



**Induced Morphological Changes in Native Prey  
Species Following the Introduction of *Cichla  
monoculus***

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# **Induced Morphological Changes in Native Prey Species Following the Introduction of *Cichla monoculus***

Ilke Geladi and Rachael Ryan

Host Organization: STRI, Diana Sharpe  
*Naos Laboratories, Torchin Lab*

As one of the leading research institutions in the world, the Smithsonian Tropical Research Institute is dedicated to understanding biological diversity in the tropics; more specifically the Torchin Lab works in the fields of marine ecology, disease and parasite ecology and invasion biology.

Humans have become the driving force of environmental change at local and global scales, altering habitats and threatening biodiversity of ecosystems. Ecosystems are essential for human life as they provide services such as drinkable water, food, and high levels of biodiversity which are important to increase ecosystem resilience to environmental changes. One of the major causes of loss of biodiversity is the introduction of non-native species. Invasive species are free from natural predators and parasites in their new habitat, and therefore can have a great impact on the native biodiversity. Conservation of biodiversity is important to sustain ecosystems, and thus there is interest in researching how adaptable natural populations are to anthropogenically-induced disturbances, and whether contemporary evolution and phenotypic plasticity can lead to the recovery of disturbed species after an invasive species is introduced.

In Lake Gatún, the piscivorous predator fish *Cichla monoculus*, or the Peacock Bass, was introduced in 1967 and decimated the native prey fish populations. Two prey species have persisted to the present day, *Astyanax ruberrimus* and *Roeboides guatemalensis*. This project explores whether these prey species have undergone specific morphological changes in response to predation by the Peacock Bass in Lake Gatún. Eye size, caudal fin area, caudal fin spot size and intensity, maximum depth, depth at operculum, and general body shape are investigated as each feature is predicted to change in a specific way to avoid predation.

Two different comparisons were made for each genus of fish. The first is a temporal comparison between *A. ruberrimus* and *R. guatemalensis* populations in Lake Gatún in 1935 before the introduction of the Peacock Bass and Lake Gatún present day. The second comparison is between contemporary populations of similar species, *Astyanax fasciatus* and *Roeboides occidentalis*, in Lake Bayano, a lake without Peacock Bass, and the aforementioned species in Lake Gatún. Specimens from 1935 had been previously photographed at the Smithsonian in Washington. Modern specimens had been caught in the field within the last three years and stored in ethanol. Photographs were taken of all modern specimens, and a database of photos and accompanying information, if the fish were previously unidentified, was compiled. Geometric morphometric analysis was performed using TPSDig2, and the results analyzed in RStudio using ANOVAs in order to visualize and detect changes in body shape between populations. Trait measurements were done in ImageJ and analyzed in RStudio using ANCOVAs.

The results of the trait measurements produced a mixture of expected and unexpected results. Between lakes, Lake Bayano populations are bigger (greater maximum and operculum depths) than Lake Gatún populations of *Astyanax* and *Roeboides*. Furthermore, *Astyanax* in Lake Bayano have bigger caudal spots, more intense caudal spots, bigger eye sizes, and bigger caudal fins. All of the morphological differences between the populations in the two lakes are contrary to predictions, except for the decrease in eye size, suggesting that other factors besides predation are putting selective pressures on the populations. The temporal comparisons yielded only one significant morphological change between past and present populations of *A. ruberrimus*: a

decrease in body depth at operculum over time. Between past and present populations of *R. guatemalensis* in Lake Gatún, the present population has a greater body depth, however the past populations have bigger caudal fins, caudal fin spots, and more intense caudal fin spots. The only predicted change was the increased depth, the other morphological changes were unexpected.

Geometric morphometric analysis revealed some significant changes in body shape, most noticeably populations of *A. ruberrimus* and *R. guatemalensis* in present day Lake Gatún have slightly smaller heads and deeper caudal regions, a body pattern strategic for faster burst speeds. This is possible evidence for morphological adaptations to avoid predation.

It is evident from this study that there are morphological differences between populations of *Astyanax* and *Roeboides* across temporal and spatial scales, however these changes cannot directly be attributed to the presence of the Peacock Bass. There are many other factors to consider such as habitat structure, abiotic factors, the adaptations associated with going from riverine species to lake species, and different kinds of adaptations to predation (life-history traits) that may weaken selection for advantageous morphological traits. Contemporary evolution has occurred in these fish species, however the direction and selective pressures it is responding to are not well understood. A lesson from this study is that the interactions and different factors working in an ecosystem are complex and it is difficult to isolate a single predator-prey system. Integration of current studies looking at other features of these specific prey fish would be useful to develop a better understanding of how species are able to survive after the introduction of such a dominant predator.

## **Cambios Morfológicos inducidos en especies de presas nativas después de la introducción de *Cichla monoculus*.**

Ilke Geladi y Rachael Ryan

Organización anfitriona: STRI, Diana Sharpe  
*Laboratorio Marino de Naos, Laboratorio Torchin*

Como una de las principales instituciones de investigación en el mundo, el Instituto Smithsonian de Investigaciones Tropicales se dedica a enriquecer el conocimiento sobre la biodiversidad biológica de los trópicos; en particular el Laboratorio Torchin trabaja en el campo de ecología marina, ecología de enfermedades y parásitos, y la biología de invasiones.

Humanos se han convertido en la fuerza principal de cambios ambientales a la escala local y ambiental, alterando hábitats y amenazando la biodiversidad de distintos ecosistemas. Ecosistemas son esenciales para la vida humana ya que proveen servicios como agua potable y comida. La biodiversidad es importante para incrementar la resiliencia del ecosistema hacia cambios en el ambiente. Una de las causas principales de la pérdida de la biodiversidad es la introducción de especies no-nativas. Especies invasoras no son amenazadas por depredadores naturales o parásitos en su nuevo hábitat, y así pueden tener un gran impacto sobre la biodiversidad nativa. Ya que la conservación de la biodiversidad es importante para sostener ecosistemas, hay interés en hacer investigaciones acerca de la adaptabilidad de poblaciones hacia perturbaciones inducidos por causas antropogénicas, y si la evolución contemporánea y la plasticidad fenotípica pueden ayudar la recuperación de las especies nativas que han sido perturbadas después de la introducción de una especie invasiva.

En el lago gatún, el pez depredador piscívora *Cichla monoculus*, o el sargento, fue introducido en 1967 y diezmo la población nativa de peces. Dos especies nativas todavía persisten hasta hoy en día: *Astyanax ruberrimus* y *Roeboides guatemalensis*. Este proyecto explora si estas especies de presa han experimentado cambios morfológicos específicos en respuesta a la depredación de *C. monoculus* en el lago gatún. Investiga el tamaño de ojo, la área de la aleta caudal, el área de la mancha en la aleta caudal, la profundidad máxima del cuerpo, profundidad del opérculo y la forma general del cuerpo ya que es esperado que cada característica haya cambiado de una manera específica para mejor escapar la depredación.

Dos distintas comparaciones fueron hechas para cada genii de pez. La primera fue una comparación sobre tiempo entre poblaciones *A. ruberrimus* y *R. guatemalensis* en el lago gatún de 1935, antes de la introducción de *C. monoculus*, y de poblaciones contemporáneas. La segunda comparación fue entre las especies contemporáneas del lago gatún y especies similares en lago bayano, *Astyanax fasciatus* y *Roeboides occidentalis*, en donde no se encuentra el depredador *C. monoculus*. Los especímenes de 1935 fueron fotografiados en el Smithsonian en Washington. Los especímenes contemporáneas fueron capturados en el campo dentro de los últimos tres años y guardados en ethanol. Fotografías fueron tomadas de todas los especímenes contemporáneas y una base de datos de fotos junto con información sobre los peces fue compilada. Un análisis de morfometría geométrica fue hecha usando TPSDig2, y los resultados fueron analizados en RStudio usando ANOVAs para visualizar y detectar cambios en la forma del cuerpo entre poblaciones. Las medidas de los rasgos fueron hechas usando ImageJ y analizadas en RStudio usando ANCOVAs.

Los resultados de las medidas de los rasgos produjeron una mezcla de resultados esperados y no esperados. Entre los lagos, las dos poblaciones, *Astyanax* y *Roeboides*, del lago

bayano son más grandes (una más grande profundidad máxima del cuerpo y profundidad del opérculo) que los del lago gatún. Es más, *Astyanax* en lago bayano tiene manchas en su aleta más grandes y más marcadas, ojos más grandes y aletas caudales más grandes que *Astyanax* en lago gatún. Todas las diferencias morfológicas entre las poblaciones de los dos lagos son lo contrario a lo que fue esperado, menos la reducción en el tamaño de ojo, lo cual sugiere que otros factores, además de la depredación, están actuando como una presión selectiva sobre las poblaciones. Las comparaciones sobre tiempo produjeron solamente un cambio morfológico que fue significativo entre las poblaciones de *A. ruberrimus* de 1935 y del presente. Contrario a lo esperado, la profundidad del opérculo ha disminuido en esta especie sobre tiempo. Entre las poblaciones de *R. guatemalensis* del pasado y presente en el lago gatún, la población del presente tiene el cuerpo más profundo, pero las poblaciones de 1935 tienen aletas caudales más grandes y manchas caudales más grandes e intensas. El único cambio que fue esperado era el aumento en la profundidad del cuerpo en la población contemporánea de *Roebooides* en lago gatún, los demás cambios morfológicos fueron inesperados.

El análisis de la geometría morfométrica reveló algunos cambios significantes en la forma del cuerpo de estos peces. Más notablemente, las poblaciones *A. ruberrimus* y *R. guatemalensis* del lago gatún de hoy en día tienen cabezas más pequeñas y una región caudal más profunda, lo cual es un modelo de cuerpo estratégico para una descarga más rápida para escapar de su depredador. Es posible que esto sea evidencia para adaptaciones morfológicas para mejor escapar la depredación.

Es evidente de este estudio que hay diferencias morfológicas entre las poblaciones de *Astyanax* y *Roebooides* a través del tiempo y entre lugares. Sin embargo, estas diferencias no pueden ser directamente atribuidas a la presencia del depredador *C. monoculus*. Hay muchos otros factores que considerar como la estructura del hábitat, parámetros físicos, las adaptaciones necesarias para completar la transformación de ser una especie ribereña a una especie de aguas abiertas y diferentes tipos de adaptaciones a la depredación (e.j. rasgos reproductivos) que pueden debilitar la selección para rasgos morfológicos que sean ventajosos. La evolución contemporánea ha ocurrido en estas especies de peces, sin embargo, la dirección y las presiones selectivas a las que responden estos rasgos, no son bien comprendidos. Una lección de este estudio es que las interacciones y factores diferentes que trabajan en un ecosistema son complejos y es muy difícil de aislar un sistema en donde hay un solo depredador. La integración de los estudios corrientes que estudian otros aspectos de estos peces en específico, sería útil para desarrollar una mejor entendimiento de como especies sobreviven después de la introducción de un depredador tan dominante.

## HOST INSTITUTION

The Smithsonian Tropical Research Institute (STRI) is a bureau of the Smithsonian Institution based outside of the United States of America and is dedicated to further understanding biological diversity in the tropics. It began in 1923 as a small field station and is now one of the leading research institutions in the world. Within Panama itself, the institute encompasses 11 principal research stations all over the country including 3 marine facilities on both the Pacific and Atlantic coasts. Although STRI is based in Panama, STRI's scientists are conducting research in over 40 different tropical countries around the world. STRI employs about 40 permanent staff members and dozens of postdoctoral fellows and associates as well as hosts about 900 visiting scientists and student each year.

STRI's facilities provide a unique opportunity for long-term ecological studies in the tropics which enables in-depth investigations which attract many elite scientists and visitors. One of the most remarkable conservation sites in the world is Barro Colorado Nature Monument (BCNM) which includes 5,600 hectares of conserved forest and wildlife. Sites like these have allowed for long-term projects such as the 50-hectare permanent tree plot which was established in 1980 and a census is taken every 5 years to study normal forest dynamics as well as extreme events such as global climate change and El Niño.

Diana Sharpe, our supervisor, is a Fonds québécois de recherche sur la nature et les technologies (FQRNT) Postdoctoral Research Fellow at STRI. She is a biologist whose interests include aquatic ecology and conservation, contemporary evolution, fisheries-induced evolution, fisheries management, invasive species ecology and life history evolution in fishes. Her current research consists of 2 main investigations. The first is a study of long-term ecological and



evolutionary consequences of the introduced Peacock Bass in Panama. The second is a study of trophic ecology and functional morphology of weakly-electric fishes in Panama.

## **Introduction**

### **BIODIVERSITY: IMPORTANCE & THREATS**

In the age of the Anthropocene, humans have become the driving force for environmental change on a global scale. Many people are disconnected from the idea that humans are responsible for ecological changes and resource depletion through the actions of pollution, habitat destruction, climate change, and the introduction of non-native species (Holdren & Ehrlich, 1974). Common misconceptions the general population hold are that the growth of the human population is unrelated to escalating ecological problems, and that science and technology can enable the rapid consumption of natural resources without consequences (Holdren & Ehrlich, 1974). Additionally, people often do not associate local environmental problems with global change. The connection between global change and small scale environmental issues is important to recognize because no environmental impacts are isolated.

This is especially true when looking at ecosystems around the world. Ecosystems are communities of organisms that interact with each other and their physical environment (Töpfer et al, 2000). Ecosystems provide essential services for human life, however this reliance on them is not reflected in the treatment and understanding of them. Freshwater ecosystems are an important ecosystem as they provide drinking water, as well as a food supply in the form of freshwater fish. Unfortunately, 20% of the world's freshwater species have become extinct, endangered, or threatened due to human impacts (Töpfer et al, 2000). High levels of biodiversity are crucial for ecosystems because having more species creates more linkages and interactions, which influences ecosystem stability (Töpfer et al, 2000). Biodiversity can increase an ecosystems resilience to stresses such as climate change, which directly feeds back to the human populations that rely on them, whether directly or indirectly (Töpfer et al, 2000).

The key role that biodiversity has in protecting ecosystems worldwide makes conservation of species a priority. Conservation is only possible with baseline studies and long term records of systems to establish what is natural, and how to best manage these systems (Willis & Birks, 2006). It is especially important to monitor long-term impacts of rapid changes to ecosystems in order to understand how certain species and systems react to changes and what to do to maintain and manage impacted populations.

Biologists are interested in populations after ecological disturbances because they often result in selection pressures that lead to rapid contemporary evolution (Hendry & Gonzalez, 2008). An anthropogenic disturbance such as climate change or the introduction of a non-native species shifts the phenotypic optimum for a species, effectively shifting the adaptive peak; thus, the mean phenotype for the population is no longer well adapted and there is a sharp initial decrease in the population fitness (Hendry & Gonzalez, 2008; Palkovaks *et al.*, 2011). Studies have documented the trajectories of phenotypic change after an environmental change and have shown that as the adaptive peak for a population shifts in the new environment, there is a strong selection gradient that drives rapid phenotypic change in the direction of the new adaptive peak (Hendry & Gonzalez, 2008). Many cases have shown that after an environmental disturbance caused by humans, the shift in adaptive peak through contemporary evolution or phenotypic plasticity has led to human-induced trait change (Palkovaks *et al.*, 2011). Human-induced trait change is starting to be monitored, however there is still little attention paid to managing the ecological consequences of it (Palkovaks *et al.*, 2011). Contemporary evolution thus can be a mechanism for populations to recover from environmental disturbances and adapt to be able to survive in the new environment (Hendry & Gonzalez, 2008). Herein lies the importance of

contemporary evolutionary studies, to be able to answer the question of how adaptable natural communities are to human impacts.

## BIOLOGICAL INVASIONS

Human impacts have altered ecosystems across the globe, with the most significant threat to indigenous biota being introduced exotic species (Hall and Mills, 2000). A definition of introduced species, which includes both non-indigenous and exotic species, is “any species intentionally or accidentally transported and released by man into an environment outside its native range” (Welcomme, 1998). Worldwide, the introduction of non-native species has dramatically altered many natural ecosystems. However, it is debated whether these introduced species pose a real threat to their introduced habitats or if they provide more societal benefits (Vitule *et al.*, 2009). It is argued that intentional fish transplantations are beneficial to the aquaculture trade, sport fishing interests and that fish with a high market value and a historical record of little to no ecological impact should be supported for further introductions (Gozlan, 2008). On the other hand, it is argued that there have been many occasions where introduced species have had a detrimental impact on the resulting environment whether it be through habitat degradation, competition with native species for spawning grounds, hybridization threatening species integrity or predation on native prey species thus changing food web dynamics and food availability (Vitule *et al.*, 2009).

Invasive species and their benefits or consequences are of great importance to conservation biologists who try to predict and manage the structure of specific biological communities (Carroll, 2007). Mitigating impacts of invasive species are thus important for conservation. Through an analysis of models of species distribution, it was found that reducing

impacts of invasive species is the most beneficial management option for the conservation of biodiversity (Conlisk *et al.*, 2013).

When species are introduced, the successfulness of this species to colonize its new habitat can range from being a failure (e.g., brown trout introduction into Lake Titicaca) to actually being successful such as the introduction of the Nile Perch in Lake Victoria (Hall & Mills, 2000). It is important to keep in mind that exotic species in large lakes or areas of the world are not limited to larger animals such as fish and mammals, but plants, invertebrates, pathogens and parasites are also part of the web of introduced species. Exotic, or invasive, species are introduced for a variety of reasons which Hall and Mills (2000) divided into 6 major categories: (1) Sports or recreation; (2) Aquaculture; (3) Ecological Manipulation; (4) Control of unwanted organisms; (5) Ornaments and (6) Accidental Transfers.

The impacts of invasive species on native species can be wide-ranging and significant. They do not only have direct and indirect effects on the structure of aquatic and/or terrestrial ecosystems, they also have economic impacts, can impact food supply and human health (Hall & Mills, 2000). Ecological impacts of introduced species include habitat alteration, competition with native prey species and predation of native prey species, the introduction of new pathogens and diseases the native population might be susceptible to, and hybridization and the deterioration of the gene pool (Hall & Mills, 2000). This directly relates to the idea that invasive species are homogenizing earth's biota. For example, for plants, exotic species typically represent only 10-30% of the species composition of most regions, but within each region they may comprise 90% or more of the plant biomass (Drake *et al.*, 1989). Socio-economic impacts are both long-term and short-term including (1) the costs of prevention and control, (2) economic costs and benefits and (3) sociological impacts (Hall and Mills, 2000). Freshwater ecosystems,

especially lakes, are impacted more than terrestrial ecosystems by introduced species as species are introduced intentionally (for example, fish stocking) but also often unintentionally through ballast water of ships (Sala *et al.*, 2000). As a result of these introductions, freshwater biodiversity is declining faster on a global scale than affected terrestrial systems (Sala *et al.*, 2000).

#### PREDATOR INTRODUCTIONS: DRIVERS OF RAPID PHENOTYPIC CHANGE?

Of all biological introductions, those of novel top predators tend to have the strongest ecological impacts (Vega-Trejo *et al.*, 2014). Furthermore, they can expose native prey to novel selection regimes and thus drive contemporary phenotypic changes. Predators can alter selection on prey populations, whether it be through lethal or non-lethal interactions, driving evolutionary change and testing the limits of phenotypic plasticity (Vega-Trejo *et al.*, 2014). In response to increased predation pressures often exerted by introduced species, the morphology of prey populations is often adapted to allow for a quicker and more efficient escape response in hopes of enhancing survivorship. In addition to influencing the morphology of their prey, predators can also influence the evolution of behaviour, coloration, physiology and life-history traits such as timing and size of maturation, the number and size of offspring and the amount of energy invested in reproduction (Vega-Trejo *et al.*, 2014). This may result in phenotypic differentiation among populations of the same species depending on the predation pressures exerted on that population.

For example, a comprehensive study on an introduced predator, the Nile Perch (*Lates niloticus*), as a stressor on native fish prey, the African cyprinid fish (*Rastrineobola argentea*), was conducted in the basin of Lake Victoria, Africa (Sharpe *et al.*, 2012). Lake Victoria, the

largest tropical lake in the world, has experienced a dramatic decline in many native prey species and the extinction of hundreds of its endemic haplochromine cichlids as a result of multiple anthropogenic stressors including overfishing, eutrophication and the introduction of several non-native fish species, most notably, the predatory Nile Perch (Sharpe *et al.*, 2012). Contrary to expectations, the small pelagic cyprinid *Rastrineobola argentea* has thrived in this new environment. It was found that in lakes where the Nile Perch was present, *Rastrineobola argentea* were smaller in mean body size, matured at smaller sizes and had increased reproductive efforts (Sharpe *et al.*, 2012). This provides support to the idea that the introduction of non-native predators, in addition to other anthropogenic factors such as commercial fishing, are important drivers for contemporary life history and morphological changes in native prey populations.

In general, the major question for native prey species following the introduction of a new predator is thus whether evolution can protect populations from extinction through morphological and behavioural antipredator adaptations (Carroll, 2007). Identifying the key traits that contribute to fitness and their response to selection, as well as testing the limits of plasticity and genetic adaptation, are instrumental in understanding how to manage species populations (Hendry *et al.*, 2008). Our research sought to address these broad questions, using the introduction of the predatory Peacock bass, *Cichla monoculus*, as a model system.

## PEACOCK BASS: AN INTRODUCED PREDATOR IN PANAMA

The introduction of the Peacock Bass (*Cichla monoculus*) in 1967 into Lake Gatún in Panama, has affected the ecosystem in and around the lake. In 1973, Zaret and Paine conducted a study on the shifts in the food web of Lake Gatún as a result of this piscivorous predator,

however few studies have followed up on the long-term effects of the introduction of *C. monoculus*.

*C. monoculus* is a strictly piscivorous predator native to the Amazon River and its tributaries and was introduced in Panama to the Chagres River around 1967 (Zaret & Paine 1973). Ineffectively opposed by any natural competitors or predators, it traveled down the Chagres River and effectively spread into Lake Gatún (Zaret & Paine 1973). In 1973, Zaret and Paine found it had effectively eliminated six of the eight previously common fish species and drastically reduced a seventh (Zaret & Paine 1973). It has thus greatly simplified the food web in Lake Gatún. *C. monoculus* most directly affects the secondary consumers which in turn result in second and third order effects throughout other trophic levels.

#### MORPHOLOGICAL ADAPTATIONS IN RESILIENT NATIVE PREY?

In general, our goal is to understand the extent to which *C. monoculus* may be driving adaptive evolution in resilient native prey. Recent fish community surveys show that *Roeboides guatemalensis* and *Astyanax ruberrimus* are the most abundant native prey species that have persisted since the introduction of *C. monoculus* (Sharpe, De Leon, González and Torchin, unpubl. data). Therefore, we asked: what morphological adaptations have these prey species, *R. guatemalensis* and *A. ruberrimus* undergone over time, if any, to survive and mitigate predation by *C. monoculus*?

To investigate this question, we compared the morphology of historical specimens from before the introduction to that of contemporary specimens from Lake Gatún over the past 3 years. Furthermore, we compared morphologies of contemporary specimens from Lake Gatún to contemporary specimens from Lake Bayano, which is a non-invaded lake also in Panama. We examined a suite of morphological characters, including body depth, caudal fin size, eye size,



caudal spot intensity and caudal spot size. We hypothesized that there would be several changes in these traits as a direct effect of native prey species adapting to increase their ability to successfully avoid predation where Peacock Bass is present.

## MORPHOLOGICAL PREDICTIONS

We predicted that body depth of the native prey species would increase as a defense mechanism since *C. monoculus* is a gape-limited predator. A bigger body depth would thus make it harder for this predator to swallow its prey. This expectation is similar to other studies that have been done, such as studies on the crucian carp, where a deep body was induced as a result of the presence of pike (*Esox lucius*), another gape-limited predator (Domenici *et al.*, 2008). Furthermore, it has also been shown that a larger body depth misdirects strikes from the center of mass area thus increasing the probability of the prey escaping (Webb, 1986). Additionally, an increase in body depth has been shown to increase speed, acceleration and the turning rate of an individual (Domenici *et al.*, 2008). This enhances a prey's performance in escape swimming, thus enhancing its ability to evade its predator.

We expected that caudal fin size would increase as larger caudal regions enhance fast-start escape performance, thus reflecting an adaptation for avoiding predation (Langerhans, 2009; Webb, 1977). Studies have shown that the caudal fin is important for propulsion and other general patterns of fin motion and performance (Flammang & Lauder, 2008; Webb, 1977). It is recognized, however, that this is a trade-off since the ability to continue swimming away from predators is reduced since smaller caudal fin sizes are more efficient for long-distance swimming.

Caudal spots, or false eyespots, are dark circles usually surrounded by a lightly coloured ring thought to represent an iris around a pupil, mimicking the appearance of an eye, in this case,

on the caudal fin (Carroll *et al.*, 2004). Although the adaptive significance of false eyespots in prey has been long debated among ecologists, the size of eyespots is plastic and one hypothesis (deflective hypothesis) states that it is used to deflect predators as a diversion technique, drawing the attack of predators to non-vital regions of the body (Carroll *et al.*, 2004). We thus hypothesized that the caudal spot size on *R. guatemalensis* and *A. ruberrimus* would increase in attempt to enhance its chances of detection over the actual eye thus directing attacks away from the head region, and giving the prey species a greater chance to escape (Carroll *et al.*, 2004; McPhail, 1977).

In relation to the size of the caudal spot, we further predicted that the intensity of the colouration of the caudal spot would increase. In previous studies, it was found that predators were more likely to direct their attacks towards conspicuous eyespots (Carroll *et al.*, 2004). A darker colouration would indeed make the caudal fin spot more obvious, thus increasing the attack rate to the caudal region rather than the head, making this a beneficial adaptation for survival.

We further hypothesized that eye size in prey species would decrease. Studies have found that prey from predator treatments have significantly smaller eyes (Carroll *et al.*, 2004). This is because the combined effect of a larger eyespot in the caudal area together with a smaller eye in the head region give the predator the impression that the true eye is present on the posterior of the body, confusing the predator about the orientation of the prey (Carroll *et al.*, 2004).

In terms of overall body shape, a general model for body shape adapted for burst-swimming performance has been developed by Langerhans and can be applied to predict shape changes in prey species after the introduction of a new predator (Langerhans, 2010). Burst-swimming is beneficial for fish that require a fast escape, and it has been proven that a deeper

caudal region, along with a smaller head region, enables a fish to have a faster burst speed (Langerhans, 2010; Walker & Bell, 2000). Thus, we hypothesized that populations from Lake Gatún after the introduction of *C. monoculus* would fit this morphological model better than those before introduction and in Lake Bayano.

This study is important because it seeks to identify morphological adaptations of *A. ruberrimus* and *R. guatemalensis* and thus contribute to a greater understanding of the food web dynamics in Lake Gatún after the introduction of the Peacock Bass. Ongoing studies of swim and escape speeds of the prey fish, as well as studies examining life history trait changes, complement the present project. Most introductions of non-native species are irreversible, and thus the role of adaptation is even more important for native populations as it may be their only option for survival. Integrating all of these studies will make it possible to start understanding how contemporary evolution can lead to species recovery after disturbances, which has implications for conservation of biodiversity in ecosystems worldwide.

## Methodology

### STUDY SITES

Fish were sampled from Lake Gatún and Lake Bayano. Lake Gatún was created in 1910 by damming the Chagres River in order to provide a water bridge for ships crossing the Panama Canal (Hall and Mills, 2000). Introduced in 1967, *C. monoculus* has severely decimated the natural fish populations in the lake. Lake Bayano is a shallow reservoir formed in the 1970s by the damming of the Bayano River as part of a hydroelectric project (Marmulla, 2001). It is the second largest lake in Panama after Lake Gatún, and does not contain *C. monoculus*, although there are non-native marine species and tilapia (Marmulla, 2001).

### SAMPLES

Specimens of *A. ruberrimus* and *R. guatemalensis* collected in 1935 from Lake Gatún, before the introduction of *C. monoculus*, were stored in 70% ethanol in the Smithsonian Natural History Museum in Washington, D.C.. Records of the dates, locations and measurements are available, however the method of capture of *A. ruberrimus* and *R. guatemalensis* specimens was not recorded. Modern samples of *A. ruberrimus* and *R. guatemalensis* from lakes Gatún and Bayano were collected over a span of three years, from 2013 to 2015, and were caught with either gill nets or cast nets. The samples were fixed in 10% formalin and then preserved in 70% ethanol.

### MORPHOLOGICAL ANALYSES

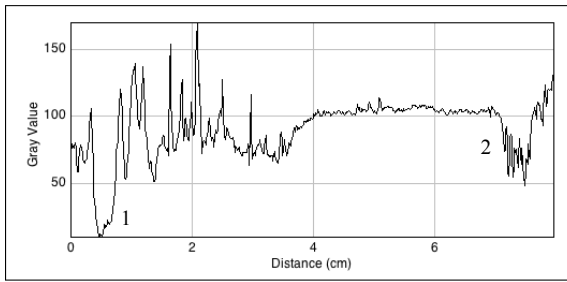
Similar to the methodology used by Langerhans (2009) to study caudal region size in mosquito fish, fish specimens were photographed for analysis. All specimens were dead and had

been preserved in 70% ethanol in order to standardize for historic samples. Photographs of the historic specimens were taken by Sharpe, Giancarlo Gerud and Luis Fernando De Leon in Washington, D.C. using Canon DSLR cameras and a ruler as a reference length. In an effort to replicate the photos of the historical specimens, the same DSLR camera model was used to photograph modern specimens from Lake Gatún and Bayano. Each picture was taken with a macro lens, an ISO of 100, and with a ruler in the frame. Focus was adjusted, as was height of the camera on an upside down tripod, depending on the size of the specimen. All photos and additions to a master database are available for Sharpe and any other researchers for future reference and research.

## IMAGEJ ANALYSIS

The program ImageJ, a tool used for image processing and analysis in java, is useful to measure area on photographs amongst other uses (Carroll *et al.*, 2004). For this study, we used ImageJ to measure the area of the caudal fin size, caudal spot size and eye size. Using the paintbrush tool, the area of interest was simply traced and calculated by the program.

Additionally, we used ImageJ to take a measurement of the caudal spot colour intensity. To do this, a line was drawn from the tip of the nose, through the eye of the fish to the tail of the fish. This is important as the dark coloration of the iris was used in comparison to the colouration of the caudal spot, thus accounting for differences in lighting between photographs. This line was analyzed through a plot profile. From the data points of the plot profile, the average grey value at the downwards eye peak (1) and the average grey value at the downwards caudal spot peak (2) was obtained (Figure 1). The ratio of the average grey values of the eye to the average grey value of the caudal spot was then taken as a measurement for caudal spot colour intensity.

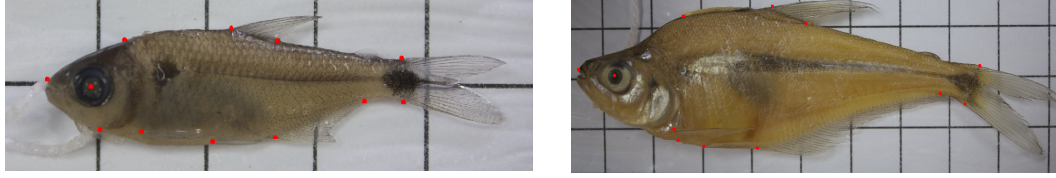


**Figure 1: A plot profile of the average grey values of the caudal fin spot**

## GEOMETRIC MORPHOMETRIC ANALYSIS

Geometric morphometric analysis is beneficial because it can describe and statistically evaluate differences in shape among and between groups of specimens, which is useful for identifying adaptive changes after an ecological disturbance such as the introduction of a non-native species (Cooke & Terhune, 2015). Geometric morphometrics have been used to investigate morphological changes after the introduction of the predator and the descriptive results achieved with this methodology demonstrate its benefits as a tool (Sharpe et al., in review).

The software used to digitize the specimens was TPSDig2 (Rohlf, 2015). There were on average 30 specimens from contemporary Lake Gatún, Lake Gatún in 1935, and contemporary Lake Bayano for both genera. In TPSDig2, we placed 12 landmarks on each fish, and measured standard length, maximum body depth and depth at operculum. Between each genera the landmarks were at the same homologous points, except for the operculum. For *Astyanax* species, a landmark was placed where the head plate ended, and another at the bottom of the head where the operculum joined the head. For *Roeboides* species, due to the unusual and characteristic hump, a landmark was placed where the hump peaked, and then directly below that point on the bottom of the fish. This was measured using right angle and straight line tools to maintain consistency. The landmarks were meant to capture overall body shape, fin positions, and eye position.



**Figure 2: Twelve landmarks placed on homologous places for all *Astyanax* (left) and *Roeboides* (right) specimens.**

The analysis of the shape information started with TPSrelw software, which created a consensus shape for all *Astyanax* and *Roeboides* specimens and calculated relative warp scores for both. The relative warp scores were then imported into Excel, and the relative warp scores that explained the majority of variation were transported into RStudio. Diagrams of the extreme points of the significant relative warps were captured.

## STATISTICAL ANALYSIS

To analyze the data collected from ImageJ and the linear measurements from TPSDig2, an Analysis of Covariance (ANCOVA) for each specific trait was used to compare between groups (Rohlf, 2014). Standard length was used as the covariate for each ANCOVA, and the dependent variable was a measured trait (eye size, caudal fin size, maximum body depth, depth at the operculum, caudal spot size, caudal spot intensity in relationship to eye size). The grouping factor was either lake, when comparing between Bayano and Gatún, or time period, when comparing modern specimens and specimens from 1935. This statistical test was chosen because an ANCOVA uses linear regression to examine the relationship between standard length and the dependent variable, can identify a significant difference of the dependent variable between groups, and test for an interaction between standard length and the grouping variables in their effect on the dependent variable. For a relationship to be significant it must have a p value of less than 0.05. Twenty-four ANCOVAs were performed to test for significant differences in

morphological traits between past and present *A. ruberrimus* and *R. guatemalensis*, and between the different lakes.

To analyze the differences in the major relative warps (defined as explaining greater than 10% variation) between groups, Analyses of Variance (ANOVAs) were performed. An ANOVA was run for each comparison (Lake Bayano and Lake Gatún *Astyanax*, for example) on the major relative warps for *Astyanax* and *Roeboides* to identify any changes in body shape between populations. A p value of 0.05 or less had to be achieved to determine if there were significant deviations of the relative warps from the consensus shape.

## LIMITATIONS

Limitations in this study include time, differences in species, technology, integration of other studies, and difficulties in fieldwork. The time limitations for this study made it difficult to measure a broader variety of morphological traits, such as caudal peduncle area and aspect ratio, both of which could have contributed to our understanding of the adaptations undergone by the native prey species. In addition, there are different species in Lake Bayano and Lake Gatún of both genera, which may have resulted in some variation in morphological traits that were not controlled for in the analyses. Technological problems were encountered when the preferred program for digitization of the fish photographs, Geomorph, did not have the right updates for the computers being used. Alternate software was used, TPSDig2, that was ultimately extremely effective, however many days were spent trying to solve the technological issues, making it a major setback in the project. Finally, the project had a very isolated feeling associated with it. Although this project is meant to be integrated into a larger body of work and contribute to a greater overall understanding, the short time given to the project and the intensive time demands detracted from the amount of collaboration between other current studies, such as the comparison



of life-history traits and the swimming speed analyses being done by other students. Greater collaboration between students would make the projects more consistent and add to the knowledge and understanding of all the students. Lack of familiarity with some of the sites, such as Lake Bayano, also hindered discussion of results and conclusions made from this project. This was an unforeseen limitation, as nets used for fieldwork were damaged and stolen during the first trip to Lake Gatún. Overall this project went smoothly and was a great experience, with time being the most limiting factor.

## ETHICAL CONSIDERATIONS

This project followed any ethical considerations deemed necessary by the McGill Code of Ethics. We euthanized live fish from the field humanely using clove oil, and any fish not required for studies were released on site. No information was taken from human subjects; the entire project was carried out without interaction or intervention from local people. The fish species of interest are not used as food sources for any people around Lake Bayano or Lake Gatún, and the sites fish were collected from were not well known fishing sites, eliminating any interference with human activities.

## Results

### GAÚTN 1935 - GATÚN PRESENT

Trait	Prediction of largest measurement	Specimen that was found to increase	Specimen that was found to increase
		<i>Astyanax ruberrimus</i>	<i>Roeboides guatemalensis</i>
Max. Body Depth	Contemporary	NSD	Contemporary**
Body Depth at operculum	Contemporary	Historical **	NSD
Caudal Fin Size	Contemporary	NSD	Historical*
Caudal Spot Size	Contemporary	NSD	Historical**
Caudal Spot Colour Intensity	Contemporary	NSD	Historical*
Eye Size	Historical	NSD	NSD

**Table 1: Predictions and Results for Historical and Contemporary comparisons**

\*\* = statistically significant

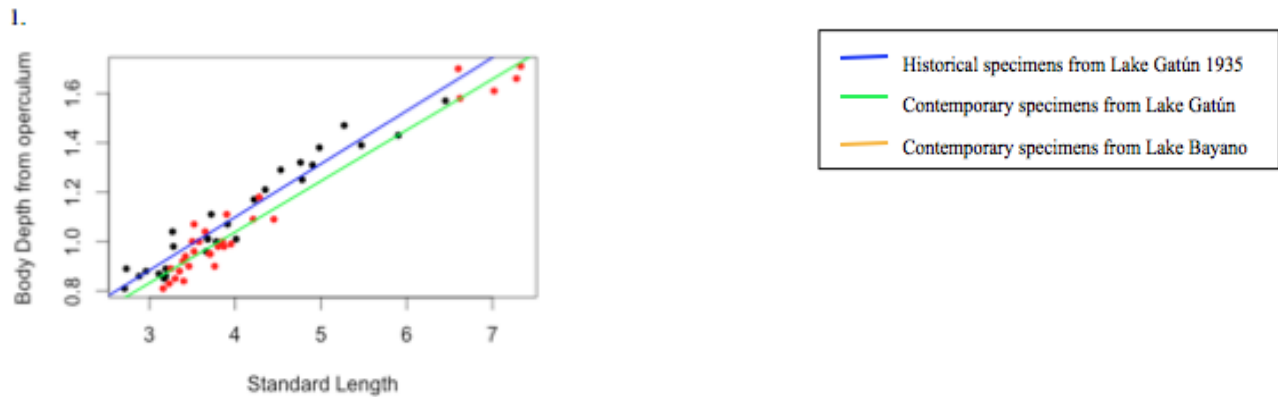
\*= statistically significant, with interaction between groups

NSD= no statistically significant difference

#### *Astyanax ruberrimus*

The comparison between *A. ruberrimus* before and after the introduction of *C. monoculus* yielded only one significant morphological difference. There was a significant difference between time periods for the depth at the operculum ( $F_{1,54}=16.18$ ,  $p=0.00018$ ) without a significant interaction ( $F_{1,54}=0.411$ ,  $p=0.52397$ ). The depth at the operculum was greater in fish from 1935 Lake Gatún compared to contemporary fish from Lake Gatún (See Fig.3, Graph 1).

There was no significant difference between the fish specimens from Lake Gatún 1935 and contemporary Lake Gatún specimens for all other traits: maximum body depth, caudal fin size, caudal spot size, caudal spot colour intensity and eye size.



**Figure 3: Differences between historical and contemporary fish specimens of *Astyanax ruberrimus***

### *Roeboides guatemalensis*

The comparison between *R. guatemalensis* before and after introduction of *C. monoculus* yielded four significant morphological differences, out of the six traits measured.

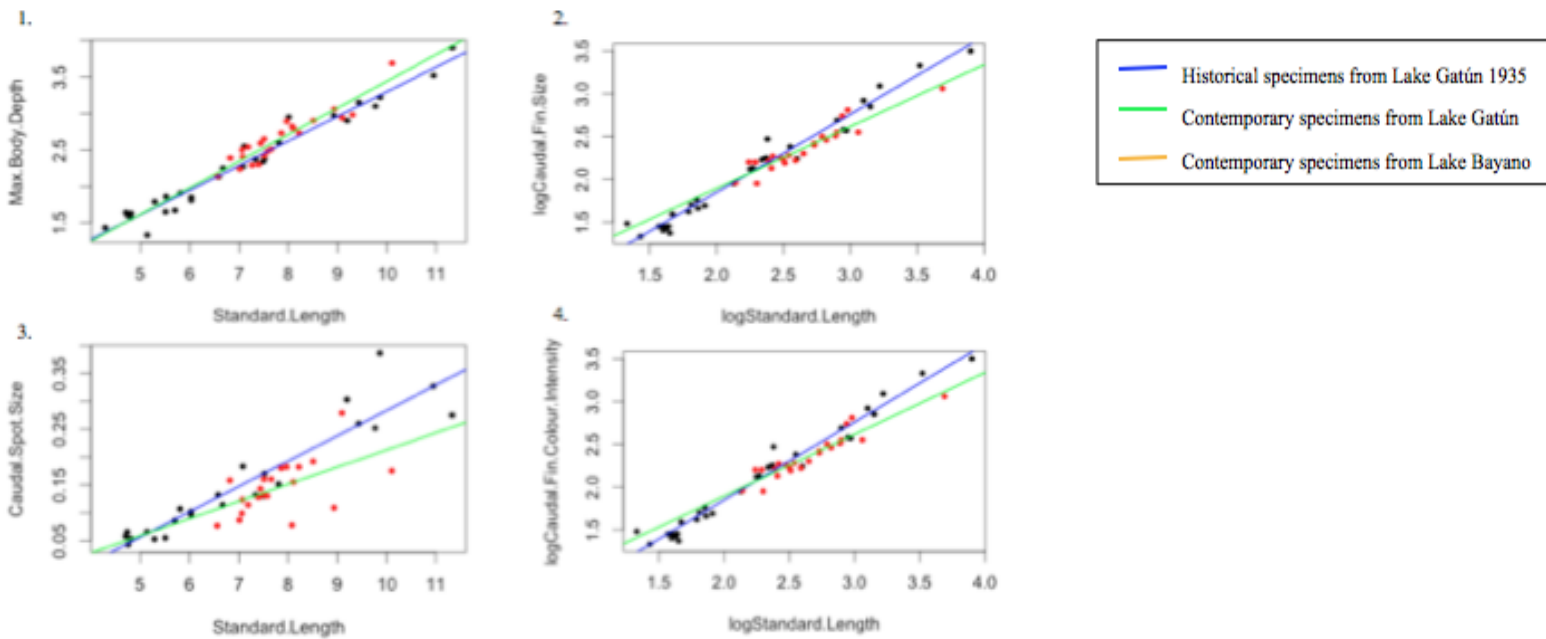
Maximum body depth has increased significantly since 1935 in Lake Gatún ( $F_{1,49}=4.671$ ,  $p=0.0356$ ), a finding enhanced by the fact that there was no significant interaction ( $F_{1,49}=0.674$ ,  $p=0.4155$ ) (See Fig.4, Graph 1). The maximum body depth was thus significantly greater in the contemporary Lake Gatún population than the historical Lake Gatún population.

The caudal fin was bigger in the historical Lake Gatún population ( $F_{1,49}=5.019$ ,  $p=0.0296$ ) than the contemporary Lake Gatún population. However, there was an interaction between the groups ( $F_{1,49}=7.672$ ,  $p=0.0079$ ). Nevertheless, this is still an indication that the caudal fin area in *R. guatemalensis* has decreased significantly since the Peacock Bass was introduced (See Fig.4, Graph 2).

Caudal spot size was also significantly bigger in the 1935 Lake Gatún specimens ( $F_{1,41}=10.370$ ,  $p=0.00251$ ) than the modern Lake Gatún specimens. Furthermore, there was no significant interaction ( $F_{1,41}=1.901$ ,  $p=0.17545$ ) (See Fig.3, Graph 3).

The colour intensity of the caudal spot was significantly darker compared to the eye iris in the 1935 Lake Gatún specimens ( $F_{1,49}=5.019$ ,  $p=0.0296$ ) than the contemporary Lake Gatún specimens. However, there was a significant interaction ( $F_{1,49}=7.672$ ,  $p=0.0079$ ) (See Fig.4, Graph 4).

There was no significant difference in eye size or the body depth at operculum between the historical and contemporary fish specimens from Lake Gatún.



**Figure 4: Differences between historical and contemporary fish specimens of *Roeboides guatemalensis***

## GATÚN PRESENT - BAYANO PRESENT

Trait	Prediction of largest measurement	Specimen that was found to increase	Specimen that was found to increase
		<i>Astyanax ruberrimus</i>	<i>Roeboides guatemalensis</i>
Max. Body Depth	Gatún	Bayano*	Bayano**
Body Depth at operculum	Gatún	Bayano*	Bayano**
Caudal Fin Size	Gatún	Bayano**	NSD
Caudal Spot Size	Gatún	Bayano**	NSD
Caudal Spot Colour Intensity	Gatún	Bayano**	NSD
Eye Size	Bayano	Bayano**	NSD

Table 2: Predictions and Results for Lake Gatún (invaded) and Lake Bayano (non-invaded) comparisons

\*\* = statistically significant

\*= statistically significant, with interaction between groups

NSD= no statistically significant difference

### *Astyanax*

All traits compared in *Astyanax* between the invaded Lake Gatún and the non-invaded Lake Bayano were significantly different.

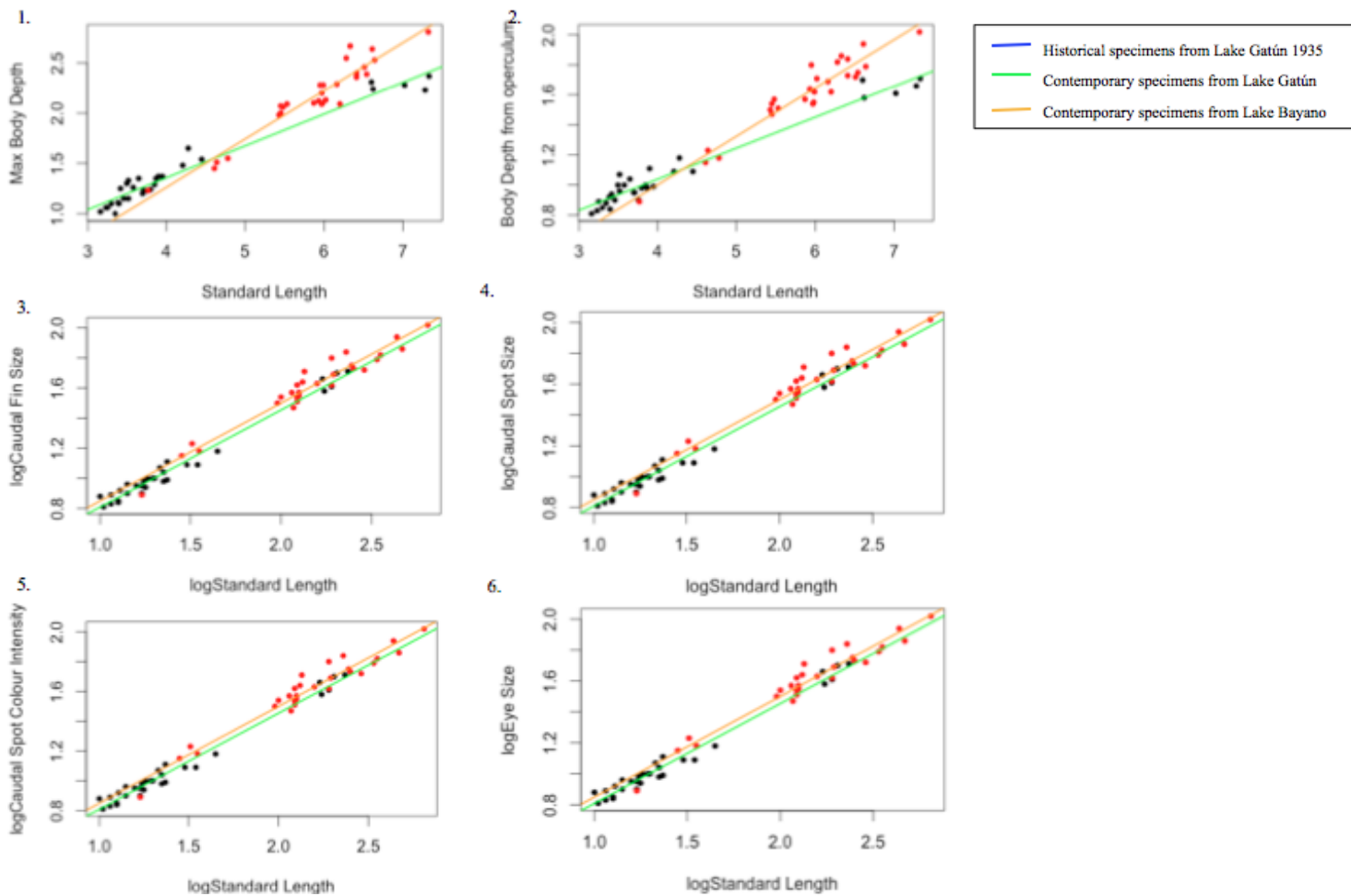
Maximum body depth was found to be significantly bigger in Lake Bayano ( $F_{1,54}=19.99$ ,  $p<0.001$ ) than Lake Gatún (See Fig.5, Graph 1). However, there was a significant interaction ( $F_{1,54}=31.82$ ,  $p<0.001$ ). Similarly, body depth measured from the operculum was found to be significantly bigger in Lake Bayano ( $F_{1,54}=37.16$ ,  $p<0.001$ ) than in Lake Gatún but once again, with a significant interaction ( $F_{1,54}=35.71$ ,  $p<0.001$ ) (See Fig.5, Graph 2).

The fish had a significantly greater caudal fin area in Lake Bayano than in Lake Gatún ( $F_{1,54}=5.809$ ,  $p=0.0194$ ) (See Fig.5, Graph 3). Furthermore, there was no significant interaction ( $F_{1,54}=0.018$ ,  $p=0.8937$ ).

The caudal spot size on the fish in Lake Bayano were significantly bigger ( $F_{1,54}=5.809$ ,  $p=0.0194$ ) (See Fig.5, Graph 4) and had a more intense colouration ( $F_{1,54}=5.809$ ,  $p=0.0194$ ) (See

Fig.5, Graph 5). There was no significant interaction for the caudal spot size ( $F_{1,54}=0.018$ ,  $p=0.8937$ ) or for the caudal spot colour intensity ( $F_{1,54}=0.018$ ,  $p=0.8937$ ).

Furthermore, the fish in Lake Bayano had a significantly bigger eye size ( $F_{1,54}=5.809$ ,  $p=0.0194$ ) than in Lake Gatún (See Fig.5, Graph6). There was no significant interaction ( $F_{1,54}=0.018$ ,  $p=0.8937$ ).



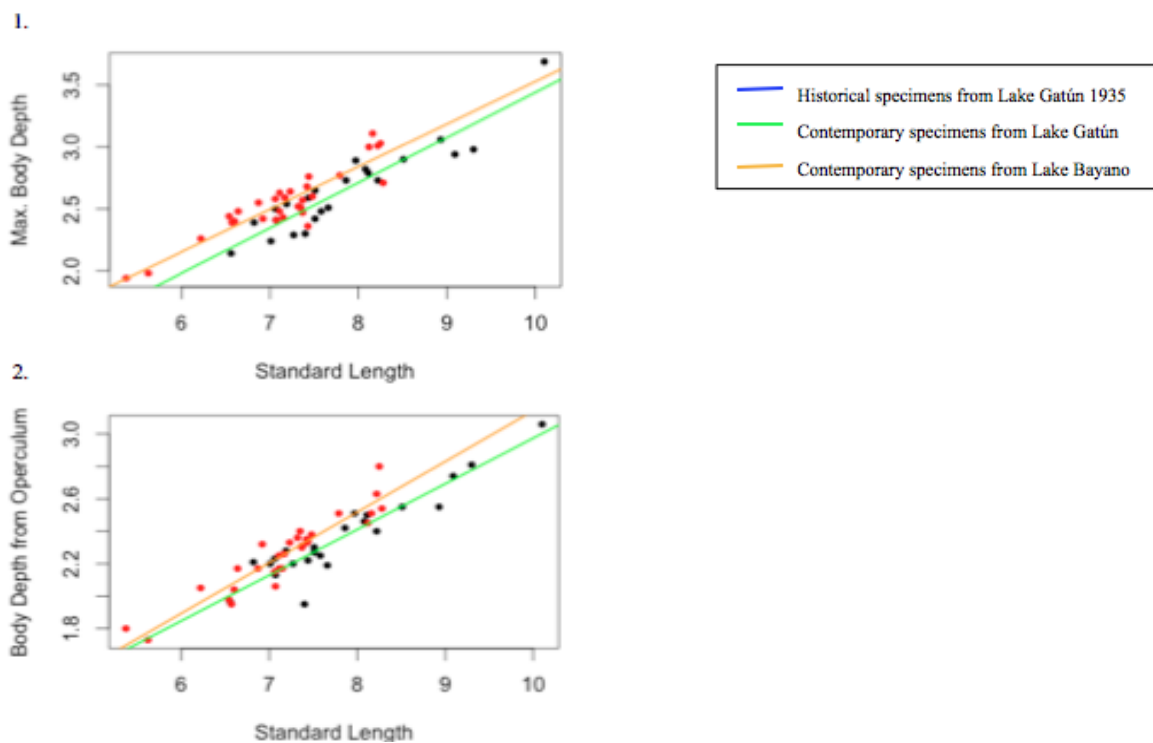
**Figure 5: Differences between Lake Gatún (invaded) and Lake Bayano (non-invaded) fish specimens of *Astyanax ruberrimus***

## *Roeboides*

The comparison of *Roeboides* between Lake Gatún, which has Peacock Bass and Lake Bayano, where the Peacock Bass is absent, yielded 2 significantly different results between these two sites, out of the 6 traits examined.

The maximum body depth was significantly deeper in Lake Bayano ( $F_{1,49}=15.367$ ,  $p<0.001$ ) than in Lake Gatún (See Fig.6, Graph1). To further this result, there was no significant interaction ( $F_{1,49}=0.241$ ,  $p=0.625323$ ).

The body depth from the operculum was also significantly deeper in Lake Bayano ( $F_{1,49}=11.751$ ,  $p=0.00124$ ) than in Lake Gatún (See Fig.6, Graph2). There was also no significant interaction ( $F_{1,49}=0.916$ ,  $p=0.34320$ ).



**Figure 6: Differences between Lake Gatún (invaded) and Lake Bayano (non-invaded) fish specimens of *Roeboides guatemalensis***

## GEOMETRIC MORPHOMETRICS

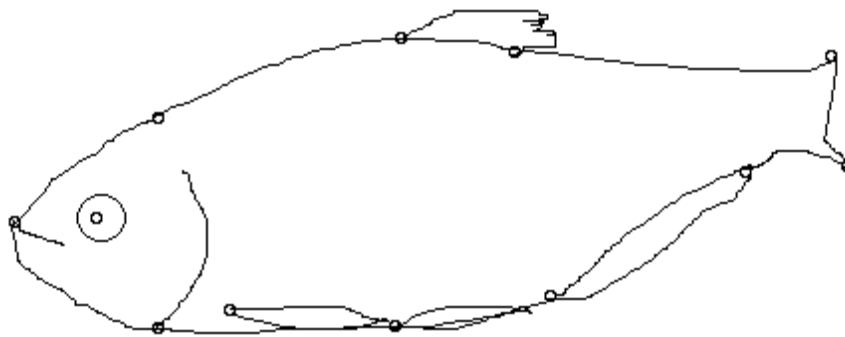
### *Astyanax*

A consensus shape was found for all the *Astyanax* specimens across the three groups (Figure 7). The relative warp score report for the *Astyanax* consensus in TPSrelw revealed that first four relative warps explained 59% of variation and thus were deemed most important (Figures 8,9,10,11). Table 3 shows the results of analysis in RStudio, which revealed that relative warps 2 and 3 differed significantly between populations from Lake Gatún 1935 and Lake Gatún, as well as populations from Lake Gatún and Lake Bayano (Figures 9 and 10). Relative warp 4 significantly differed between populations in Lake Bayano and Lake Gatún (Figure 11).

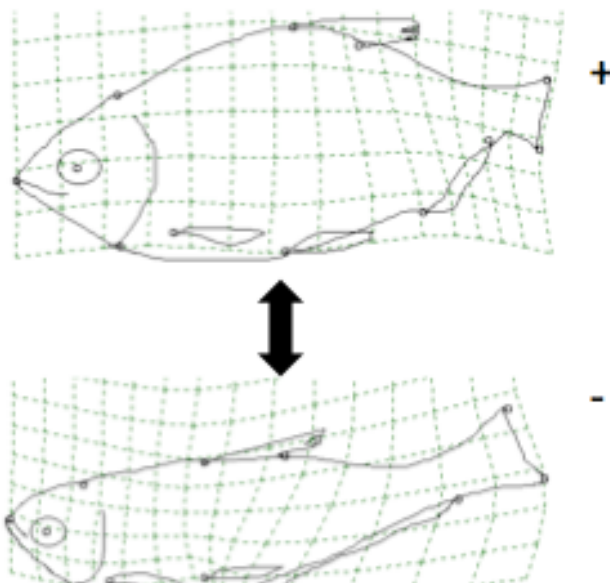
We used geometric morphometrics to detect significant changes in body shape and positions of major features between populations before and after the introduction of the predator *C. monocus* and between populations of invaded and uninvaded lakes. Relative warp one was found to explain 20% of variation among all individuals in the positioning of anal fins and length of the caudal peduncle area, however there were no significant differences in shape between different populations. This could be due to variation in body size, body condition, or variation between the sexes. Relative warp 2 explained 16% of variation between specimens, and relative warp 3 explained 12% of variation. Lake Bayano populations appeared to be much deeper at the head and middle of the body compared to the other two populations. These results are the same as those from the ANCOVAs, which was expected as the same measurements were used for both. In addition, Lake Gatún 1935 populations had dorsal fins and pectoral fins located further back on their body compared to the consensus shape, which implies that present Lake Gatún populations have had their dorsal and pectoral fins move forwards along their body. Eye position also varied; it is evident from the diagrams of the extreme relative warps that the eye position of



the fish in the Lake Bayano populations is higher up on the head, and closer to the tip of the fish head. The eye position in populations from Lake Gatún 1935 appears slightly lower on the head than the consensus shift. Relative warp 4 was significantly different between Lake Bayano and Lake Gatún populations and provides further evidence for Lake Bayano populations being deeper at the head and middle, although it only explains 11% of variation. It also shows that the present Lake Gatún population has receding pectoral and dorsal fins as compared to the consensus figure, which contrasts the evidence from relative warp 2 which shows Lake Gatún 1935 having receded dorsal and pectoral fins compared to present Lake Gatún populations.



**Figure 7: Consensus shape of *Astyanax* across all three populations.**



**Figure 8: Extreme forms of relative warp 1, showing variation among individuals across all three populations. No significant differences found between populations.**

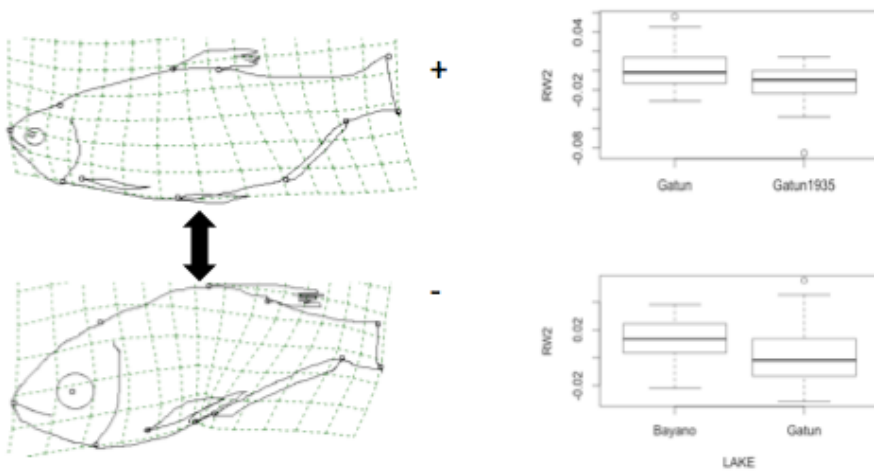


Figure 9: Extreme forms of relative warp 2 and the corresponding graphs showing significant differences in the body forms between Lake Gatún and Lake Bayano, and Lake Gatún past and Lake Gatún present. Overall, the extreme negative shape corresponds best with past Lake Gatún fish, while the extreme positive shape corresponds best with present day Lake Bayano fish.

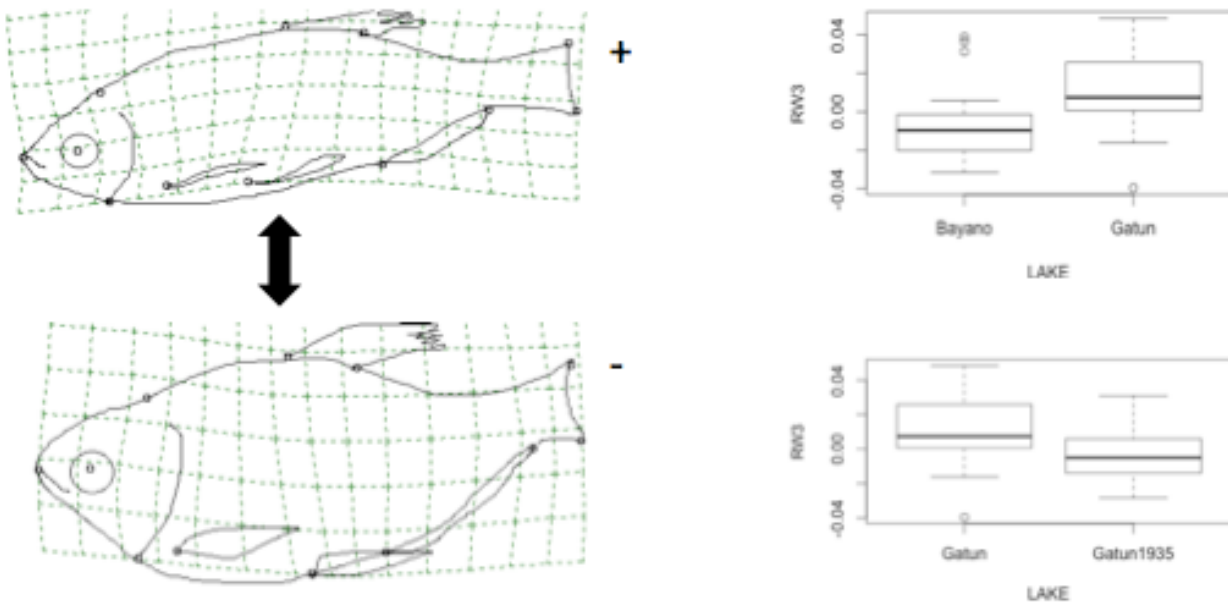


Figure 10: Extreme positive and negative forms of relative warp 3 and the corresponding graphs showing significant differences in the body forms between Lake Gatún and Lake Bayano, and Lake Gatún past and Lake Gatún present. Overall, the extreme negative shape corresponds best with Lake Bayano, while the extreme positive shape corresponds best with present day Lake Gatún fish.

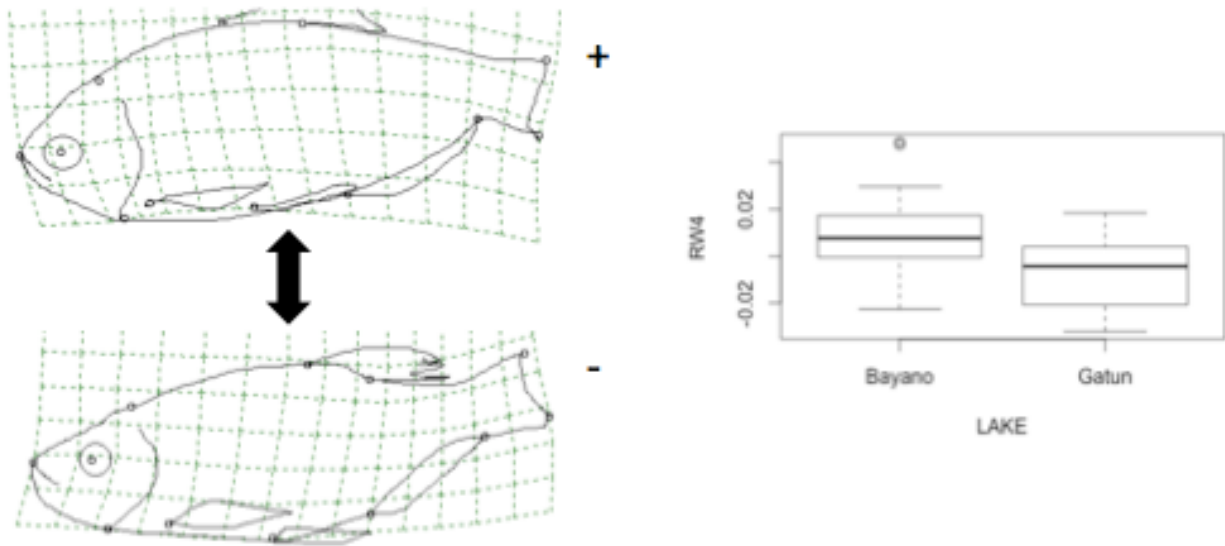


Figure 11: Extreme forms of relative warp 4 and the corresponding graphs showing significant differences in the body forms between Lake Gatún and Lake Bayano. Overall, the extreme negative shape corresponds best with present Lake Gatún fish, while the extreme positive shape corresponds best with present day Lake Bayano fish.

Table 3: ANOVAs performed on major relative warps to detect differences between populations of *Astyanax*.

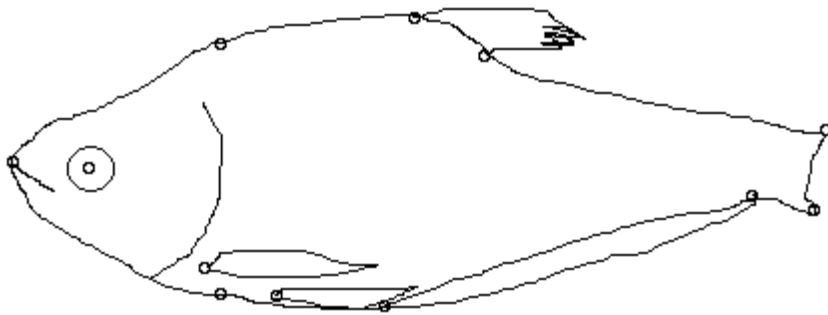
Relative Warp	Lake Gatún present/Lake Gatún 1935	Lake Gatún/Lake Bayano	Percent variation explained
RW1	no significant difference	no significant difference	20%
RW2	$F_{1,56}=8.46$ ; $p=0.0052$	$F_{1,56}=5.895$ ; $p=0.0184$	16%
RW3	$F_{1,56}=9.524$ ; $p=0.00315$	$F_{1,56}=13.73$ ; $p<0.001$	12%
RW4	no significant difference	$F_{1,56}=19.08$ ; $p<0.001$	11%

## *Roeboides*

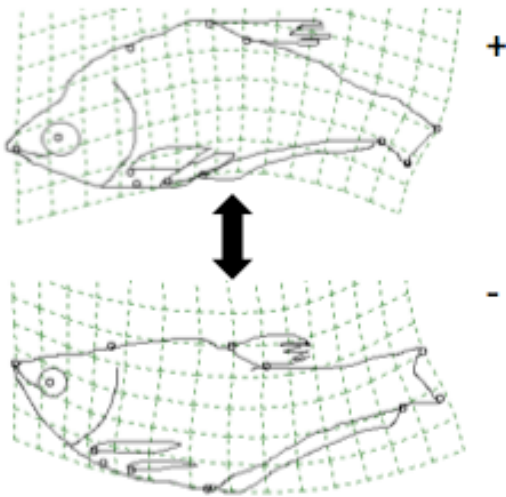
The consensus shape for all specimens of *Roeboides* after the three groups can be seen in Figure 12. The first three relative warps explained 63% of the variation from the consensus; these three relative warps were used for further analysis (Figures 13,14,15). The results of ANOVAs performed for each group comparison for each significant relative warp can be seen in Table 4. Relative warp 3 changed significantly from 1935 to present (Figure 15), and relative warp 2 significantly differed between populations from Lake Bayano and Lake Gatún (Figure 14).

The first relative warp explained variation at an individual level, clearly showing that the fish shape depended heavily on the way it was preserved. The first relative warp is the most important as it explains 34% of the variation among specimens. Many fish had bent upwards or downwards upon preservation and thus caused lots of variation in landmark positioning that had little to do with the morphological differences between populations. Relative warp 2, which explains 17% of variation, showed a significant difference between Lake Gatún and Lake Bayano populations, proving Lake Bayano specimens to be deeper, with a less pronounced hump, eyes closer to the tip of the head and higher up, a smaller anal fin, and pectoral fins placed further back on the body. Relative warp 3 accounts for 11% of variation and differed significantly between past and present Lake Gatún populations; Lake Gatún populations without predation had a smaller hump, with their pectoral fins placed further up on their body than the consensus figure, a more slender caudal peduncle area, and had a greater body depth. From the results of the relative warp analysis, it can be concluded that present populations of *R. guatemalensis* have a more prominent hump but still have a smaller body depth than Bayano populations of *R. occidentalis*, although they have increased in mid-body depth since the

introduction of the peacock bass. Additionally, the caudal peduncle area appears to have increased in the last 80 years, but remains smaller than that of Lake Bayano populations. This feature was not directly measured, and is only inferred from diagrams drawn from the relative warp analysis. Eye position and pectoral fin position are also different among populations, with the consensus figure agreeing more with present Lake Gatún populations.



**Figure 12: Consensus shape of *Roeboides* across all three populations.**



**Figure 13: Extreme forms of relative warp 1, showing variation among individuals across all three populations was due to specimen bending through preservation. No significant differences found between populations.**

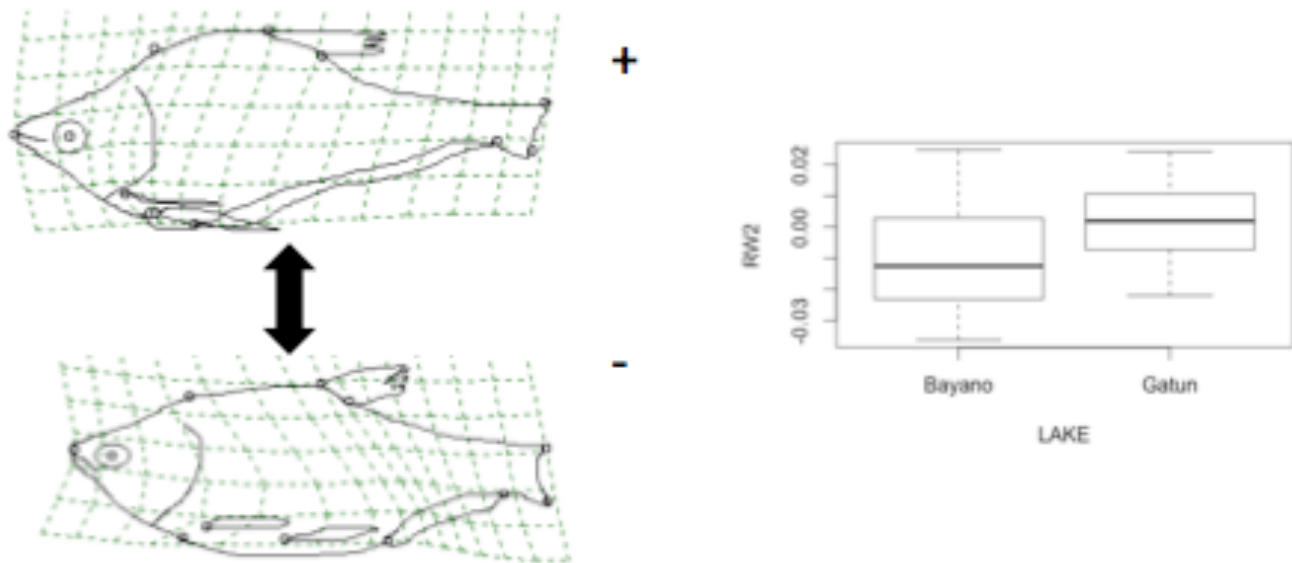


Figure 14: Extreme positive and negative forms of relative warp 2 and the corresponding graphs showing significant differences in the body forms between Lake Bayano and Lake Gatun present. Overall, the extreme negative shape corresponds best with Lake Bayano, while the extreme positive shape corresponds best with present day Lake Gatun fish.

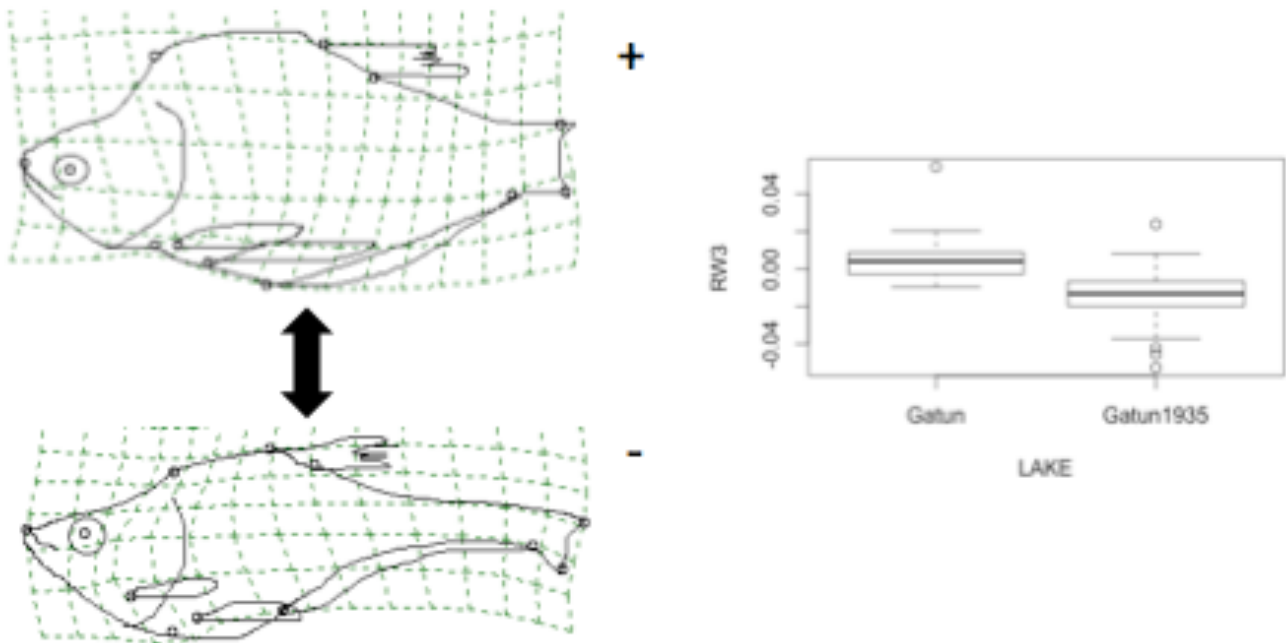


Figure 15: Extreme positive and negative forms of relative warp 3 and the corresponding graphs showing significant differences in the body forms between Lake Gatun past and Lake Gatun present. Overall, the extreme negative shape corresponds best with past Lake Gatun fish, while the extreme positive shape corresponds best with present day Lake Gatun fish.

**Table 4: ANOVAs performed on major relative warps to detect differences between populations of *Roeboides*.**

<b>Relative Warp</b>	<b>Lake Gatún present/Lake Gatún 1935</b>	<b>Lake Gatún/Lake Bayano</b>	<b>Percent variation explained</b>
RW1	no significant difference	no significant difference	34%
RW2	no significant difference	$F_{1,51}=4.399$ ; $p=0.0409$	17%
RW3	$F_{1,52}=25.88$ ; $p<0.001$	no significant difference	11%

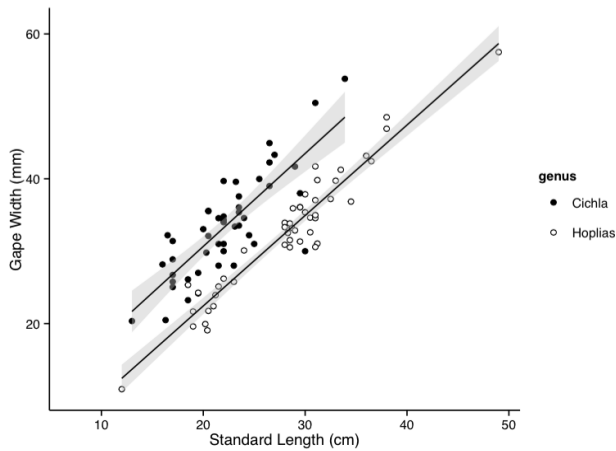
## Discussion

The goal of this study was to examine if there had been morphological adaptations in native prey species in Lake Gatún species after the introduction of a novel top predator. We expected increases in body depth, caudal fin area, caudal spot size and intensity, a decrease in eye size, and a change in general body shape in Lake Gatún contemporary species as compared to historical Lake Gatún species and contemporary species in Lake Bayano.

### GATÚN 1935 - GATÚN PRESENT

We found that the maximum body depth of *R. guatemalensis* was significantly bigger in the contemporary Lake Gatún specimens than in the historical specimens. This is consistent with the *a priori* prediction since, as mentioned previously, studies have shown that an increase in body depth increases speed and acceleration in addition to being a defense mechanism against gape-limited predators. On the other hand, in *Astyanax*, the body depth measured at the operculum was lower in modern specimens. These are conflicting results. However, according to a preliminary study seen in Figure 16, gape-width limitation might not influence the body depth of *Astyanax* or *Roeboides*, as was expected (Sharpe, in prep). Most body depth measurements of *Astyanax* were between 15-25mm and of *Roeboides* between 20-30mm. The gape-width of both *Cichla monoculus*, Lake Gatún's major predator, and *Hoplias*, Lake Bayano's major predator, is bigger than the body depths of both species in both lakes. This means that an increase or decrease in body depth, whether it was maximum body depth or from operculum, in *Astyanax* and *Roeboides* has not been in response to gape-width since the change in body depth has not been advantageous as a defense mechanism against gape-width predators, as was thought to have been.





**Figure 16: Gape-width of major predator species, *Cichla* and *Hoplias* (Sharpe, in review)**

Contrary to our previous predictions, caudal fin size was smaller in contemporary (post-invasion) species. A possible explanation can be found in the transition these species underwent from being riverine species to open-water species. A study found that species inhabiting high-velocity waters, such as rivers, had larger caudal fins than those inhabiting low-velocity waters, such as lakes (Irme *et al.*, 2002). Therefore, it is possible that the decrease in caudal fin size since 1935 is attributed to the species adapting from a high-velocity to a low-velocity environment.

Caudal spot size decreased significantly over time, also contrary to previous predictions. A possible explanation is offered by a study by Kjærnsmo on sticklebacks, who found that the size of the eyespot is only relevant in relation to the fish's true eye (Kjærnsmo and Merilatita, 2013). If the caudal spot is smaller than the fish's own eye, it effectively draws the attack of the fish towards them (Kjærnsmo and Merilatita, 2013). This study found little support for an intimidating effect by larger eyespots (Kjærnsmo and Merilatita, 2013). Furthermore, caudal spot colour intensity became less dark over time which was not consistent with the *a priori* predictions. The same study by Kjærnsmo found evidence that the conspicuousness of eyespots were not related to the latency and attack rate of the predator (Kjærnsmo and Merilatita, 2013). This study provides conflicting results with many other studies (Carroll *et al.*, 2004), but

supports the above findings. Clearly, more work remains to be done to uncover the adaptive significance of caudal spots in fishes.

#### GATÚN PRESENT - BAYANO PRESENT

*A. ruberrimus* had smaller eyes in Lake Gatún (invaded) than *A. fasciatus* in Lake Bayano (uninvaded). This is in accordance with predictions as a smaller eye size in Lake Gatún is expected as a technique to make the caudal spot more conspicuous thus more effectively diverting predators, such as the Peacock Bass, away from the head region. On the other hand, all other results: the maximum body depth and body depth at operculum in *Roeboides* and these two traits, in addition to caudal fin size, caudal spot size and caudal spot colour intensity in *Astyanax* were smaller in Lake Gatún compared to in Lake Bayano. All these results were contrary to predictions. In addition to studies mentioned previously which can support findings which do not agree with previous predictions, it is important to keep in mind other factors that can influence morphology (see below). These include differences in habitat, other important morphological diversification factors such as DO concentrations, light availability, flow regimes and differences in predation pressures.

#### MORPHOLOGICAL CHANGES DUE TO PREDATION PRESSURE?

The strongest evidence for morphological changes between *Astyanax* populations from the morphometric analysis is the larger body and head depth in the Lake Bayano populations. The same is true for analysis of morphological differences in *Roeboides* populations. The body depth of the Lake Bayano populations is the greatest, and slightly larger in present than past populations in Lake Gatún. The causation and implications of this phenomenon were previously discussed above, although not the overall pattern of depth between populations.

The shape pattern that emerged for *Astyanax* from relative warp analysis follows a general model of a smaller head and slightly deeper body for present Lake Gatún populations in both genera compared to before. This pattern is consistent with predictions that fish that require a fast burst speed will have a deep middle region and smaller anterior region. However, as the caudal regions appeared to be similar for all populations, we cannot conclusively say that predation is the sole selection pressure for body shape.

In *Roeboides* populations, Lake Gatún populations had smaller heads and smaller caudal peduncle areas compared to Lake Bayano populations. Fish from Lake Bayano had a larger caudal peduncle area, but they were also larger at the head and mid-region. Further standardization to control for weight should be used in order to confirm a significant increase in caudal peduncle area. There appeared to be a slight increase in caudal peduncle area and body depth in predated contemporary populations from non-predated past populations, which provides some evidence for selection of a body form equipped for C-starts to escape predators. An alternative hypothesis is that *R. guatemalensis* do not escape from predators through burst speed, and instead prefer hiding in dense vegetation as an anti predator strategy (Zaret & Paine, 1973). Swim performance trials done during an ongoing study by an intern at STRI are revealing that *R. guatemalensis* exhibits an unusual swim behaviour when trying to escape from predators, instead of the classic C-start getaway (Valverde *et al.*, in prep). This phenomenon could explain why the body shape of the populations studied here did not change in the way we expected.

The differences in eye position and dorsal and pectoral fin positioning between Bayano and Gatún populations of *Astyanax* are similar to the shifts in eye position and pectoral fin positioning in *Roeboides* populations. The results are complicated by the fact that Lake Bayano populations are different species, *Astyanax fasciatus* and *Roeboides occidentalis* (whereas

species in Lake Gatún are *Astyanax occidentalis* and *Roebooides guatemalensis*), and thus while the differences between species are extremely minimal, it is difficult to determine which differences are caused by speciation or predation pressures. There is evidence in the literature that a shift downward in eye position is advantageous in prey species in order to enhance detection of predator strikes from below, however considering that eye position only differed between lakes and not over time, other factors other than predation may be responsible for the differences (Langerhans *et al.*, 2004). The specific location within the water column that fish feed from has the ability to influence mouth and eye position (Willis *et al.*, 2005). Lake Bayano is considerably shallower than Lake Gatún, which could influence the feeding behaviour and locations of *A. fasciatus* and *R. occidentalis* in the water column, thereby resulting in a difference in eye position compared to fish in Lake Gatún (Marmulla, 2001).

Differences between populations in fin position highlights one of the weaknesses of geometric morphometrics. This method can identify and describe morphological differences between individuals and populations, however there is no link between morphology and function (Cooke & Terhune, 2015). It is difficult to associate small changes in fin position with a functional purpose for the fish, especially if trying to relate a certain selective pressure. The minor shift back in pectoral fins for both genera in Lake Gatún after predator introduction could have consequences for swimming performance unrelated to predation as Lake Bayano fish have their pectoral fins positioned even further back on their body without predation pressures from the peacock bass. Both lakes were created from rivers, and therefore this morphological change could be associated with the habitat switch from river to lake. Further studies would be required to investigate the consequences of these morphological changes.

## OTHER FACTORS THAT MAY INFLUENCE MORPHOLOGY

### *Bayano - Gatún*

Perhaps the most obvious other factor when comparing morphologies of species between Lake Bayano and Lake Gatún is the difference in habitat due to the difference in location. Lake Bayano is a shallower reservoir created about 60 years later than Lake Gatún, in an area that used to be only agricultural fields (Marmulla, 2001). As a result, it is currently still surrounded by agricultural fields on either side and has more aquatic plants. Furthermore, Lake Bayano is a low-elevation reservoir as a result of decomposition of organic material making it a more productive lake (Marmulla, 2001). Lake Gatún on the other hand, is better protected, surrounded by trees and has less vegetation in the water. This difference in habitat can explain the need of these species to adapt their morphologies in order to survive in their particular environment. It has been shown that complex habitats, or microhabitats, can be responsible for different traits such as eye position, body depth and fin aspect ratios to better manoeuvre within their habitat such as around dead wood or aquatic vegetation (Willis *et al.*, 2005). Furthermore, complex habitats may also reduce mortality due to predation, weakening adaptive pressures (Willis *et al.*, 2005).

The difference in the environment between Lake Bayano and Lake Gatún can also mean that there are differences in abiotic conditions that can influence morphology. Studies have shown that dissolved oxygen concentrations in the water, light availability, and flow regimes are all important morphological diversification factors that affect fishes (Franssen *et al.*, 2013).

Furthermore, evidently Lake Bayano's food web is quite different from that in Lake Gatún as the Peacock Bass has not been introduced. Nevertheless, it is important to keep in mind that other predators in Lake Bayano, such as *Hoplias*, may have different selection pressures on

native prey species that have driven selection pressures in a different or similar manner compared to in Lake Gatún.

### *1935 Lake Gatún - Present Lake Gatún*

It is important to consider changing conditions over time in Lake Gatún, other than the introduction of *C. monoculus*, when looking at morphological changes over time. In the past 70 years, the environment on earth has undergone major changes such as a drastic increase in carbon dioxide levels and an increase in global temperature (NASA, 2014; NCAR, web). Furthermore, specific to Lake Gatún, there has been continuous dredging which severely impacts populations living in the surrounding areas, and increased boat traffic as annual traffic has risen from about 1000 ships in 1914 to 14702 vessels in 2008 (“The Panama Canal”, web). All these changes to the lake and added stressors from 1935 to the present day have probably changed many of the abiotic factors of the lake and lake composition thus also affecting native prey species such as *A. ruberrimus* and *R. guatemalensis*. These may have played a role in morphological adaptations of these species.

### ADDITIONAL FACTORS

There are other factors that may also be influencing morphology of native prey species in both spatial and temporal comparisons. Firstly, native prey species may be responding to predation pressures, but simply through other means than morphology. For example, they may be adapting life-history traits leading to weaker selection on morphology. Secondly, both of these bodies of water are reservoirs and the native prey species both used to be riverine species. Changing from a riverine to an open-lake species requires many morphological adaptations due to this change in environment, water flow, higher predation pressure, alteration of dissolved

oxygen and light levels (Franssen, 2013). We can not be confident that morphological adaptations observed in these native prey species are due solely to predation pressure, when many other factors can also influence the optimal morphology. Thirdly, responses to selective pressures can be species-specific, thus comparing *Astyanax* and *Roeboides* phenotypic trait changes to other papers is not always a good representation and can perhaps help explain why most of our results do not correspond to our predictions.

## FUTURE RESEARCH

Little is known about the behaviours of these particular prey species; further research into their feeding habits, habitat use, behaviours, and physical differences between Lake Bayano and Lake Gatún could provide more insight into why some morphological differences unexplained by predation are present between populations. Specifically, burst swimming trials to test the response of *Roeboides* and *Astyanax* between Lake Gatún and Lake Bayano could help link body shape patterns to their function. Furthermore, the morphological changes observed could be due to phenotypic plasticity instead of genetic adaptation, and if this is the case, the question is whether the fish are changing morphology in a temporary way to cope with environmental variations, or are these morphological changes legitimate adaptations to long term environmental pressures? Even if there is a genetic basis for morphological changes, this is not sufficient enough to prove adaptive evolution (Merilä & Hendry, 2014). Genetic drift, gene flow, and inbreeding are all processes that influence genetic makeup of a population and could contribute to maladaptive morphological changes depending on the environmental stress and the genetic material available (Merliä & Hendry, 2014).

An interesting study to better understand our results, would be to conduct an experiment comparing morphologies of riverine species to species in the lakes. The rivers feeding into Lake

Gatún and Lake Bayano are free of *Cichla monoculus* thus it would be interesting to see if there has been a morphological change in the native prey species between Lake Gatún and the rivers feeding into it. As a control, a morphological comparison would need to be drawn between the native prey species in the rivers feeding into Lake Bayano and the native prey species in Lake Bayano. This would provide a better understanding as to morphological changes due to the transition these prey species underwent from being riverine species to open water species.

A highly useful method to detect morphological adaptation to a certain pressure is a reciprocal transplant experiment (Hendry & Gonzalez, 2008; Merliä & Hendry, 2014). The idea of this method is to transplant live specimens from a control habitat into the treatment habitat, and then reciprocate the experiment. The expectation is that the treatment specimens should have higher fitness in their treatment habitat than the control specimens placed there, demonstrating that changes in the treatment specimens have allowed for increased survival in their altered environment. In this case, it would be interesting to place fish from Lake Bayano in an environment with the predator *C. monoculus* and the Lake Gatún fish, to see if Lake Gatún fish have higher fitness. A higher fitness would prove that morphological changes between the populations identified in this study are adaptations to the novel predator introduced into Lake Gatún. To reciprocate the experiment, Lake Gatún fish would have to be transplanted into the Lake Bayano environment, predator-free, and their fitness measured against the Lake Bayano fish. An adaptation to a predator should have meant fitness trade-offs in other areas, and thus the Lake Gatún fish should theoretically have lower fitness than Lake Bayano fish. This experiment would be a great way to test for adaptive morphological changes in prey fish species under predation pressure, and the results from this study could be used in conjunction with this experiment.



Escape from predation could potentially induce morphological changes in the caudal fin aspect ratio and the caudal peduncle area, both features which were not explicitly measured in this study. Caudal fin aspect ratio looks specifically at the height and surface area of the caudal fin, and has implications in the swim performance of fish. This would directly link to escape speed, an increase in which would be beneficial with increased predation pressure, as mentioned earlier in this paper. An increase in caudal peduncle area would also benefit prey species when escaping predators, and although there seemed to be a slight increase in caudal peduncle length and area in present Lake Gatún populations of *R. guatemalensis*, it was not directly measured and analysed. More focused studies on this aspect may produce interesting results for adaptive changes in Lake Gatún fish in response to predation.

Morphological changes may be in conflict with life-history traits that may be changing in order to adapt to the introduction of *C. monoculus* and therefore a current study being done on comparing life-history traits between Lake Bayano and Lake Gatún populations by Lina Ortiz would be interesting to integrate with this study to develop a greater understanding of the selective pressures acting on the prey species and how they are adapting.

## IMPLICATIONS OF THIS STUDY

It is evident from our study that native prey species have the ability to adapt their morphologies to environmental pressures, possibly including the introduction of a novel predator. However, there are many other factors influencing morphology, such as habitat, abiotic conditions, and conflicting selection for other adaptations to predation such as selection for altering life history traits. It is difficult to determine which morphological changes are directly a result of predation pressures because the freshwater ecosystems of the lakes studied are highly complex, with many interactions between biotic and abiotic factors that make it difficult to

isolate systems such as predator-prey relationships. This is encouraging as ecosystem complexity is important to maintain ecosystem stability and resilience.

This study complements Diana Sharpe's current work on the long-term impacts of the introduction of the Peacock Bass, which is a thorough follow-up to Zaret and Paine's initial paper. Sharpe is repeating community fish surveys in Lake Gatún, and her results may be able to be explained by these smaller studies in local adaptations that have enabled species to survive. The example of the Peacock Bass is just one of many introductions of non-native predators globally, thus our study, as a part of Sharpe's work, has the potential to help understand and predict impacts of invasive predators in order to better manage and conserve biodiversity.

A better understanding of the impact the Peacock Bass has had on native prey species and its interaction with its environment is not only important for conservation of the ecosystem and maintenance of biodiversity, but also has important social and economic implications. With the introduction of the Peacock Bass in 1967, many stocks were founded that were conducive to fishery exploitation (Marmulla, 2001). Many cooperatives were formed that invested in fishing gear, ground transportation and marketing (Marmulla, 2001). Additionally, many fishers not affiliated with cooperatives sell their fish on the roadside. Recreational sport fishing also evolved and nowadays peacock bass fishing tournaments have become a big attraction of Lake Gatún. The socio-economic health of the communities around Lake Gatún is reliant on the Peacock bass, and thus in order to ensure a sustainable industry there must be an emphasis on the protection of the biodiversity within Lake Gatún. Biodiversity ensures the health of an ecosystem and creates many links and interactions within the food web that decrease the ecosystem's susceptibility to abnormal environmental fluctuations. The prey species studied here are part of

that food web, and the recovery and maintenance of their populations are important to sustain the populations of peacock bass and other organisms in and around the lake.

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