity alter the gut physiology of wild Trinidadian guppies (*Poecilia reticulata*), assessing whether variation in digestive traits correspond with enhanced nutrient assimilation under a pronounced dietary shift. We examined gut enzyme activity, and gut size and mass of wild guppies from both high-predation (HP) and low-predation (LP) habitats when reared in the laboratory and fed on high- or low-quality diets designed to reflect their dietary differences previously found in nature. After 10 weeks on the experimental diets, HP guppies maintained shorter and lighter guts than LP guppies on either diet. Guppies also differed in their digestive enzymatic profiles, more often reflecting nutrient balancing so that increased enzyme expression tended to correspond with more deficient nutrients in the diet. LP guppies had increased somatic phosphorus at the end of the experiment, possibly related to the higher alkaline phosphatase activity in their guts. Our results suggest that differences in gut physiology exist among populations of Trinidadian guppies that may reflect local adaptation to their disparate environments.

Keywords Digestive enzymes · Gut morphology · Local adaptation · Nutrient balancing · *Poecilia* · Stoichiometry

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Introduction

Shifts in dietary niche driven by environmental change can create strong selection for phenotypic divergence, favoring novel traits that increase the acquisition, digestion and assimilation of energy and nutrients in dietary items (Grant and Grant 2006). Many studies have investigated traits for capturing and ingesting novel resources (Schluter 1995; Parsons and Robinson 2007), but there are relatively few studies that focus on the digestive tract. Because the structure and physiology of the digestive tract can alter how energy and nutrients are extracted from resources (Karasov and Martinez del Rio 2007), the gut forms a critical link between an organism's diet and the fitness benefits derived from its diets. Digestive traits and the mechanisms by which they vary can, therefore, be central to ecological and evolutionary interactions.

Intestinal morphology and enzyme activity reflect a balance between the benefits obtained from digesting various dietary items (e.g., energy, nutrients) and the costs of maintaining metabolically expensive digestive tissues and enzymes. These traits can vary both among and within species depending on diet (Horn 1989; Horn et al. 2006; Karasov and Martinez del Rio 2007; Wagner et al. 2009; German et al. 2010) in order to enhance assimilation of required nutrients and energy. Diets lacking in nutrients can also drive changes in an organism's body stoichiometry [i.e., elemental composition of phosphorus (P), nitrogen (N) and carbon (C)] and constrain organism fitness and life history (Elser et al. 2000; Sterner and Elser 2002; Frost et al. 2010; Demott et al. 1998). In the context of trophic niche shifts, altered digestive traits may mediate the impact of diet quality on organism body nutrient content, and ultimately life history characteristics. Understanding the roles of both phenotypic plasticity and population-level divergence on digestive traits and body nutrient composition is essential to describe how animals face the challenges of rapidly changing environments (Ghalambor et al. 2007; Palkovacs et al. 2012).

We explored how diet-induced plasticity and population-level divergence influence digestive traits and body nutrient composition in a model system for evolutionary ecology, the Trinidadian guppy (*Poecilia reticulata*). The trophic niche of omnivorous Trinidadian guppies varies considerably across populations in freshwater montane streams of Trinidad (Zandonà 2010). Within the streams of the Northern Range of Trinidad, populations of guppies have been categorized into two phenotypes (see Electronic Supplementary Material, ESM, Table S1 for summary of traits differences across streams). A high-predation (HP) phenotype guppy occurs in downstream habitats where it is exposed to predation by a number of piscivorous fishes, and a low-predation (LP) phenotype is found upstream of barrier waterfalls that have prevented dispersal of predatory fish (Reznick 1982; Magurran 2005). HP guppies consume more high-quality (i.e., high N and P content) invertebrates and less low-quality (i.e., low N and P content) detritus and algae than LP guppies (Zandonà et al. 2011; El-Sabaawi et al. 2012). HP guppies are also more selective for high-quality dietary items, even while in common garden conditions (Bassar et al. 2010; Zandonà et al. 2011). Frequent predation on guppies in HP sites maintains their low densities, leading to high per capita resource availability but limited opportunity to feed due to predation risk (Fraser et al. 2004). In upstream LP sites, guppies that are largely free from predation risk spend more time foraging. In these LP sites, guppy densities are also several folds higher suggesting increased competition for high-quality resources in LP sites (Grether et al. 2001; Zandonà et al. 2011). Although the role of food quantity has been investigated in guppy evolution (Reznick 1982; Arendt and Reznick 2005; Auer et al. 2010), the physiological and ecological effects of food quality have not been explored empirically.

In this study, we describe how the intestinal traits of Trinidadian guppies, specifically gut enzyme activity and morphology, respond to dietary shifts observed in their native habitat by comparing gut traits of an HP-LP population pair laboratory-reared from maturity on high- or low-quality foods. We also describe body nutrient composition to assess potential linkages between enzyme activity and elemental homeostasis. We assess (1) the role of population divergence on these traits by comparing HP and LP guppies, (2) plastic trait change by comparing all guppies reared on the two diet treatments in the laboratory, and (3) the effect of population divergence on plasticity by comparing the response to the food treatment of each population.

We formed a number of predictions for guppy gut morphology and physiology based on the diets of the wild guppy populations and their experimental diets in the laboratory (Table 1). Because LP guppies have a lower-quality diet in the wild, we expected that they would maintain longer and heavier guts—as would laboratory-reared guppies fed the lower-quality experimental diet. A longer and more voluminous gut helps herbivores maintain sufficient retention times in order to digest more nutrient-poor diets (Sibly 1981; Kramer and Bryant 1995; German and Horn 2006; German et al. 2014). Our hypotheses regarding enzymes were based on the adaptive modulation hypothesis, which predicts that an increase in substrate concentration within an animal's diet will be matched by an increase in the corresponding digestive enzyme to maximize the digestion of available material (Karasov and Hume 1997; German et al. 2004; Karasov and Martinez del Rio 2007; German et al. 2010; Day et al. 2011).The decreased selectivity of LP guppies and increased ratio of detritus, algae and diatoms in their diet means that they consume more cellobiose, a intermediate product of

Table 1 Summary of enzymes, their ecological function, substrate proxy, concentration used, experimental expectations, and results

Enzyme	Enzyme function	Substrate	Concentration used (μM)	Expectations under AMH[a]: population differences[b]	Expectations under AMH: dietary differences	Reasoning for hypothesis under AMH:	Experimental results: population differences	Experimental results: diet
α-glucosidase (AG)	digests startch degradation products	4-Methylumbelliferyl-α-D-glucopyranoside	2000	Elevated in LP	Elevated in low-quality diet	LP diets of algae and detritus and a low quality diet of spinach have more degradation products from startch and cellulose and increased substrate for AG and BG	Elevated in HP	No difference
β-glucosidase (BG)	digests cellulose degradation products	4-Methylumbelliferyl-β-D-glucopyranoside	2000	Elevated in LP	Elevated in low-quality diet		Elevated in HP	No difference
N-acetyl-β-D-glucosaminidase (NAG)	digests chitin degradation products	4-Methylumbelliferyl-N-acetyl-β-glucosaminidase	2000	Elevated in HP	Elevated in high-quality diet	HP diets of increased invertebrate consumption and a high quality diet of brine shrimp have more chitin degradation products and phosphorus and increased substrate for NAG and AP	No difference	Elevated in low-quality diet
Alkaline phosphatase (AP)	releases phosphorus from organic compounds	4-Methylumbelliferyl-phosphate	400	Elevated in HP	Elevated in high-quality diet		Elevated in LP	No difference

[a] AMH is the Adaptive Modulation Hypothesis, which posits that as substrate availability increases, enzymatic activity correspondingly increases

[b] Population differences refers to the two populations used in the experiment, which were derived from different habitats within the Aripo River with varying levels of predation pressure (*HP* high predation, *LP* low predation). The two populations are known to differ in a variety of phenotypic traits

cellulose decomposition composed of two glucose molecules with β (1→4) bonds and digested by β-glucosidases (BG). The LP guppy diet of more algae and detritus also has more starch and exopolymeric substances, which is composed of glycoproteins and soluble polysaccharides with α (1→4) linkage bonds (German et al. 2010) and are hydrolyzed, in part, by α-glucosidases (AG). Because of increased AG and BG substrates in LP guppy diet, we expected LP guppies to show higher expression of both enzymes. Alkaline phosphate (AP), on the other hand has been found to be elevated in fish with more carnivorous diets (German et al. 2004), and N-acetyl-β-D-glucosaminidase (NAG) degrades the disaccharide, N-acetyl-β-D-glucosaminide, an intermediate product of chitin degradation, which we would expect to be elevated in fish that consume more arthropods. Therefore, we expected an increased AP and NAG activity in HP guppies and those consuming more invertebrates. Our results provide insight on the role of digestive traits in a niche shift that may interact with variation in life history and sexually selected traits in this model system.

Materials and methods

Experimental set up

In April 2011, we collected juvenile guppies (<4 weeks post-parturition) from one HP (Aripo) and one LP (Naranjo, a tributary of the Aripo) stream reach within the Caroni Drainage of Trinidad's Northern Range. Fish were transported to Cornell University and acclimated to laboratory conditions for 6 weeks (at the end of which period all fish were mature adults). At the start of the diet manipulation, all female guppies were anesthetized with MS-222, measured for length and weight, and assigned randomly to tanks, with each tank containing three female guppies and a single male guppy, all from the same population (i.e., all HP or LP).

Treatments were blocked across space (vertical location on shelving units), with each of the four blocks being fully factorial for diet quality (high and low) and guppy population (HP and LP) treatments. Males were included in the tanks because females decrease energy assimilation in the absence of male guppies (Reznick 1983), but only measurements of females were used in this study. We used the ratio of one male to three females, which reflects the natural occurrence for most wild guppy populations to be female-biased (Rodd and Reznick 1997; Arendt et al. 2014) and also reduced male harassment of females kept in laboratory aquaria. The diet manipulation commenced on June 7, 2011 and proceeded for 10 weeks.

Two experimental dietary treatments were tested: one composed primarily of spinach and one primarily of

invertebrates. The spinach diet was designed to mimic a low-quality food, composed of small numbers of invertebrates imbedded in a detrital and algal matrix, a typical diet of guppies in LP environments (Zandonà et al. 2011). The invertebrate diet was designed to reflect the high invertebrate content, high-quality diet with small amounts of detritus typical of HP environments in the dry season (2011) (see ESM, Tables S2–S4 for additional details on diet preparations). We fed fish equal calories of the two diets (i.e., 1.6 × more spinach diet by dry mass) at a level in excess of estimated daily caloric demands (Reznick 1983). The invertebrate diet had a carbohydrate/lipid/protein ratio of approximately 1:1:2.8 and the spinach-based diet had a carbohydrate/lipid/protein ratio of 1:0.1:0.6 (ESM, Table S2).

Tissue preparation

At the conclusion of the experiment, fish were not fed for approximately 20 h preceding the measurements of their gut enzymes to ensure that enzymatic activity was from endogenous origin rather than from dietary items (Dabrowski and Glogowski 1977). Fish were euthanized with MS-222 (Sigma) following IACUC protocol 2008–0106 Cornell University, and then measured standard length (SL) and weighed. The removed gut was laid out without stretching, and a digital photograph was taken of whole intestinal tracts for subsequent measurements using Image J (http://rsb.info.nih.gov/ij/). Gut tissue was weighed within 1 min of dissection then homogenized with 25 mM Tris-HCL buffer (pH 7.5) with a dilution factor of 100 volumes (v/w). The homogenates were centrifuged at 9,400*g* for 2 min, and the supernatant was collected in small aliquots (400 uL) and stored at −80 °C until just before the fluorometric analysis.

Fluorometric enzyme assays

All assays were measured at pH 7.5, the approximate pH of the guppy gut (assessed with ColorpHast® pH Test Strips; EMD Millepore, USA) and at 25 °C (the temperature at which fish were kept during the experiment). We measured brush-border enzyme activities, which are enzymes secreted from the intestinal microvilli—or intestinal brush-border, because they can be measured using highly sensitive fluorometric substrates and small volumes of gut homogenates. On small guts such as guppies, where it would be impossible to separate intestinal mucosal layer from whole gut, we used intestinal homogenates, which is often done in similar studies (Harpaz and Uni 1999; Zemke-White and Clements 1999; German et al. 2010). We focused on two C-acquiring enzymes [α-glucosidase (AG) and β-glucosidase (BG)], one N- and C-acquiring enzyme [N-acetyl-β-D-glucosaminidase (NAG)], and one P-acquiring enzyme [alkaline phosphatase (AP)] to obtain an overview of enzymatic activity in the guppy gut with respect to different nutrients. A fish gut in the enzyme assays was smaller than half the size of any other samples and was not run due to the lack of homogenate produced. Therefore, three experimental blocks were included in the enzyme models to maintain a balanced design.

Preliminary trials with guppy guts were used to determine saturating concentrations for all substrates. Fluorescence intensities were measured on a Bio-Tek Fluorescence plate reader, with excitation set at 360 nm and emission set at 460 nm. Enzyme activities were calculated using methods outlined by German et al. (2011).

Body elemental composition analysis and reproductive allotment

We examined whether diet or population affects how guppies incorporate C, N and P into tissues. At the conclusion of the experiment, guppy egg masses were dissected, weighed, and dried at 55 °C to constant mass. Reproductive allotment was calculated as the ratio of reproductive tissue dry mass to somatic tissue dry mass.

Dried guppies and eggs were ground into homogenous powder. Subsamples of 2 mg of homogenized tissue were weighed to the nearest thousandth of a mg and assayed for percent C and N using a Carbo Erba elemental analyzer (ElementarVario EL III; Elementar Analysensysteme, Hanau, Germany; see ESM for details). The remainder of the guppy somatic tissue and entire egg masses were ashed in Pyrex vials at 500 °C for 2 h and digested in 1 N HCl at 105 °C to facilitate dissolution of P. Soluble reactive phosphate of the resulting solution was then quantified using serial dilutions and the molybdate blue method (Parsons et al. 1984). The total amount of C, N and P in each fish and egg mass was estimated by multiplying the dry weight by the percent of the dry weight composed of each element.

Statistics and data analysis

We used linear mixed-effects models and likelihood ratio tests to explore the effects of population (HP vs. LP), diet (brine shrimp vs. spinach) and their interaction on enzyme activity, gut length, gut weight and body nutrient composition. For the nutrient composition analysis, gut morphology analysis and enzyme analysis, all models were constructed and compared using corrected Akaike information criterion (AIC) scores. Statistical analyses were conducted using the *lme4* package in R v.2.15.1 (R Development Core Team 2012). We included block as a random effect, and fish standard length was used as a covariate for gut length, gut weight and body nutrient composition. One fish per tank was measured for the enzyme analysis. The average

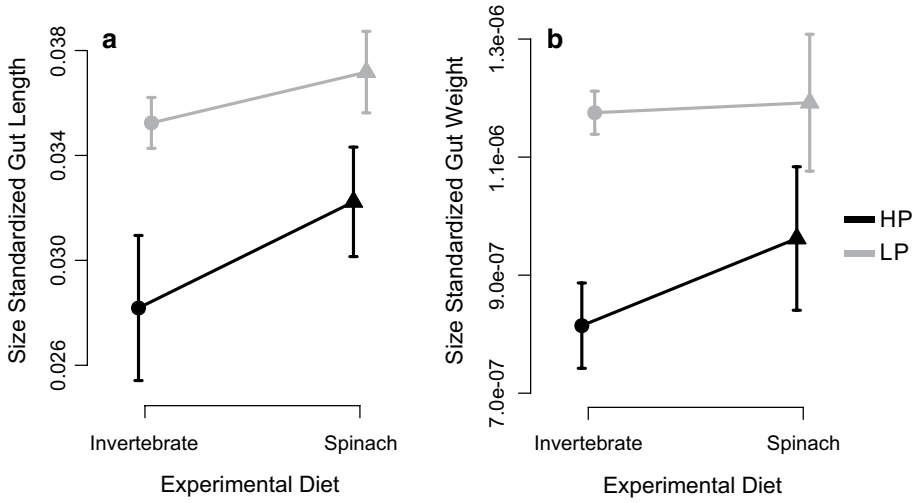

Fig. 1 Size standardized gut length and weight comparison of guppies, *Poecilia reticulata*, on spinach (*triangle*) and invertebrate (*circle*) diets ($n = 4$)

of the three female fish in each tank was used for analysis of gut morphology, body nutrient composition, growth, and reproduction. We regressed (log-transformed) whole-body nutrient content and gut length and weight against log-transformed standard length for the nutrient composition analysis and gut morphology analysis, respectively, prior to using information criteria-based model selection and χ^2 likelihood ratio tests to assess the role of diet and population as drivers of change in the amount of each nutrient present at a given fish length. We checked for the assumptions of normality and homoscedasticity using Q–Q plots and Tukey-Anscombe plots. Bartlett tests were used to confirm the assumption of homoscedasticity for all linear models of all response variables. Except where otherwise discussed in the results, all models conformed to this assumption.

We analyzed the enzyme activity data with multivariate statistics using principal component analysis (PCA) to assess co-regulation among enzymes. After log-transforming the data to meet the assumptions of linearity and standardizing each enzyme activity to the mean of that enzyme, the PCA was performed on a correlation matrix of enzymatic activity. Only PCs with eigenvalues that were larger than 1 and loadings that were greater than 0.3 were included in the analysis. The multivariate analyses were preformed using JMP (v.10) for Macintosh computers.

Size standardizations to visualize the results of gut morphology and account for allometry (Brown et al. 2002; Torres and Vanni 2007) were made by calculating the size corrected gut length through the power function:

$$Y_0 = \frac{Y}{X^b}$$

where Y_0 is an individual's size standardized gut length, Y is the gut length of that individual, and X is the standard length of that individual. The scaling exponent, b, was calculated from the relationship between the log standard length and the log gut length of the population. The same standardization procedure was done with gut weight.

Results

Gut morphology and gut enzyme activity

At the conclusion of the experiment, the guppy population (HP vs. LP) and fish standard length significantly affected gut length and weight (Fig. 1; Table 2). The best-fit model for both gut length and weight included only fish standard length and population. Neither gut length nor weight differed among diet treatments (spinach and brine shrimp), and the interaction between diet and population was not significant. In addition, the allometry of gut length or weight to standard length was not variable among HP and LP fish in the diet treatments.

AG and BG activities differed significantly between HP and LP guppies (Fig. 2). Likelihood ratio tests and model comparison indicated population alone best explained variance in AG and BG activities, and including diet or a diet × population interaction did not significantly improve explanatory power for models of AG or BG (Table 2). Variances in BG activities were not equally distributed (Bartlett test, $p = 0.013$), but the non-parametric, two-tailed Wilcoxon Rank Sum test (Population: $W = 31$, $p = 0.041$; Diet: $W = 13$, $p = 0.485$) also supports that population, but not diet, influences BG activity.

Mass-specific gut AP activities were affected strongly by population and weakly by diet (Table 2). AIC-score-based model comparison indicated the best model for AP activity included both diet and population effects, but removing the diet treatment from this model only marginally reduced model explanatory power. Removing population significantly reduced the explanatory power of a model

Table 2 Comparisons of fits of models for gut morphology and enzyme activities and their liklihood ratio tests at the conclusion of the dietary manipulation experiment

Model number	Model terms	Deviance	AIC	ΔAIC	Relative likelihood	w_i	Model simplification significance test			
							Models compared	Term removed	$\chi 2$ (likelihood ratio test statistic)	p (df = 1)
Gut morphology										
Gut length										
1	SL + diet + population + diet × population	−26.7	−12.7	2.97	0.00	0.003				
2	SL + diet + population	−25.7	−13.7	1.97	0.02	0.019	1,2	Diet × population	1.01	0.320
3	SL + population	−25.7	−15.7	0.00	1.00	0.974	2,3	Diet	0.03	0.850
4	SL	−15.5	−7.5	8.17	0.00	0.000	3,4	Population	10.16	0.001
5	Population	−21.0	−13.0	2.70	0.00	0.004	3,5	SL	4.70	0.030
Gut weight										
1	SL + diet + population + diet × population	−19.3	−5.3	1.40	0.06	0.053				
2	SL + diet + population	−17.5	−5.5	1.20	0.09	0.078	1,2	Diet × population	1.86	0.173
3	SL + population	−16.7	−6.7	0.00	1.00	0.864	2,3	Diet	0.81	0.368
4	SL	−3.2	4.8	11.48	0.00	0.000	3,4	Population	13.45	<0.001
5	Population	−12.2	−4.2	2.53	0.01	0.005	3,5	SL	4.45	0.034
Gut enzymes										
α-Glucosidase (AG)										
1	Diet + population + diet × population	97.27	109.30	1.20	0.09	0.076				
2	Diet + population	99.22	109.20	1.10	0.11	0.092	1,2	Diet × population	1.95	0.163
3	Population	100.10	108.10	0.00	1.00	0.832	2,3	Diet	0.90	0.343
4	Diet	107.40	115.40	7.30	0.00	0.000	2,4	Population	8.23	0.004
Alkaline phosphatase (AP)										
1	Diet + population + diet × population	121.90	133.90	2.00	0.02	0.015				
2	Diet + population	121.90	131.90	0.00	1.00	0.845	1,2	Diet × population	0.00	0.961
3	Population	124.80	132.80	0.90	0.17	0.140	2,3	Diet	2.83	0.093
4	Diet	131.80	139.80	8.00	0.00	0.000	2,4	Population	10.01	0.002
β-Glucosidase (BG)										
1	Diet + population + diet × population	150.80	162.80	3.10	0.00	0.002				
2	Diet + population	151.00	161.00	1.30	0.07	0.069	1,2	Diet × population	0.26	0.610
3	Population	151.70	159.70	0.00	1.00	0.929	2,3	Diet	0.70	0.404
4	Diet	157.80	165.80	6.10	0.00	0.000	2,4	Population	6.74	0.009
N-acetyl-β-D-glucosaminidase (NAG)										
1	Diet + population + diet × population	90.00	102.00	0.20	0.67	0.332				
2	Diet + population	92.44	102.40	0.60	0.30	0.149	1,2	Diet × population	2.44	0.118
3	Population	96.39	104.40	2.60	0.01	0.003	2,3	Diet	3.95	0.047
4	Diet	93.85	101.80	0.00	1.00	0.496	2,4	Population	1.41	0.236

Population indicates whether the guppy originated from populations collected in high (HP) or low (LP) predation habitats while *Diet* corresponds to diet differences during experiment, where guppies were either fed spinach- or invertebrate-based diets. SL is the fish standard length. All models include block as a random effect

AIC Akaike's information criterion, *ΔAIC* difference in AIC between the *i*th model and the best model, w_i Akaike's weight

Table 3 Summary of principal coordinates analysis of gut enzyme data; the eigenvectors included in the principal component analysis are italicized

	Principal component			
	I	II	III	IV
Eigen value	2.0373	1.1799	0.6128	0.17
Percentage of total variance	50.932	29.498	15.32	4.251
Cumulative percentage of variance	50.932	80.429	95.749	100
$\chi 2$ value	17.768	10.016	4.434	0
Degrees of freedom	5.256	4.307	1.83	–
p	0.004	0.0493	0.0937	–
Eigen vectors				
NAG	*0.45769*	*0.39571*	−0.7962	−0.0006
AG	*0.63557*	0.06903	0.40015	−0.65663
BG	*0.62029*	−0.30327	0.20532	0.69363
PHOS	−0.04267	*0.86411*	0.40471	0.29617

for mass-specific gut AP activity. The best model for NAG activity included only an effect of diet. Including effects of population or the diet × population interaction did not significantly improve the explanatory power of the model. Removing diet from the model weakly but significantly reduced model explanatory power, indicating our diet treatment did influence NAG activities.

Growth and reproduction

Guppies on the low-quality diet had strongly reduced tank-average growth rates ($F_{12,1} = 35.6$, $p = <0.01$), but neither the population nor the diet × population interaction explained a significant proportion of variation in growth rates ($F_{12,1} = 0.58$, $p = 0.46$; $F_{12,1} = 0.16$, $p = 0.71$, respectively; ESM, Figure S1A). The lack of a difference in growth rate between HP and LP guppies during our experiment is not entirely surprising considering that growth rate only sometimes differs between HP and LP populations and is a plastic trait for which resource availability has been described to influence more than predation regime (Arendt and Reznick 2005). Over the course of the experiment, LP guppies allocated significantly less of their overall tissue growth to reproduction ($F_{12,1} = 7.2$, $p = 0.02$), but neither diet nor its interaction with population significantly altered allocation to reproduction during the experiment ($F_{12,1} = 1.0$, $p = 0.34$; $F_{12,1} = 0.1$, $p = 0.76$, respectively; ESM, Figure S1B).

Multivariate analysis of digestive enzymes

The PCA decomposed the data onto two PCs (with eigenvalues larger than 1), which together explained ~80 % of the data (Table 3). PC1, which explained ~51 % of the variance, was generated by positive loadings of NAG, BG and AG. In contrast, PC2, which explained ~29 % of the variance, resulted primarily from positive loadings of AP. The majority of the variance in the data was attributed by differences in enzymatic activity between the two guppy populations, while a smaller proportion of the variance was associated with experimental diet. This pattern is illustrated in the biplot generated from the PCA (Fig. 3). The extent of these trends are evident along component 1 in that 6/7 HP guppies had a score of greater than 0.5 along PC1, while 8/8 of LP guppies had score of less than 0.5 along it.

Body nutrient content

Body length explained a significant amount of variance in whole-body mass of N, P and C, (Fig. 4; ESM, Tables S5–S7), and no interactions between body length and any treatment were significant, indicating the slope of the relationship between whole-body nutrient mass and length was not affected by diet treatment or population (ESM, Tables S5–S7). Fish on the invertebrate diet averaged more C at a given length than fish on the spinach diet ($\chi^2 = 14.1$, $p < 0.01$; Fig. 4; ESM, Table 5). Whole-body N content was best explained by a model with only length (Fig. 4; ESM, Table S6) and including diet in the model did not significantly increase the explanatory power ($\chi^2 = 2.6$, $p = 0.11$). Whole-body P was best explained by length and population background, as LP guppies had significantly elevated whole-body P content at a given length ($\chi^2 = 7.5$, $p < 0.01$; Fig. 4; ESM, Table S7). Analysis including only somatic tissues produced comparable results (ESM, Tables S5–S14). Analysis of percent of tissue composed of each element and stoichiometric measures (C:N, C:P, N:P) are included in the ESM, Tables S9–S14. Guppies in this experiment had slightly different tissue composition than found in wild guppies from natural streams (ESM, Figure S2), which is most likely due to the altered resource, predation risk, hydrological and social environment of the laboratory environment compared to natural streams.

Discussion

We found that guppies from HP and LP stream sites, reared in a common garden experiment from before maturity, exhibited differences in gut morphology and physiology. LP guppies were previously found to have longer guts than HP guppies in the wild (Zandonà 2010), and we found LP guppies maintained longer guts on the experimental diets. This finding supports our hypothesis that LP guppies, which consume lower-quality diets in the wild, invest more in gut mass, even in common garden and when reared on

controlled diets. We expected guppy enzyme activity to follow the adaptive modulation hypothesis (Table 1), showing a positive correlation between enzyme activities and substrate concentrations in the diets associated with each population (e.g., carbohydrate- and cellulose-rich LP diets and protein- and chitin-rich HP diets). Instead, we found that HP guppies had higher activities of carbohydrate and cellulose digesting AG and BG, and LP guppies had higher activities of phosphorus cleaving AP. Our diet treatment did not significantly affect gut morphology or enzyme activity. We consider these results in the context of plastic effects and population divergence on traits in response to environmentally induced trophic niche shifts.

Effect of plasticity versus population divergence on gut morphology

Gut morphology varies with food quality, and animals that consume lower-quality diets require higher levels of intake to meet nutritional and energetic requirements (Sibly 1981; Kramer and Bryant 1995; Clements and Raubenheimer 2006). Higher rates of intake accelerate the flux of food through the gut, thereby reducing the gut residence times of dietary items. Because gut residence time is important for digestion and absorption of nutrients, longer guts are one adaptive response to the enhanced gut passage rates required by low-quality food (Karasov et al. 2011). The high energy cost of gut tissues, however, demands large energetic or nutrient returns for investment in incremental gut length (Cant et al. 1996). The patterns we observed corroborate previous observations that fish from an environment with lower-quality resources have longer guts (Zandonà 2010; Zandonà et al. 2011). Our low-quality diet treatment directionally increased gut length (Fig. 1), though not significantly.

Predation pressure can also dramatically reduce dietary intake (Werner et al. 1983) and, thus, impact gut length. In the wild, HP guppies feed less frequently than LP guppies and are more likely to have empty guts, likely due to restricted foraging under predation risk (Fraser et al. 2004; Zandonà, unpublished data). This reduced consumption may contribute to shorter gut lengths in HP guppies by lowering the rate of intake and enabling longer gut transit times without investment in energetically expensive digestive tissue. Previous work in tadpoles indicates that predation risk induced shorter guts, while competition increased their gut length (Relyea and Auld 2004), allowing the competition-stressed tadpoles to retain food for sufficient lengths of time, increase their absorption of nutrients and energy, and grow faster. HP guppies may, thus, have shorter, lighter guts because of behavioral changes induced by predation risk while the lower dietary quality of LP guppies or the presence of increased competition in a more crowded conspecific environment may have led to the longer gut exhibited in LP guppies.

Although gut length can be subject to plasticity, it can also vary through genetic mechanisms and be the target of natural selection, as variation among individuals is heritable (Charo-Karisa et al. 2007; Wagner et al. 2009) and affects fitness in the context of both food quality (German et al. 2010) and predation risk (Relyea and Auld 2004). Our results demonstrate that intraspecific variation in guppy gut length is maintained even when the two populations are reared in common garden and on grossly different food qualities. Therefore, population divergence appears to have a greater effect on gut length than short-term changes in dietary quality within guppies.

Effect of plasticity versus population divergence on enzyme activity

We found that HP and LP guppies express different gut enzymatic profiles. We based our expected results on the adaptive modulation hypothesis, which posits that animals enhance expression of enzymes for the most abundant substrates in dietary items in order to maximize uptake of the most abundant dietary nutrients (Karasov et al. 2011). An alternative regulatory strategy is that of nutrient balancing, wherein animals secrete digestive enzymes to target nutrients that are deficient in the diet in order to maintain homeostasis (Clissold et al. 2010). These explanations have been employed to describe both plastic and evolved responses of enzyme expression to different diets (Caviedes-Vidal et al. 2000; German et al. 2004).

If our results were in line with the adaptive modulation hypothesis, the enzymatic activity would follow the expectations outlined in Table 1, where up-regulation occurs for enzymes whose substrates were most abundant in the diet. In our experiment, we expected to see up-regulation either in response to the short-term, dietary manipulation used during the treatment period or to the diet of the ancestral HP or LP population from which each fish was derived. We found a number of deviations from this expectation in our results. For example, BG and AG are carbohydrates, and increases in their activities enhance the breakdown and assimilation of energy-yielding carbon compounds from starch and cellulose (Stevens and Hume 1995; Karasov and Martinez del Rio 2007). We expected LP guppies to have increased AG and BG activity to match the greater proportion of starch and cellulose in their more algal and detrital diet, but instead we found that HP guppies had higher AG and BG activity. A possible explanation for these observations is that, because HP guppies consume high N and P content diets and are more likely to encounter predator-restricted calorie intake (Fraser and Gilliam 1992) than LP guppies, they are more prone to energy limitation than

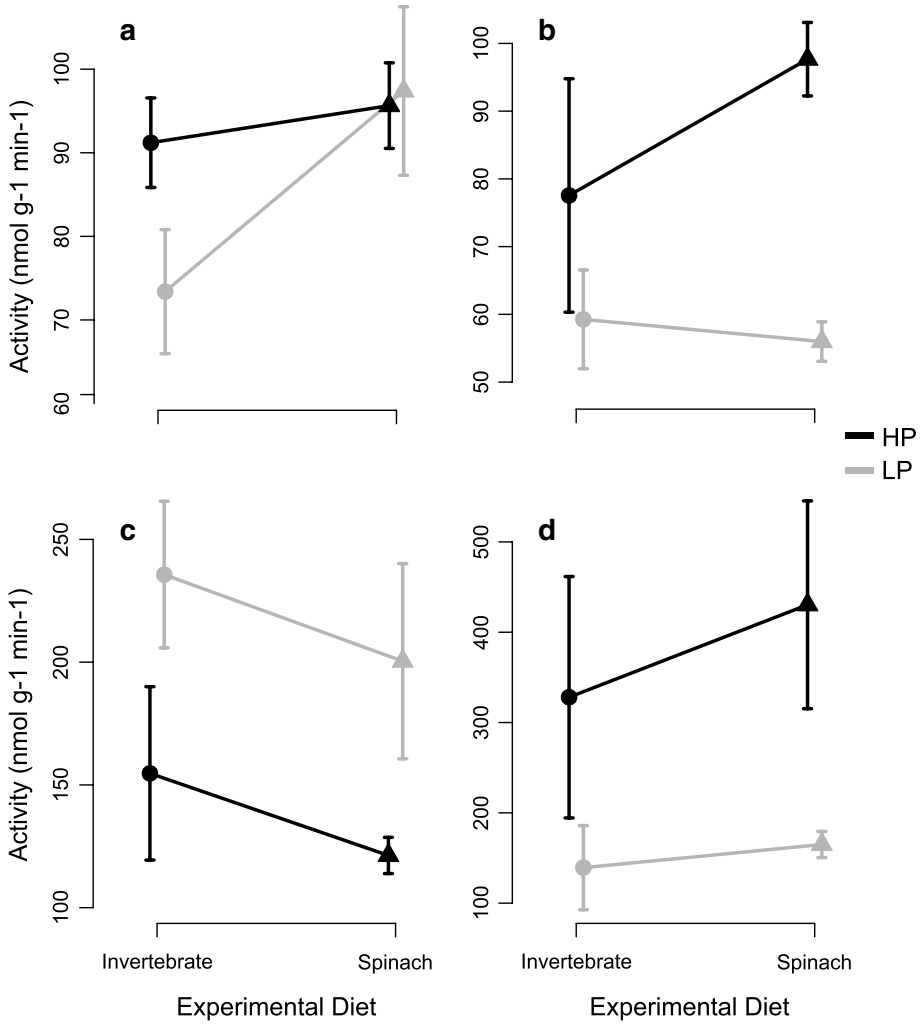

Fig. 2 Enzyme activity per gram of gut per minute for guppies, *Poecilia reticulata,* from low-predation (*LP*) and high-predation (*HP*) habitats for **a** N-acetyl-β-D-glucosaminide (NAG), **b** α-glucosidase (AG), **c** alkaline phosphatase (AP), **d** β-glucosidase (BG). *Circles* represent invertebrate-reared fish and *triangles* represent spinach-reared fish ($n = 3$)

nutrient stress (El-Sabaawi et al. 2012). In this context, HP guppies may obtain greater nutritional benefit from increased access to energy-yielding C compounds through increased AG and BG activity and, thus, compensate for the relative lack of C in their diet, a pattern suggestive of the nutrient-balancing hypothesis.

AP was higher in LP guppy guts, and it appears to be regulated independently of the other enzymes in guppies (indicated by its loading on PC2, Fig. 3). These observations deviate from the patterns of AG and BG and from our hypothesis that HP guppies would have increased AP activity because their diet contains a greater proportion of P based on the adaptive modulation hypothesis. Instead, we found LP guppies to have greater AP activity, which is consistent with nutrient balancing (Clissold et al. 2010) regulation, where organisms increase enzymes to help acquire a nutrient in deficit in their diet (Koch 1985). It should also be noted that intestinal AP can have broad functions, including promoting gut homeostasis, protection against pathogenic bacteria endotoxin detoxification, and regulation of lipid absorption (Poelstra et al. 1997; Bates et al. 2007; Lallès 2010). It can also be correlated with general nutrient levels in diet (German et al. 2004) and differ based on the interaction of genetic background and food level (Hakim et al. 2006). A possible explanation for the population differences we found could be that, because LP guppies consume material that is less balanced with their tissue nutrient requirements (El-Sabaawi et al. 2012), they are more likely to be limited by P than HP guppies. It has been suspected that AP helps P-deprived *Daphnia* obtain P from their diets (McCarthy et al. 2010), and higher expression of AP in LP guppies may enhance acquisition of limited P. Therefore, it may be a trait under positive selection to accommodate their low P diets; however, more investigation is needed to test this hypothesis directly.

From our analyses, HP and LP guppy populations from the Aripo River appear to have strong differences in their gut physiology and morphology. Because we did not test assimilation efficiency directly in this experiment, a main question still remains: do these differences in gut length

and enzymatic activities result in modification of guppy biology and, potentially, their fitness? To gain insights into the potential impact of these physiological processes, we analyzed body nutrient content of the guppies after the experimental manipulation.

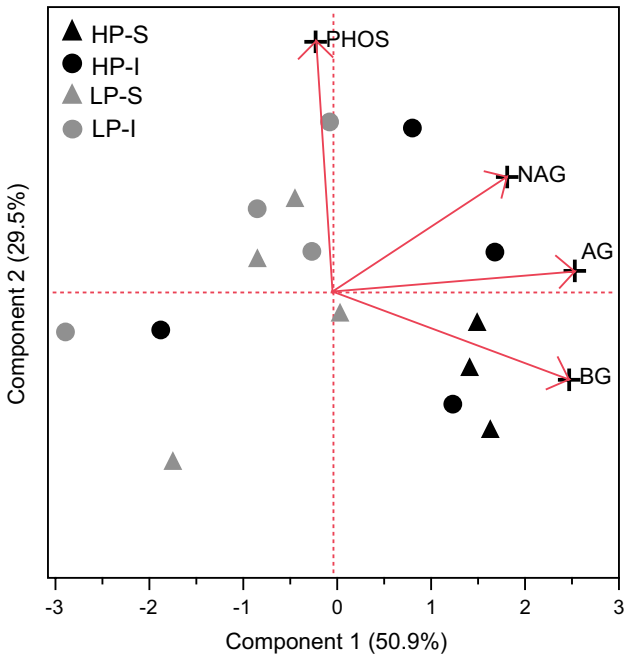

Fig. 3 Principal component analysis (PCA) of enzymatic activity in guppies, *Poecilia reticulata*. *Circles* represent invertebrate-reared fish (*I*) and *triangles* represent spinach-reared (*S*) fish. The *scalars* represent loadings of each variable onto each component. The *percent values* indicate the proportion of the variance explained by each component

Relationship of gut enzyme activity to body nutrient composition

Fish fed the invertebrate diet had higher C relative to N content, presumably due to increased levels of C-rich lipids, but there were no differences in tissue C or N between populations. LP guppies in this experiment maintained higher, length-specific whole-body P mass (Fig. 4) than HP guppies. The differences in HP and LP body nutrient composition are likely related to structural differences in skeletal investment related to body shape between HP and LP guppies (Hendrixson et al. 2007; Torres-Dowdall et al. 2012). Variation in whole-body P content within species, however, can also reflect the availability of P in the diet (Ketola and Richmond 1994; Baeverfjord et al. 1998). The higher body P of LP guppies might indicate increased P acquisition due to the higher AP expression observed, as suspected to occur in *Daphnia* (McCarthy et al. 2010). The relationship between dietary elements and gut enzyme activity may influence nutrient homeostasis and could be one mechanism through which organisms can adapt to a stoichiometrically imbalanced world.

Potential for ecosystem consequences

Our analyses reveal that population-level differences in enzyme activities and elemental content exist in HP and LP Trinidadian guppies collected from the wild. Phosphatase activity differs substantially between populations and may contribute to the variation in body nutrient composition observed at the end of our dietary manipulation experiment. Such a difference in nutrient processing and retention at the whole-population-level could have impacts on nutrient cycling, and so digestive enzymatic activity and

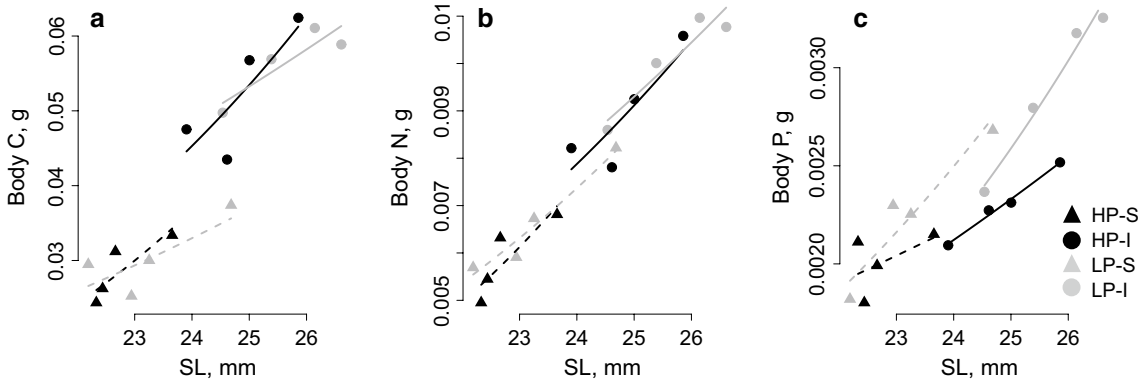

Fig. 4 The average fish nutrient content per tank by the average fish standard length per tank for tank for carbon (**a**), nitrogen (**b**) and phosphorus (**c**). LP guppies are shown in *open gray* symbols and HP in *filled black*. *Circles* represent invertebrate-reared (*I*) fish and *triangles* represent spinach-reared (*S*) fish. *Trend lines* are the best fit of a simple linear model of Log(Body Nutrient) as a function of Log(SL) for each treatment group presented in untransformed space, with *solid lines* denoting invertebrate diet, *dashed lines* denoting the spinach diet, *black* representing HP guppies, and *gray* indicating LP guppies ($n = 4$)

digestive capacity could be ecosystem effect traits (Matthews et al. 2011) on which selection could act. Fish play a significant role in nutrient cycling and can also affect river reaches by creating biogeochemical hotspots and altering nutrient flows (Taylor et al. 2006; McIntyre et al. 2008). This study suggests that, in addition to species identity, fish phenotype—ranging from dietary selection to gut size and digestive physiology—should be considered as a factor in nutrient cycling as nutrient retention is a trait that can vary within recently diverged populations of the same species.

Conclusions

We found variation in gut enzyme activity and morphology between Aripo LP and HP guppies, and we speculate that these differences are related to the diet quality of their environments of origin. Our diet treatment affected the activity of NAG and marginally affected the activity of AP, though these effects were relatively weak compared to the pronounced effect of population on the expression of AG, BG, AP and gut morphology. Short-term differences in diet were also met with changes in somatic growth and altered body carbon content, while somatic P varied most strongly according to population.

Our findings suggest that guppy population may be a stronger determinant of gut length and enzyme activity than short-term change in dietary quality. This work indicates that there is a possibility LP and HP guppies may have adapted to their diverse resource environments and feeding regimes through differential gut morphology and enzyme activity, which may enhance nutrient assimilation. Two important caveats are necessary to this adaptive interpretation. First, we compared one HP–LP population pair, so we cannot rule out the hypothesis that the difference we observed was not causally related to predation environment. However, a number of other traits related to differences in consumption and gut morphology have been found in different streams and across years (ESM, Table S1), suggesting the patterns we observed may be more widespread. Second, because the guppies in our experiment were collected from the wild in their first few weeks of life, it is possible that the observed gut differences were not genetic but induced early in development, and the use of second-generation laboratory-born fish would be the best way to test for this possibility.

Guppies are not the only animals that have exhibited population-level differences in enzyme activities and gut morphology (Tracy and Diamond 2005; Horn et al. 2006; German et al. 2010), suggesting that such physiological divergences may be adaptations to resource availability and diet. Our study provides additional support that adaptive responses in gut morphology and gut enzyme activity may accommodate dietary differences and that these traits may also be linked to alterations in nutrient processing.

Knowledge of the ways in which organisms adapt to new diets is especially relevant today given that ecosystems are experiencing an unprecedented loss of biodiversity due to human modification of the environment (Thomas et al. 2004). As ecosystems are altered, dietary shifts may help populations survive. As such, it is crucial to understand the capacity of both individual organisms and evolutionary lineages to make such shifts, including the adaptive dynamics of gut physiology (Karasov and Martinez del Rio 2007; Karasov et al. 2011) and interaction of digestive features, body condition and fitness.

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