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Geometric Morphometric Analysis of ecomorphs of freshwater fish of Eastern Panama; *Bryconamericus emperador* and *Astyanax aeneus*

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Presented to:

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EXECUTIVE SUMMARY

Geometric Morphometric Analysis of *Bryconamericus emperador* and *Astyanax aeneus* ecomorphs freshwater fish of Eastern Panama

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With INDICASAT - Edificio 219, Ciudad del Saber Clayton, Panamá, Rep. de Panamá

Morphology lies at the crossroads of genetics and the environment - the genes expressed by an organism determine that organism's phenotype, although expression of that gene can be influenced by external environmental factors. We aimed to determine whether environmental pressures can transcend genetic species barriers in the determination of organismal morphology. We studied two morphologically similar, common, and co-occurring species; *Astyanax aeneus* and *Bryconamericus emperador*. Specifically, we compared these species' morphology in samples from different locations across eastern Panama. We hypothesized that environmental pressures *do* transcend species barriers. In other words, we predicted that when found in the same location, these two species would be more similar to each other than to conspecifics found in a different location. This is based on two findings in the literature: first, both have similar diets, and second, they can school together. The first is important because diet is a main determinant of ecological niche, such that species with very similar diets can be said to have overlapping niches. The second is telling because schooling dynamics dictate that dissimilar fish have higher risks of predation, making morphological similarity an advantageous trait. This would result in our two species of interest looking very similar to each other.

Our methods involved sampling different rivers in central and eastern Panama with a trolling net, identifying adult individuals from our species of interest, and performing geometric morphometric analysis where sub samples included one species from one location. Geometric morphometrics consists of digitizing photographs of specimens, tagging twelve landmark features (anatomical reference points) in the free software TpsDig, and processing the landmark coordinate data with TpsRelw to extract information about variation in morphology between and among samples. This software extracts principal axes of variation, and ANOVAs allow the identification of any significant differences that exist between sub-samples.

Our result suggest that local environmental pressures *can* transcend species barriers, and result in sympatric individuals being more similar to each other, regardless of species, than to allopatric conspecifics. This result highlights the importance of regional effects of species morphology.

However, further research, such as a common garden experiment, is needed to determine whether this is a result of genetic factors or phenotypic plasticity.

RESUMEN EJECUTIVO

Análisis morfométrica geométrica de ecomorfos de *Bryconamericus emperador* y *Astyanax aeneus*, dos peces de agua dulce del Este de Panamá

Naomi Robert & Marika Hirtle-Lewis
Con INDICASAT - Edificio 219, Ciudad del Saber Clayton, Panamá, Rep. de Panamá

La morfología esta a la esquina de la genética y el medio ambiente - los genes exprimados por un organismo determinan su fenotipo, aunque la expresión de estos genes está influida por factores externos. Tuvimos como meta de establecer si el medio ambiente de un organismo pueda superar las barreras entre las especies en la determinación de la morfología de organismos. Estudiemos dos especies comunes, que tienen morfología similar y que ocurran juntas; B*ryconamericus emperador* y *Astyanax aeneus*. Concretamente, comparemos la morfología de múltiples muestras de cada especie, proviniendo de lugares diferentes del este de Panamá. Hicimos como hipótesis que las presiones ambientales pueden superar barreras interespecíficas. En pocas palabras, predecimos que cuando se encontran las dos especies en el mismo lugar, la morfología de ellas se parecería más entre ellas que con muestreas de la misma especie de otro lugar. Nuestra hipótesis está basada en dos pruebas encontradas en la literatura: primero, las dos tienen la dieta similar, y luego, se pueden encontrar las dos especies en los mismos bancos. El primer punto es importante porque la dieta es un determinante principal de los nichos ecológicos, de tal forma que se puede decir de especies con dietas similares que ocupan nichos superpuestos. El segundo es un punto clave porque las dinámicas de bancos muestran que peces distintos tienen mayor riesgo de ser capturados por depredadores, pues hay ventajas por las dos especies a ser morfológicamente similares. El resultado de esta presión sería un homogeneidad morfológica.

Nuestros métodos incluyeron muestrear diferentes ríos en Panamá Este y Central con una red de pesca de arrastre, identificar los individuos adultos de las especies queridas y hacer una análisis de morfométrica geométrica. Cada muestra en el análisis representa una especie de un lugar. La morfométrica geométrica consiste en digitalizar las fotografías de los especímenes y de etiquetarlos con 12 puntos de referencias anatómicos. Esto se hace con el software gratis de TPSdig. TPSrelw procesa las coordenadas de los puntos de referencias y extraer información sobre la variación entre y dentro las muestras. El software extrae los ejes principales y las pruebas ANOVA permitían la identificación de diferencias significativas entre las muestras.

Nuestros resultados sugieran que el medio ambiente local puede ser más importante que las barreras entre las especies para determinar la morfología de individuos, lo que resulta en individuos simpátricos que tienen más similitudes entre ellos, a pesar de que no sean de la misma especie, que con conspecíficos allopátricos. Este resultado acentúa la importancia de efectos regionales sobre la morfología de especies.

Sin embargo, más investigaciones, como 'common garden experiment', son necesarias para determinar su esto resulte de factores genéticos o plasticidad fenotípica.

PRELIMINARY INFORMATION

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Host Institutions

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INDICASAT

INDICASAT dedicates itself to the advancement of science and technology in Panama through research in fields such as biotechnology, immunology, neuroscience and toxicology. INDICASAT has recently added an ecological component to their research domain. It is in this portfolio that our supervisor, Luis Fernando de Leon Reyna is conducting his research.

The Smithsonian Tropical Research Institution

The Smithsonian Tropical Research Institution (STRI), a branch of the United States based Smithsonian Institution, is dedicated to the understanding of biological diversity. Established in Panama in 1923, STRI has expanded and become one of the world's leading research institutions. STRI aims to facilitate research throughout the global tropics through the provision of facilities, training and funding for tropical biologists (STRI, 2012)

Project Hours

Number of full days spent on the project: 40
Number of full days spent in the field: 3

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INTRODUCTION

Both genetic diversity and phenotypic plasticity influence the phenotypic variation that exists among natural populations (Stearns, 1989, Sharpe et al. 2008). The former is due to the characteristics of individual environments applying selective pressures on resident organisms (Endler, 1986). On an evolutionary time scale, these selective pressures can contribute to the natural selection of individuals with adaptive traits, resulting in organismal communities genetically adapted to their environment. The second factor contributing to phenotypic variation, plasticity, describes the ability of a single genotype to produce different morphological, physiological or behavioral phenotypes as a result of environmental conditions (Gause, 1947; Bradshaw,1965; West-Eberhard, 1989, Mittelbach et al. 1999). Thus, phenotypic plasticity results in morphological divergence by altering gene expression in response to environmental pressures, not through natural selection. Consequently, morphology, being the visual representation of multiple phenotypes, is the intersection of these two processes.

Heterogeneous environmental conditions and associated selective pressures have resulted in ecomorphs; local varieties of species whose geographic differences in morphology are the result of ecological variation (Langerhans et al., 2006). Our study, based in the freshwater riparian environment of eastern Panama, examines ecomorph populations of two Characidae species; *Astyanax aeneus* and *Bryconamericus emperador.* We aim to understand what type of morphological variation exists among the ecomorphs of these species and answer the following question: Can morphology among ecomorphs of these species differ to the extent that sympatric species share greater similarities than allopatric conspecifics? This occurs in Anolis lizards,

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where ecomorphs have diversified independent of the degree of relatedness of species, as well as in certain fish and birds (Langerhans et al. 2006). Given that *A.aeneus and B. emperador* are morphologically similar and *A. aeneus* is known to display a large degree of phenotypic plasticity (Ornelas-Garcia et al. 2008), we hypothesize that sympatric species will be more similar than allopatric conspecifics. Such a result would highlight the importance of regional effects on an organism's morphology over species barriers.

METHODS

Study species

Characidae are found in the Americas from Southern Texas, USA, to Patagonia, and the family is said to have up to 885 families (Bussing, 2002). Several genera of the same family can also be found in Africa. Of the Characidae family, *Astyanax aeneus* and *Bryconamericus emperador* were selected for this study because they are abundant in Panama, are very similar in appearance and exist in the same bodies of water. Thus, they lend themselves well to questions of ecological dynamics between ecological equivalents. In addition *A.aeneus* is the most widely distributed Characidae in the Americas and is characterized by both a high degree of phenotypic plasticity (Ornelas-Garcia et al. 2008) as well as the capacity to adapt to diverse habitats (Dowling et al. 2002, Strecker et al. 2003).

Study sites

Eight sites were selected throughout eastern Panama (figure 1). The individual site description information is summarized in table 1. Fish samples were collected from two rivers in Parque Nacional Soberanía; Rio Frijoles and Rio Frijolitos. Rio Mamoni was sampled close to its

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intersection with the Panamerican Highway. Sampling in Quebrada Hoya was done approximately 100m from the banks of Mamoni. Our supervisor, Luis Fernando, collected samples for our analysis from two locations in the province of Darién including Rio Chacunaque and Laguna Aguabuena. Samples from Rio Ukupti and Agua Clara were taken from the fish collection located at the Naos Island Laboratories of the Smithsonian Tropical Research Institute. Consequently, descriptive environmental data could not be obtained for these two locations.

	Width	Depth	LAI	Substrate	Current
Rio Frijoles	3-6m	30cm-1m	50-90%	sand/leaf debris	alternating riffles and pools
Rio Frijolitos	3-6m	30cm-1m	50-90%	sand/leaf debris	alternating riffles and pools
Rio Mamoni	80m	50 cm	0	rocky	relatively swift current
Quebrada Hoya	6m	60cm-1m	90%	clay	stagnant
Laguna Aguabuena	small lake (2 ha)		0%	clay	stagnant
Rio Chacunaque	30 m	6m	10	mud/clay	slow flowing

Table 1. Qualitative and estimated quantitative environmental descriptive data of study sites sampled. Both Quebrada Hoya and Laguna Aguabuena are seasonally disconnected in the dry season from adjacent water bodies. As a result water at these sites was stagnant at the time of sampling.

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Fig 1. Sampling sites in eastern Panama. A. Rio Frijoles (9.15N 79.73W) B. Rio Frijolitos (9.15N 79.73W) C. Rio Mamoni (9.20N 79.07W)D. Quebrada Hoya (9.25N 79.71W). E. Laguna Aguabuena (8.63N 77.95W). F. Rio Chacunaque (8.25N 77.72W) G. Rio Ukupti (8.82N 77.72W) H. Rio Agua clara (9.32N 78.69W).

Sampling

All sampling was completed in the dry season during the months of January and February 2012. Transects of 200m were marked as fields of study in each river location. Each environment was qualified according to water velocity, width, depth, LAI (leaf area index) and river bed substrate.

Fish were captured by manually dragging a trawling net upstream along segments of the transect. This was accomplished by two people, on opposite sides of the net, walking upstream at a moderate pace, each as close as possible to the river bank and ensuring that the lower, weighted part of the net dragged on the riverbed. After approximately 10m, the net was brought to the

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riverbank, while the lower weighted part was gathered in to prevent escape. For shallow sections (approximately shallower than 2 feet), a smaller weighted net was used. In deeper water, a large pocketed net was used. Each section was sampled twice. For statistical significance, sample sizes have a minimum of 30 individuals. One sample represents a single species from a single location (e.g. the first sample is *A.aeneus* from Rio Frijolitos, and the second is *B. emperador* from the same location). To avoid shape bias due to ontogenic development, juveniles were returned to the river. All mature individuals of the species of interest were euthanized and preserved in 70% ethanol. We sampled Rio Frijolitos (n = 31 *A.aeneus*, 41 *B. emperador*), Rio Frijoles (n = 34 *A.aeneus*, 68 *B. emperador*), Rio Mamoni (n = 33 *A.aeneus*), Quebrada Hoya (n = 31 *A.aeneus*), Laguna Aguabuena (n = 30 *A.aeneus*), Rio Chacunaque (n = 33 *A.aeneus*), (n = 36 *B. emperador*) and Rio Agua Clara (n = 32 *B. emperador*). The rios Frijoles and Frijolitos are geographically close to each other, as are Rio Mamoni and Quebrada Hoya, and rios Agua Buena and Chacunaque.

We were aware of McGill University's *Code of Ethics* during the completion of this internship.

Processing and Morphological data

Collected individuals were returned to the lab, separated by species and tagged with thread through the mouth and gills. Tags were marked with a number using waterproof paper and samples were weighed to the 0.1g and photographed (right side of the body) on 1x1 cm gridded paper (Panasonic Coolpix GF3).

The geometric morphometric analysis was performed with the TPS suite software developed by James Rohlf available as a free download (http://life.bio.sunysb.edu/morph/).

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TpsDig [v. 2.16] was used to record the XY coordinates of twelve landmarks (Figure 2a/2b) used to describe fish morphology (Bookstein, 1991). Pictures were landmarked by two people blinded to the origin of each specimen by random ordering. TPSrelw [v. 1.49] extracted principal components, partial warps and relative warps explaining the variation in landmark positions among all samples (method explained by Rohlf and Slice, 1990). The morphological features represented by each principal component (relative warp) were identified using the relative warp visualizer graphic user interface. Additionally TPSrelw calculated the centroid size which was used as a measure of body size, a possible covariate with relative warp scores. The centroid size is calculated as the the square root of the sum of squared distances of a set of landmarks from their centroid (Slice et al, 1998). Many studies have documented using this method to track morphological variation among populations (Toline & Baker, 1993; Zúñiga *et al.*, 2007; Langerhans, 2003, Sharpe et al., 2008).

Fig.2a Photo after landmarking in TpsDig. Red circles indicate landmarks.
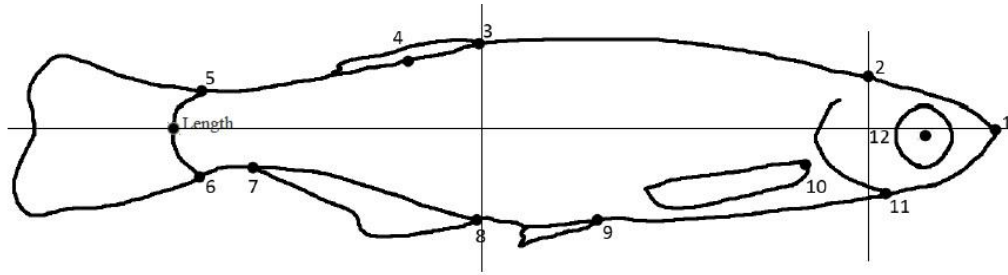
Fig.2b: Landmark map showing the 12 morphological landmarks measured on each individual. (1) Anterior tip of the upper jaw, (2) Tip of *the nasal bone /Insertion of the operculum* (3), anterior insertion of the first dorsal fin ray, (4) posterior insertion of dorsal fin membrane, (5) origin of caudal fin membrane on dorsal midline, (6) origin of caudal fin membrane on ventral midline, (7) posterior insertion of anal fin membrane on ventral midline, (8) anterior insertion of first anal fin ray on ventral midline, (9) insertion of pelvic fin on ventral midline, (10) anterior insertion of pectoral fin ray (11) and centre of the eye (12).

Statistical Analysis

All statistical analyses were performed in GraphPad [v. 3] unless otherwise stated. After the principal components analysis, the samples were separated according to location and species, with a total of 10 sub-samples. The relative warp scores do not correlate to centroid size ($R^2 <$ 0.003) and therefore analyses of covariance were not performed. Instead three one-way ANOVAs (one for RW1, RW3 and RW3) were performed with all samples to determine if there were significant differences in relative warp scores between the 10 sub-samples. Post-hoc Tukey tests were performed to determine which samples differ from each other. Because the scores for RW2 and RW3 did not meet the assumption of normality, the Kruskal-Wallis test, which does not assume normality or homoscedasticity was performed to confirm the results of the ANOVA and Tukey tests.

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RESULTS

Relative warps

RW1 captured 28.3% of the total variation in all samples. This principle component relates to body depth, where increasingly positive scores reflect a more streamlined body, and an increasingly negative score translates to a deeper body (Fig. 3a). RW2 captures 12.31% of total variation and is related to the position of the pelvic fin insertion and a slight effect on orientation of snout and tail relative to the horizontal axis. Increasingly positive score yielded individuals with posteriorly inserting pelvic fins and upward-oriented snout and tail, while negative score resulted in individuals displaying more anteriorly inserted fins and downward-oriented snout and tail (Fig. 3b). RW3 explains 10.77% of total variation and describes tail angle relative to the horizontal axis, where a more positive score describes an upward-oriented tail, and vice-versa (Fig. 3c). Summary stats for RW1, RW2 and RW3 can be found in the annex.
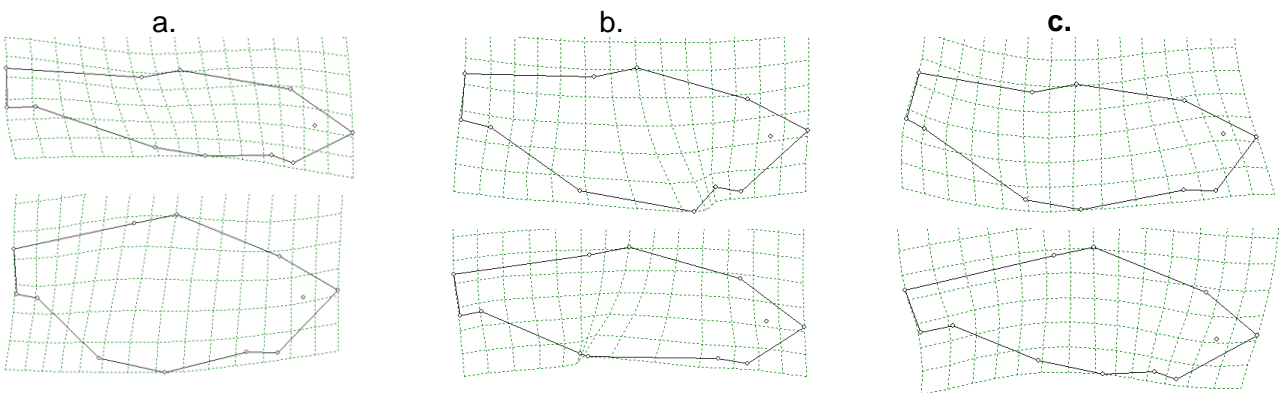
Fig.3 Thin Plate Spline deformation for RW1 (a), RW2 (b) and RW3 (c). Highest observed scores are on upper row, and lowest observed score, on the lower.

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ANOVA results
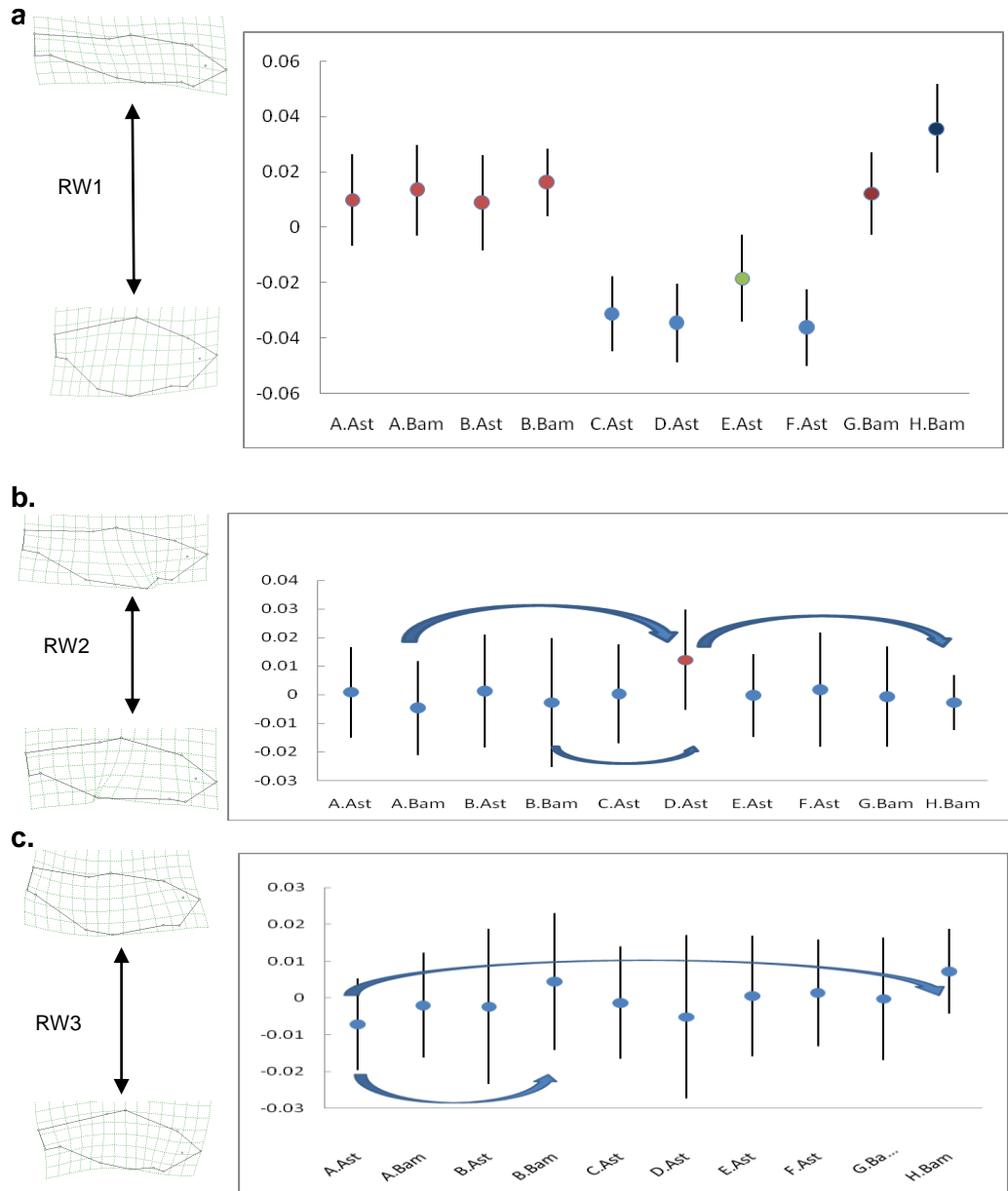
Fig. 4. Variation in relative warp values among all samples. (a) shows mean values of RW1, where values of the same color are not significantly different from each other. (b) shows mean values for RW2, where arrows show which samples are significantly different from each other and (c) shows the mean for RW3 and significant differences are shown by arrows (± standard deviations).

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Post-hoc Tukey test results

Post-hoc Tukey tests revealed that body depth (RW1) does not vary significantly between any samples of locations A and B, which are geographically very close, while they are significantly different from all other samples except *B. emperador* from location G(Rio Ukupti) ($p<0.05$) (Tab.1.a). Variation along RW2 was revealed between the sample of Astyanax in location D and Bryconamericus from A, B and H locations ($p<0.05$) (Tab.1.b). Values of RW3 varied significantly between the sampled Astyanax from location A and sampled Bryconamericus from locations B and H ($p<0.05$) (Tab.1.c.).

a.

Sample	A.Ast	A.Bam	B.Ast	B.Bam	C.Ast	D.Ast	E.Ast	F.Ast	G.Bam	H.Bam
A.Ast	---	ns	ns	ns	***	***	***	***	ns	***
A.Bam	---	---	ns	ns	***	***	***	***	ns	***
B.Ast	---	---	---	ns	***	***	***	***	ns	***
B.Bam	---	---	---	---	***	***	***	***	ns	***
C.Ast	---	---	---	---	---	ns	*	ns	***	***
D.Ast	---	---	---	---	---	---	**	ns	***	***
E.Ast	---	---	---	---	---	---	---	***	***	***
F.Ast	---	---	---	---	---	---	---	---	***	***
G.Bam	---	---	---	---	---	---	---	---	---	***
H.Bam	---	---	---	---	---	---	---	---	---	---

Note: Bam = *B. emperador*, Ast = *A. aeneus*

ns = not significant ($p > 0.05$), * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

b.

Sample	A.Ast	A.Bam	B.Ast	B.Bam	C.Ast	D.Ast	E.Ast	F.Ast	G.Bam	H.Bam
A.Ast	---	ns	ns	ns	ns	ns	ns	ns	ns	ns
A.Bam	---	---	ns	ns	ns	**	ns	ns	ns	ns
B.Ast	---	---	---	ns	ns	ns	ns	ns	ns	ns
B.Bam	---	---	---	---	ns	**	ns	ns	ns	ns
C.Ast	---	---	---	---	---	ns	ns	ns	ns	ns
D.Ast	---	---	---	---	---	---	ns	ns	ns	*
E.Ast	---	---	---	---	---	---	---	ns	ns	ns
F.Ast	---	---	---	---	---	---	---	---	ns	ns
G.Bam	---	---	---	---	---	---	---	---	---	ns
H.Bam	---	---	---	---	---	---	---	---	---	---

Note: Bam = *B. emperador*, Ast = *A. aeneus*

ns = not significant ($p > 0.05$), * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

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c.

Sample	A.Ast	A.Bam	B.Ast	B.Bam	C.Ast	D.Ast	E.Ast	F.Ast	G.Bam	H.Bam
A.Ast	---	ns	ns	*	ns	ns	ns	ns	ns	*
A.Bam	---	---	ns	ns	ns	ns	ns	ns	ns	ns
B.Ast	---	---	---	ns	ns	ns	ns	ns	ns	ns
B.Bam	---	---	---	---	ns	ns	ns	ns	ns	ns
C.Ast	---	---	---	---	---	ns	ns	ns	ns	ns
D.Ast	---	---	---	---	---	---	ns	ns	ns	ns
E.Ast	---	---	---	---	---	---	---	ns	ns	ns
F.Ast	---	---	---	---	---	---	---	---	ns	ns
G.Bam	---	---	---	---	---	---	---	---	---	ns
H.Bam	---	---	---	---	---	---	---	---	---	---

Note: Bam = *B. emperador*, Ast = *A. aeneus*
ns = not significant ($p > 0.05$), * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

Table 2.
a. Results of post-hoc Tukey tests for RW1 of all samples
b. Results of post-hoc Tukey tests for RW2 of all samples
c. Results of post-hoc Tukey tests for RW3 of all samples

Thus, A. *aeneus* from Pipeline Road (locations A & B) differ in morphology (shallower body) from those in all other locations. Similarly, *B. emperador* from Pipeline Road also differ in morphology (shallow body) from its conspecifics in all other sampled locations, except in location G. With the exception of *B.emperador* from location G, both samples of both species from Pipeline Road resemble each other more (in body depth) than they do any other sample for any other location, including conspecifics. These results indicate that (1) fish from different locations differ morphologically in terms of body depth and (2) samples from the same location can be more similar among themselves than to samples of conspecifics from different locations.

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DISCUSSION

In this paper we assessed the relative impacts of shared selective pressures and unique responses on ecomorph morphology of two similar species. To do so we compared the morphology of these species between and among samples multiple locations. We hypothesized that shared environmental pressures would transcend species differences. Our results support this hypothesis; we found that samples from the same location, regardless of species, were more similar to each other along one axis of variation (RW1) than all but one other sample (*B. emperador* from location G, Ukupti).

How does such morphological similarity occur? It is widely held that convergent evolution of traits in unrelated species (as well as the parallel evolution of traits in related species such as *A. aeneus* and *B. emperador*) is the result of shared environments such as the ecological niche (Diaz et al, 1998, Lawlor , 1976). This suggests that our study species coexist in the same niche. The opposite situation, competitive exclusion, leads to niche partitioning and character displacement of similar species (Brown & Wilson, 1956). This leads to the question of how *A. aeneus* and *B. emperador,* which have similar diets, could occupy the same niche when food is one of the most important niche dimensions driving niche partitioning (Sabagh & Carvalho-e-Silva 2008, Schoener, 1974). Successful occupation of a single niche by similar species that would normally compete for resources is possible when intraspecific competition is more important than interspecific competition (Armstrong & McGehee 1980). Esteves's study showed that the niche overlap between similar species varies in response to food availability, where food scarcity translates to reduced overlap, and abundance allows for increased overlap (Esteves, 1995). Thus, an abundance of food resources would permit coexistence of these two species in

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the same ecological niche. It would be interesting to evaluate similarity of cohabiting species in relation to resource abundance. We would predict morphological similarity in high resource environments, and divergence in cases of scarce resources. Scarcity of resources would result in inter-specific competition being more important that intraspecific competition, ultimately resulting in competitive exclusion, or niche partitioning.

Another factor driving and maintaining niche coexistence is schooling behavior. The formation of schools is a widespread behavioral phenomenon in fish, with over 50% of fish species partaking in it (Viscido et al., 2004). Belonging to large swimming groups can provide a number of advantages to the individual including improved defense and avoidance of predators (Viscido and Weathley, 2002, Morgan and Godin, 1985). Antipredator benefit is achieved by reducing the risk of attack on any one individual, because the proportion of individuals that fall prey to a predator's attack is smaller as the group itself gets larger, assuming risk of detection does not increase disproportionately with an increase in group size. Interestingly, Characid schooling can involve more than one species (Parzefall, 1993, Bussing, 2002). If this is the case with *A. aeneus* and *B. emperador*, then both species would benefit from being morphologically similar to each other, 'mimicking' each other, since an individual's risk of predation decreases as it resembles its peers (Viscido et al., 2004). Thus schooling behavior might drive the development of morphological similarity.

While schooling behavior drives morphological similarity, morphological traits are the result of environmental pressures. Pressures that could influence the distinguishing trait of body depth in our samples are (1) water velocity (Langerhans, 2008) and (2) predation (Eklov & Jonnson, 2007) (3) foraging strategy (Toline & Baker 1993). Where body depth influences swimming (deep bodies allow burst/unsteady swimming and shallow bodies allow steady

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swimming), all factors affect body depth because they influence which type of swimming is optimal (steady vs. unsteady). Water velocity has an effect on body depth, where shallow bodies are advantageous in fast-flowing water, and individuals benefit from having deeper bodies in high-velocity currents (Langerhans, 2008). Our results did not prove to be consistent with this prediction, as deeper bodied fish were found in faster flowing rivers such as Mamoni (location C) and Chacunaque (location F). However, we did not collect sufficient data about predation and foraging strategy to examine their effect on body depth.

With regards to predation, many fish predators are gape-limited (i.e. limited by how wide they can open their mouth) and can't predate on deep-bodied fish, which therefore have an advantage in environments of high predation (Eklov & Jonnson, 2007).

Similarly, different body depths are best suited to different foraging strategies; catching faster prey involves ambushes and sprints and requires a deeper body, while a fish with slower prey can have a wider foraging range, with the shallower body that allows for prolonged swimming (Toline & Baker 1993).

While the there is a general agreement about the ecological process that results in convergent evolution, this can be reflected in a number of physiological processes. Morphological variation can be driven by two things: genetic divergence and/or phenotypic plasticity. Measuring the effects of each process would require a common-garden experiment (Sharpe, 2008). Tracking morphological change in the offspring of different ecomorphs in different rearing environments would lead to a better understanding of the interaction between genetic divergence and phenotypic plasticity. If on the one hand, individuals reared in a different environment from their parental generation had the same morphology, this would indicate a

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strong genetic component to morphological features. However, this does not allow to distinguish between the effects of natural selection and genetic drift.

The opposite result, where individuals raised in a new environment displayed morphology characteristic of native individuals, would suggest phenotypic plasticity was at play. More often than not, morphology results from both factors, wherein certain traits are more plastic, while others are more genetic. Moreover, phenotypic plasticity is a trait in itself, which can be advantageous in environments with variable conditions (Via & Lande, 1985).

Variation in body depth proved to explain the largest percentage of variation among all samples (28.3%) while the second (pelvic fin insertion) and third (relative head/tail position) axes represented 12.31% and 10.77% of total variation respectively. However, it is important to note that while Tukey tests yielded significant differences between at least two locations for all three relative warps, body depth variation revealed the most striking geographic patterns. This result can bring to light which traits are subject to selective environmental pressures. The variation found for traits described by RW2 and RW3, while explaining a relatively large proportion of variation among all samples, did not elucidate obvious geographic patterns among the study sites under investigation. From such a result, it may be hypothesized that body depth variations could be the subject to regional environmental pressures while fin insertion position and relative head/tail position are not and thus free to vary among the population regardless of location. Alternatively it could be said that features described by RW2 and RW3 are subject to the same environmental pressures.

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LIMITATIONS

Our findings would be more robust had both species been present in more study sites. This would have allowed us to draw more general conclusions about the ecological dynamics of the species. As it stands, having only two similar sites that have both species, we have insight on the dynamics of those particular locations, but can only make hypotheses concerning species-wide interactions. Additionally, a common garden experiment is necessary to tease apart heritability and plasticity of the traits we have studied. Finally, it would be ideal for this study would be replicated across many more study sites, and ultimately with different species. This was not possible because of time and technical constraints.

CONCLUSION

The goal of our research is to determine whether environmental pressures could transcend species barriers, causing in sympatric individuals of *A.aeneus* and *B emperador* to be more similar to each other than to their allopatric conspecifics. Results indicate that they can, and we propose this is a result of several ecological processes. We hypothesize both species can cohabit the same niche, thus experiencing the same environmental pressures. Moreover, their behavior of schooling together means that it is advantageous for them to be morphologically similar.

LESSONS LEARNED

This project presented us with many opportunities to expand our skills as researchers, specifically developing resourcefulness and organization in the planning of independent research projects. Learning geometric morphometric technique contributed to developing rigorous analytical skills. Moreover, work in the field gave us a better idea of the constraints in time and material involved in applied work in biology. It also taught us patience and tenacity. Life skills.

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ANNEX

a.

Group	Sample size	Mean	Standard Deviation	Error of Mean	Median
A.Ast	31	0.009914	0.01656	0.002975	0.0111
A.Bam	41	0.01339	0.01621	0.002532	0.0152
B.Ast	34	0.008879	0.01721	0.002951	0.00763
B.Bam	68	0.01623	0.01231	0.001493	0.01665
C.Ast	33	-0.03131	0.01348	0.002347	-0.0334
D.Ast	31	-0.03451	0.01421	0.002553	-0.0348
E.Ast	30	-0.01839	0.01563	0.002854	-0.01885
F.Ast	33	-0.03619	0.01396	0.002431	-0.0362
G.Bam	36	0.01233	0.01491	0.002485	0.0106
H.Bam	32	0.0357	0.01607	0.00284	0.034

b.

Group	Sample size	Mean	Standard Deviation	Error of Mean	Median
A.Ast	31	0.000853	0.01584	0.002845	0.000771
A.Bam	41	-0.00465	0.01644	0.002568	-0.00939
B.Ast	33	0.001232	0.01981	0.003448	0.000918
B.Bam	68	-0.0028	0.0225	0.002729	-0.00743
C.Ast	33	0.000236	0.01732	0.003014	-0.00342
D.Ast	31	0.01219	0.01749	0.003142	0.014
E.Ast	30	-0.00022	0.01452	0.002652	0.000154
F.Ast	33	0.001713	0.01997	0.003476	0.00366
G.Bam	36	-0.00066	0.01765	0.002942	0.000105
H.Bam	32	-0.00277	0.009563	0.001691	-0.00331

c.

Group	Sample size	Mean	Standard Deviation	Error of Mean	Median
A.Ast	31	-0.00717	0.01253	0.00225	-0.00551
A.Bam	41	-0.00198	0.01431	0.002236	-0.00104
B.Ast	34	-0.00234	0.02112	0.003622	-0.00112
B.Bam	68	0.004453	0.01866	0.002262	0.003685
C.Ast	33	-0.00129	0.01523	0.00265	-0.00291
D.Ast	31	-0.00516	0.02217	0.003981	-0.00696
E.Ast	30	0.000491	0.01644	0.003002	0.000888
F.Ast	33	0.001347	0.01448	0.002521	0.000916
G.Bam	36	-0.00022	0.01665	0.002776	0.002385
H.Bam	32	0.007227	0.01147	0.002027	0.008835

Table 3. Summary stats for RW1 (a), RW2 (b) and RW3 (c).

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