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PATTERNS OF MORPHOLOGICAL PLASTICITY IN *METRACLIMA ZEBRA* AND  
*DANIO RERIO* SUGGEST DIFFERENTLY CANALIZED PHENOTYPES DUE TO  
FORM-FUNCTION RELATIONSHIPS

A Thesis Presented

by

DYLAN S. JOCKEL

Submitted to the Graduate School of the University of Massachusetts Amherst in partial  
fulfillment of the requirements for the degree of

MASTER OF SCIENCE

September 2019

Organismic & Evolutionary Biology

**PATTERNS OF MORPHOLOGICAL PLASTICITY IN *METRACLIMA ZEBRA*  
AND *DANIO RERIO* SUGGEST DIFFERENTLY CANALIZED PHENOTYPES  
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## ABSTRACT

# PATTERNS OF MORPHOLOGICAL PLASTICITY IN *METRIACLIMA ZEBRA* AND *DANIO RERIO* SUGGEST DIFFERENTLY CANALIZED PHENOTYPES DUE TO FORM-FUNCTION RELATIONSHIPS

SEPTEMBER 2019

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In order to ascertain the degree of compatibility in developmental restructuring and behavioral plasticity between two fish species frequently made subject of laboratory research (*Metraclima zebra* & *Dano rerio*), alternative trophic niche exposure experiments utilizing novel three-prong feeding treatments were conducted to obtain morphometric data, which demonstrated both species do bear some degree of plasticity. The results are somewhat complicated by differences in locality of detectable restructuring, which may be due to disparity in the form-function relationship for each species' lineage. Broadly, the confluence of each is notable in differences between respective species' jaw protrusion, as it is driven by anterior kinethmoid rotation in *D. rerio*. as opposed to force imparted upon the rostral cartilage of the premaxilla's articular process in *M. zebra*. Each is markedly distinct in the pharyngeal jaw as well, as zebrafish (also toothless at the oral jaw) bear teeth only on the lower set at the posterior of the mouth, while cichlids bear teeth on all jaws and additionally possess a unique, fused lower pharyngeal jaw. However, accounting for this difference in experimental models does allow for direct comparison, both at the morphological/behavioral and potentially

the genetic level, though additional research is necessary. The evidence provided here also provides encouragement that more nuanced approaches to laboratory trophic niche exposure experiments could elucidate further evidence on the nature of phenotypic plasticity.

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## CHAPTER 1

### INTRODUCTION

Four billion years of evolution on planet earth has induced a prodigious quantity of phenotypic variation within the tree of life. Broadly, it is understood phylogenies owe this diversity to the interplay between variation of genomic content within populations, and developmental responses to environmental perturbation (Zimmer/*et al.* 2013). These responses, controlled by gene regulatory networks that modulate expression, allow traits to vary their phenotype within one organism in response to selective pressures through pathways of developmental restructuring (Moczek/*et al.* 2011).

While earlier models of evolution placed more emphasis upon ultimate, population-level allele sorting among individuals as an explanation for divergence (Gilbert/*et al.* 1996), it has become apparent (largely due to an improved understanding of any given gene's ability to be expressed according to external signaling) that the latter half of a Gene x Environment interaction should, in regard to speciation and morphological diversification, be carefully examined (Bateson & Gluckman 2011). Because the plasticity of phenotypic expression is adaptive, it is likely to fall under selection as well. Additionally, as a result of conserved patterns of gene regulation that make use of 'die-cast' chemical mechanisms, we may see a proliferation of similar patterns in genes selected for increased plasticity or robustness (Feinberg & Irizarry 2010). Thus, the various phenotypes of one organism may become repeatedly and differently canalized, both within one lifetime, or across a lineage (Westneat/*et al.* 2015). This, it has been postulated, is vital to linking proximate and ultimate mechanisms of evolution and better describing exactly how species originate. However, since the time of

Darwin, this window of evolutionary change i.e.: the inception of new species via divergence, has been difficult to conceptualize (Laland/*et al.* 2015). Natural selection, for instance, has long been understood to act on trait variation, but what leads traits to vary? Why do we see disparity in the development of phenotypes within one generation?

These are the questions that have led evolutionary theorists to a more 'Environment First' model of speciation. Essentially, this revision is not radically dissimilar from the modern synthesis, rather it simply explicitly accounts for the interplay between phenotypic disparity induced by development, and ultimate pathways of evolution. To expand, plasticity in a phenotype may enable different individuals within a population to excel at exploiting different niches, or different aspects of social behavior. Consequently, this may cause populations to begin sorting themselves according to aspects of individual fitness prior to the existence of any technical 'barrier' to gene flow (Smith/*et al.* 2016). So rather than become completely isolated, a fully interbreeding population may find selection toward divergence favors individuals who exploit specific niches within an ecosystem. Those who remain generalists experience lower fecundity proportional to energetic investment in any one ecotype. Over time, this results in isolation of sub-populations that is circumstantial to the diversity of the local environment. If the variables remain static over a long enough time-span, this may form the basis for reproductive isolation and more concrete barriers back toward introgression.

Such sorting of the population is especially obvious when examining adaptive radiations of organisms. Lake Malawi African cichlids, a collection of hundreds of fish species that diverged from few ancestors in less than 2 million years, are one of the most frequently studied of such evolutionary events (Seehausen 2006), and their degree of

morphological divergence, despite no obvious barriers to introgression beyond behavioral divergence/color morphs, is both well documented and extensive. Being that many of these fish exhibit highly plastic phenotypes as well, it has been hypothesized a low degree of canalization has enabled a veritable explosion of novelties to evolve (Gavrilets & Losos 2009). This attribute has led cichlids to enjoy more recent attention as an evolutionary model (particularly generalist “mbuna” cichlids from Lake Malawi) (Genner & Turner 2005). However, while some advancements have been made in tying these phenotypes to developmental restructuring of specific genes and genetic pathways, the newer, less familiar genomic environment of this system means much of the detected variation in cichlids cannot, as Robinson and Parsons (2002) put it, easily be declared either a, ‘...cause or a consequence of divergence’. As a result, there is some desire to reconcile the morphological diversity of the cichlid model with a second, more genetically accessible one, and, our taxonomic neighborhood being the ray-finned fish, few would seem more appropriate to utilize than the versatile laboratory familiar, *Danio rerio* (Dahm & Geisler 2006). The Albertson lab has integrated studied in these two systems for well over a decade (e.g., Albertson/*et al.* 2005; Conith/*et al.* 2018). Though, as the meristem of this multi-model approach grows outward, it is imperative to raise the question of whether the marriage of cladistically distinct subjects bears any compatibility. Does the genetic toolbox available to us in zebrafish apply to cichlids, and does documented phenotypic plasticity in cichlids apply to zebrafish? It would be prudent to identify the overlap of both development and genetics between each taxon. Ideally, in the process, we may also confirm that plasticity in each is the result of conserved genetic mechanisms.

A conserved, or at least consistent, mechanism of plasticity could have broad implications for fish diversity in that it would suggest that morphological divergence in species that use disparate environments is facilitated by plastic responses within single populations reared under similar disparity (Robinson & Parsons 2002). In short, if this is found to be so, not only does ‘ontogeny recapitulate phylogeny’, but ‘development dictates divergence’ as well. Already, there is some evidence supporting such a notion. Studies in select species have suggested that trophic generalists (food acquisition is thought to be a strong selective pressure for fish, especially in the context of craniofacial interactions) can be induced toward genetically traceable behavioral or morphological accommodation when placed in alternate feeding ecologies (Parsons/*et al.* 2016) (Hayden/*et al.* 2014). Alternatively, populations bearing more specialized feeding regimes show a more limited response when exposed to novel trophic niches, showing the signature of trait assimilation under selective pressures from similar conditions (Parsons/*et al.* 2014) (Navon 2018).

Within this larger context, my research first set out to examine the cross compatibility of two models at a morphological level and expand the realm of possibilities when experimenting with novel ecology exposure (*a lá* (Parsons/*et al.* 2016)). Feeding was deemed an appropriate variable to manipulate, in part because it is thought to be a limiting factor in recruitment of fish to novel environments (as the physical requirements of energy acquisition, along with associated costs/risks may vary and disparately apply selective pressures in disparate ecosystems) (Walters & Juanes 1993), and partially as a follow up to recent publications (e.g., Parsons/*et al.* 2018) and (Parsons/*et al.* 2016) that suggest craniofacial plasticity may be prominent in each taxon.

Additionally, due to the inclusion of third experimental trophic niche (a novel approach for this method) the subsequent work may provide the basis for more detail examinations of more extensive feeding behaviors in the future.

## CHAPTER 2

### MATERIALS AND METHODS

#### 2.1 Trophic Niche Exposure

Albertson Laboratory-raised lines of both F4/F5 *Metraclima zebra* (a pelagic-leaning “mbuna” trophic generalist (Streelman/*et al.* 2007)), bred from a wild-caught population, and AB/EW laboratory lines of *Danio rerio* were utilized. All treatments were balanced for sex differences. *M. zebra* were housed in identical 190 Liter, rectangular glass tanks. *D. rerio* were housed in identical 10-Liter, rectangular, synthetic polymer tanks. Both species were connected to a respective recirculating water conditioning system, and all tanks were stored within the same temperature-controlled space (lit by full spectrum halogen lights timed to a 14hr light, 10hr dark cycle), ensuring the quality of the environment was identical treatment-to-treatment.

During the course of each experiment, all zebrafish were fed 2mm ‘New Life Spectrum Float’ pellets (34% Crude Protein Min, 5% Crude Fat Min, 5% Crude Fiber Max) that were prepared disparately depending upon treatment. The first group was fed un-modified pellets (i.e., “pellet treatment”), which floated for a few minutes before sinking. The second group was fed with a finely pulverized version (accomplished with a conventional kitchen coffee grinder, i.e., “powder treatment”). The third was fed by mixing the feed powder with 1% Agarose, spreading the homogenous solution over ceramic ‘lava rocks’ of the variety frequently used to decorate aquaria, and allowing the food to fully dry and harden before setting at the bottom of aquaria (i.e., “rock treatment”). The treatments were identical for cichlids, however the feed utilized was a

generic 1.25cm diameter vegetable wafer (30% Crude Protein min, 7.5% Fat min, 4.0% Fiber max) marketed under multiple brands including ‘Ken’s Premium Veggie Wafers’ (being that it bore more appropriate blend of nutrients for cichlids (Erdogan/*et al.* 2012)).

Functionally, the pellet treatment was intended to engage the pharyngeal jaw of each species. Bearing no oral jaw teeth, zebrafish process most food with their ceratobranchials, suggesting accommodations for any feeding behavior may heavily involve restructuring of these bones. Cichlids, although not toothless at the anterior, do possess fused ceratobranchials, and it has been specifically hypothesized this represents an adaptation for processing harder prey (Mabuchi/*et al.* 2007). However, it is less clear how canalized this apparatus is, and it may be differently so in disparate populations. The rock treatment, conversely, was designed to force each fish to feed in a manner that imparted more force from their oral jaws, back (by comparison to feeding in the water column). Given that oral jaw shape is thought to heavily influence the tradeoff between strength and speed of jaw closure (Albertson/*et al.* 2003), it was expected this would induce noticeable consistent deformation in both clades. The powder fed treatment was intended to bias fish toward speed in this functional tradeoff, specifically toward generating suction as a means of gathering food.

Zebrafish, being more behaviorally canalized toward feeding in the water column, necessitated an entrainment period where benthic groups were fed and then monitored to ensure active and vigorous feeding. Care was taken to remove the lava rocks before the Agarose-food mixture delaminated (After about 10 minutes) to ensure the treated fish would only utilize the desired feeding behavior. Once all fish in the tank began readily feeding in a benthic manner, the practice of removing food was ceased and the lava rocks



were left to be removed later in the day after feeding behavior had ceased. For the pellet treatment, *D. rerio* were fed approximately 0.007g/individual daily, whereas *M. zebra* received roughly 0.2g/individual/day. Whole pellets used to feed cichlids were broken into smaller pieces to reduce effects of competition. Feeding quantities were adjusted up or downward depending on apparent growth rate and behavior (if fish in a particular experiment did not consume all food in a timely manner, less was added subsequently, etc.). This was done in an effort to control for both size and to reduce the possibility of food waste which would likely lead to confounding feeding behaviors. Prior to experimentation, all fish were fed a lab-standard diet (Gemma Micro 300 pellets for Zebrafish and a 3/1 dried spirulina/egg yolk flake mixture for cichlids).

Each experimental period lasted ~90 days, and two iterations of the experiment were conducted six months apart, one in the spring of 2018, another in the following autumn. The first cichlid round consisted of 10 rock fed, 9 powder fed, and 9 pellet fed fish. The first zebrafish round consisted of 28 rock fed, 30 powder fed, and 33 pellet fed fish. The second round of cichlid experimentation consisted of 11 fish in all treatments, though one pellet fed fish was later removed as an outlier. The second round of zebrafish experimentation began with 20 fish in each treatment but was subject to unexpectedly high mortality and only 7 from each treatment completed the experiment. Additionally, a single lower pharyngeal jaw in the pellet group was damaged before it could be analyzed.

## 2.2 Whole Body Clearing and Staining

Following treatment, all experimental fish were euthanized ethically according to IACUC approved practices outlined in (Silverman/*et al.* 2014), before ocular dissection and evisceration. Each specimen was then fixed in a 4% Paraformaldehyde/PBST solution before being prepared for imaging via Trypsin-KOH digest tissue clearing, Alizarin Red bone staining, and Glycerol clearing/storage following (Albertson & Yelick, 2007). The final specimens were stored in an 80%/PBST mixture.

## 2.3 Imaging, Dissection, and Morphometrics

The full head of each zebrafish, viewed laterally, was imaged in backlit 80% glycerol via a Leica M165 FC dissection microscope mounted with a Leica DFC 450 C digital camera. A stand-mounted Olympus, EOS DSLR camera was used to accomplish the same task for *M. zebra*. Following lateral imaging, all cichlids and 60 randomly selected zebrafish were dissected for their lower-right pharyngeal jaw (though the lower pharyngeal jaws are fused in cichlids, meaning this dissection often resulted in the full extraction of the apparatus), which were all also imaged (from the dorsal/anterior perspective) via the Leica M165 FC/DFC 450 C. Images were converted to high quality jpegs and digitized with continuous, morphologically distinct semi-landmark curves via the R package ‘StereoMorph’ (Olsen 2017). These curves were placed around the opercle (including subopercle in *D. rerio*), hyomandibula/interopercle, premaxilla, orbitals, and the skull profile from the anterior end of the ethmoid to the tip of the supraoccipital crest. An additional curve was placed on *D. rerio* specimens that spanned the

parasphenoid as it traversed the orbital diameter. Lateral landmarks, with the exception of the orbital bones around the eye and dorsal crest, were chosen because the bones involved play important roles in feeding mechanics. For example, the opercle, hyomandibula, interopercle, and premaxilla each represent constituent bones of the four bar linkage system responsible for the operation of jaw protrusion/closure in both zebrafish and cichlids (Diogo/*et al.* 2008)(Westneat 1990). The dorsal crest was landmarked to ascertain the degree of head depth and angle, as the morphology of benthic feeding groups should, at least in cichlids, feature deeper, more downward facing heads which are comparatively larger to general features (Liem & Osse 1975). Orbitals, meanwhile, were marked to provide an estimate of eye size/position, as water-column prey feeders require better distance acuity (Baxter 1980). Additionally, possibly because of improved benefits for energetic cost, eye size may also increase with prey size, thus it might be expected pellet fed groups possess larger orbital regions (Aksnes & Giske 1993). Finally, benthic foragers tend to have eyes positioned dorsally in the head, compared to pelagic foragers (Otten 1983) Pharyngeal jaws were selected due to their obvious relevance when it comes to prey processing for both zebrafish and cichlids. Zebrafish possess no oral jaw teeth, necessitating any actual mastication be done at the rear of their feeding apparatus (Huysseune & Sire 1998). Cichlids, in particular possess a unique, fused lower pharyngeal jaw and muscle complex specific to their morphology. It has been theorized this trait is key to their overall morphological diversity, as it has allowed for a mechanical decoupling of the oral and pharyngeal jaws (Liem 1973).

The placed morphometric curves were converted to x,y coordinates. First, Procrustes distance ANOVAs were conducted on non-size-adjusted data to assess the

relative effects of size (*i.e.*,  $\log(\text{CS})$ ), experimental round, and treatment on craniofacial and pharyngeal jaw shape. Because size typically had a significant effect on shape, the data were then corrected for allometry via a regression of shape on the log of Centroid Size (CS). Residuals from this analysis were used for all subsequent analyses. Allometry corrected shape data were utilized to conduct Procrustes distance ANOVAs for shape differences in both the pharyngeal jaw and lateral head between treatments, as well as between experimental replicates. Additionally, principal component analyses were conducted and 1-4 were plotted on cartesian planes, forming polygon plots. Since it is possible that diet treatments could influence not just shape means but also variation, morphological disparity between treatment groups was calculated. Statistical modelling made use of R's 'geomorph' package (Adams/*et al.* 2019), alongside the separate function 'polygon.plot' developed by Michael Collyer (Gilbert 2018).

## CHAPTER 3

### RESULTS

The foraging treatments employed here were intended to engage different functional units of the fish feeding apparatus and challenge each region to mount a plastic response. It was expected that *M. zebra* would be capable of more pronounced plasticity than *D. rerio*, but that each would likely exhibit some degree of accommodation for novel feeding behaviors. Due to the zebrafish's lack of oral (and upper pharyngeal) dentition and unique anatomy of the cichlid pharyngeal jaw, it was unclear how much variation would be directly shared between species, as each phenotype undoubtedly requires the employment of distinct feeding behaviors. Furthermore, the kinematics of oral jaw protrusion, which is typically involved in suction feeding, is largely distinct in these species. Protrusion in cichlids is enabled by a relatively long ascending arm of the premaxilla, which slides over the rostral cartilage as the jaw extends anteriorly (Otten 1983). Zebrafish, on the other hand, possess relatively short ascending arms, and protrusion is instead driven by the kinethmoid, which sits under and is connected to the ascending arm via a complex series of ligaments, and pushes the upper jaw anteriorly as the kinethmoid rotates forward (Hernandez/et al. 2007). How each action loads the oral jaw apparatus is largely unclear. In spite of the obvious differences in craniofacial anatomy and function between cichlid and zebrafish, we make the following generally predictions: In the benthic treatment, there should be some degree of compaction of the oral jaw in both species. To accommodate this mechanical load jaws and faces should become shorter, with a more steeping sloping skull profile. In both water column feeding treatments, the development of larger eyes is expected. In the powder treatment, we

expect to observe more gracile oral jaws. Finally, the pellet treatment groups are expected to develop robust pharyngeal jaws and perhaps deeper heads to accommodate the musculature associated with the functional apparatus.

### **3.1 Lateral Craniofacial Morphometrics**

Among zebrafish treatments little difference in shape was detected (Procrustes distance ANOVA,  $f=1.486$ ,  $z=1.4602$ ,  $r^2=0.027$ ,  $p=0.0712$ ) suggesting differences due to random chance cannot be ruled out at 95% confidence. Its relative proximity to the threshold of  $p=0.05$  can be attributed to a significant difference in shape between benthic and pelagic treatment groups ( $p=0.0138$ ), while all other differences between treatments were found to be well above 95%. Of the first six axes, only PCs 4 and 5 approached significance in an ANOVA ( $p=0.0595$  and  $p=0.0684$ , respectively), with each explaining <10% of morphological variation. Visual analysis of morphospace via polygon plots of the first four principal component axes indicated strong overlaps between all specimens, except for a slight, yet observable divergence between benthic and pellet fed groups along PC axes 2-4.

Visual analysis of mean consensus shapes for each treatment group suggested benthic fish developed a deeper and more steeply descending head, and somewhat larger interopercle/preopercle region. The opercle and subopercle region of the two water-column-feeding groups appeared relatively large. In pellet fish, eye size is somewhat larger and dorsally positioned, and the parasphenoid appears to be displaced ventrally within the orbit. While statistical support for these differences are low, aspects of shape

that do differ between groups are largely consistent with our predictions. Due to large differences in survivorship between experimental rounds, and differences in sample size, we did not test for between experiment effects on the data. A test for outliers revealed two specimens that crossed the threshold by only a slim margin, thus they were not discounted.

In contrast to zebrafish, Procrustes distance ANOVA on size-corrected, lateral *M. zebra* landmark data revealed strong differences between treatments ( $f=2.408$ ,  $z=3.0577$ ,  $r^2=0.0792$ ,  $p=0.0011$ ), with differences ( $p<0.05$ ) observed between all treatments as well. Of the first six PC axes, differences were predominately observed in the 1st axis ( $p=0.05123$ ), which accounted for 25.2% of the total variation, and the 4th axis ( $p=0.00027$ ), which explained 8% of the variation. In a test for morphological disparity, differences between pellet and benthic were larger, but still were not statistically significant. This was somewhat corroborated by visual analysis of morphospace along the first four PC axes (made possible with polygon plotting), which suggested there may be a slight expansion in morphological disparity from benthic, to pelagic, to pellet.

Visual analysis of mean consensus shapes per treatment showed a number of notably trends. In the benthic fish, the oral jaws were short, and the skull was steeply descending. This was accompanied by a more dorsal-posteriorly positioned orbital position and larger hyomandibula/preopercle/interopercle complex. In the pelagic group, animals possess larger and more ventrally positioned eyes. In addition, these fish appeared to have longer jaws and larger opercles. All in all, these differences were consistent with our expectations and similar to changes observed in other cichlid species in response to similar diet challenges (Parsons/*et al.* 2014). Notably, the pellet treatment

resulted in fish with a mosaic of benthic/pelagic skull morphologies, such as longer jaws and larger eyes and opercles similar to pelagic fish, and dorsally positioned eyes and large hyomandibula/preopercle/interopercle complexes like benthic fish.

An ANOVA estimating the relative contribution of variation from experimental round and treatment found both were significant factors, but that treatment had a greater impact ( $f=2.5370$ ,  $z=4.1680$ ,  $p=0.0001$ , compared to  $f=1.5265$ ,  $z=3.2623$ ,  $p=0.01$ ). A test for outliers revealed only one individual that was not discounted from the dataset due to its proximity to the outlier threshold.

### **3.2 Pharyngeal Jaw Morphometrics**

A Procrustes distance ANOVA for shape difference between treatments in *D. rerio* returned significance ( $f=2.7668$ ,  $z=2.9005$ ,  $r^2=0.09$ ,  $p=0.0013$ ), including pairwise differences ( $p<0.05$ ) between all treatments. Of the first six PC axes, 2-4 were significant at  $P<0.05$ , and 1 fell close at  $p=0.07374$ . No differences in morphological disparity were detected. This was reflected in relatively little difference in the size of morphospace occupied by polygon plots along PC axes 1-4. However, all three seem fairly segregated with respect to treatment when plotting  $x=PC\ 2$  to  $y=PC\ 3$ , while the benthic group is more morphologically variable along PC 4 than its counterparts, as is the pelagic group along PC 2. Visual analysis of mean consensus shape per treatment indicated pelagic and pellet groups possessed relatively larger tooth plates compared to the benthic group. In addition, each treatment possessed a dorsal muscular process which angles inward toward the concave edge of the tooth plate, and a ventral process which angles away from the



concave edge. The case is the opposite for benthic treatment fish. All of the randomly selected pharyngeal jaws were sourced from the first replicate, so no testing for differences due to experiment was conducted. Upon analyses for outliers, no specimens were returned.

For *M. zebra*, the Procrustes distance ANOVA by of size-corrected data by treatment was not significant ( $f=0.9802$ ,  $z=0.14732$ ,  $r^2=0.0333$ ,  $p=0.4471$ ), neither were any single treatment comparisons. Only PC Axis 3 approached significance at  $p=0.05747$ , and there were no differences in morphological disparity. There were also no obvious differences in mean consensus shapes per treatment, and polygon plots of the first four PC Axes only suggested a mild increase in occupied shape space in pelagic fish versus other groups. An ANOVA to estimate the relative contribution of variation from experimental round versus treatment found no significance for either but a greater effect due to treatment ( $f=1.0589$ ,  $z=0.35966$ ,  $p=0.3636$ , opposed to  $f=0.6994$ ,  $z=0.16156$ ,  $p=0.5735$ ). An outlier test revealed one individual that was not discounted due to extreme proximity to the threshold.

## CHAPTER 4

### DISCUSSION

The results garnered from repeat trophic niche exposure experiments within *D. rerio* and *M. zebra* suggest both species do indeed exhibit some degree of morphological plasticity in the face of disparate feeding regimes. However, the patterning of this restructuring is inconsistent between species. There is considerable change in the gross cranial morphology of *M. zebra* in the lateral view. In fish reared in the benthic treatment, the head angles downward and the premaxilla is shorter and more concave (possibly due to compaction), while they bear smaller eyes that are displaced dorsally. Notably, these anatomical changes predict functional shifts to accommodate the generation of greater bite force while feeding, and are similar to cichlid species that are adapted to the benthic foraging niche (Waingwright & Richard 1995)(Hulsey/*et al.* 2013). In opposition to this, *M. zebra* exhibit almost no morphological changes in the pharyngeal jaw in response to foraging treatments. This is somewhat surprising given how well documented plasticity in the cichlid pharyngeal is in the literature. Muschick/*et al.* (2011), for example, identifies differences in pharyngeal jaw due to diet. Multiple factors were measured, including geometric morphometrics which found significant differences in shape (though weight measurements were more significantly different by feeding treatment). However, the feed employed in experiment was hard-shelled snails vs. no-shell snails, which represents an arguably greater disparity in mechanotransduction than the pellets utilized here. Additionally, landmarking was conducted on the whole fused lower jaw, and made use of 8 fixed points across the shape. Methodologically, this is distinct from the previously discussed methods of this study, as only 2-3 fixed landmarks were assigned to

each specimen and these were connected via continuous, curves containing more than 8 equidistant semi-landmarks.

The application of these continuous curves was enabled via the R package ‘StereoMorph’, which allows the statistician the ability to draw a line on an organic shape and then retroactively apply evenly spaced semi-landmarks to it. This represents a putative analytical improvement over previous digitizing methods, as more organic shape variation is captured by comparison to previously developed methods, which require all landmarks to be individually placed by hand and then retroactive demarcation of ‘fixed’ or ‘semi’. The result is a theoretically more evenly balanced analysis of shape across a region in question, but this greater fidelity may also produce a ‘coastline paradox’ effect on more disparately shaped datasets. Akin to the apparent inverse proportionality of a coastline’s distance to the rule of measure employed (Pant & Pant 2013), the process of increasing landmark number per distance may reduce the ability to capture whole shape variation, as more minor variation is subsequently considered. Based upon apparent detectable differences in *D. rerio*, it is unclear this is the only factor involved, though it may play a role, and regardless may suggest greater benefit in utilizing gross measures. For example, Chapman/*et al.* (2008) noted plasticity of morphology in the lower pharyngeal jaw, by examining keel depth, a measurement with comparatively low dimensionality. Given the degree of methodological distinction between this approach, the previously discussed, and those documented in this research, it seems reasonable all conclusions may be accepted without conflict.

Notably, zebrafish exhibit the opposite trend compared to cichlids, with the plastic responses localized to the pharyngeal jaw. In fish from the pellet/pelagic

treatments, there is an apparent expansion overall size, accompanied by larger, more anterior facing muscular processes compared to benthic fed fish. Both treatments appear to exhibit a morphology that is associated with increased mechanotransduction due to chewing and subsequent bone deposition. It would be valuable to complement these anatomical findings with molecular data. For instance, are genes involved in mechanosensing and/or bone deposition upregulated in some treatments over others? It is difficult to determine the functional significance of these shape changes, and transcript data could provide insights.

Plastic responses in *D. rerio* thus far appear to be limited to the region of the skull directly associated with prey processing. That zebrafish lacked a large-scale plastic response in the lateral view could be due to a number of factors. Lack of oral jaw dentition undoubtedly presents a challenge to benthic treatment specimens, as there was no way to chew at the anterior of their jaw apparatus. Additional landmarking, or an alternate landmarking schema, may have uncovered more variation between treatments, but to retain comparability, a similar method was used for each species. Given the differences in morphology and behavior between *M. zebra* and *D. rerio*, it is not unreasonable to think variation in shape due treatments may accumulate in different manners. Behavior, in turn, could be necessitated by species specific feeding kinematics. It has been previously suggested mechanical advantages of specific feeding apparatuses do not necessarily scale with body size, so it may be incorrect to assume similar gross morphological variance bears any relevance to comparing the biophysics of trophic exploitation at the interspecific level (Hernandez 2000). Additionally, this is complicated by explicit distinctions in the anatomy of the fish in question. Oral jaw protrusion in *D.*

*rerio* is driven by a bone novel to cyprinids, the kinethmoid, which is connected posteriorly to the articular process of the premaxilla via ligament. Other ligaments spanning between the kinethmoid and palantine/maxilla/neurocranium coordinate to rotate the small bone 90° during jaw protrusion (Hernandez/*et al.* 2007). This is mechanically distinct to jaw protrusion in cichlids, which rely upon pressure applied by the vomer and anterior swing of the maxilla to occur (Otten 1983). Presumably, due to this difference, the applied forces of each system do not directly overlap in direction or magnitude, and this alternate application of mechanotransduction may manifest alternate oral jaw phenotypes in response to similar treatment. This is also the case for the pharyngeal jaw, as the lack of teeth in zebrafish anywhere but the lower pharyngeal jaw-set (Mabuchi/*et al.* 2007), distinguishes it from the cichlid, which possesses teeth on both upper and lower oral jaws, and both pharyngeal. Additionally, cichlids possess fused lower jaws which have necessarily been posteriorly anchored to novel processes at the rear of the mouth (Liem 1973). Both of these aforementioned factors represent divergence in form and function of the experimentally manipulated structures. However, in addition to the physical limitations at the morphological level, these differences may also be the product of differently canalized patterns of regulation surrounding jaw development at the molecular level. Subsequently these morphologies may be differently canalized in *M. zebra* and *D. rerio*, directing differences in the pattern or magnitude of the plastic response between species.

Thus, these results permit the proposition that not only do the distinctions in plasticity between these two species bear origins which are behavioral, anatomical, or genetic in nature, but each origin undoubtedly contributes in part to various trait liabilities.

Behaviorally, for instance, *M. zebra* appears a much better fit for exploiting novel trophic niches. They do not require training to learn exploitation of experimental trophic niches and each fish appears independently capable of learning novel feeding behaviors.

Conversely zebrafish, do not easily take to new regimes, and purportedly exhibit high degrees of variability in their learning proficiency (Bilotta/*et al.* 2005). As a result, a lower number of *D. rerio* can be expected to acclimate to new feeding behaviors on their own, and group learning is likely necessary to encourage rapid uptake of novel methods for trophic niche exploitation. Indeed, it was noted in a pilot study that ~1/3 of zebrafish reared on a benthic diet in isolation became malnourished from lack of feeding before they learned how to feed benthically. However, this apparent behavioral distinction between species is additionally complicated because it is difficult to differentiate the degree to which behavior is divorced from morphology. As has been previously discussed, each species bears distinct characteristics. Between the lack of oral jaw teeth on *D. rerio*, the fused ceratobranchials of cichlids, the general difference in body depth and the disparate gross morphology of each species' head, there are many aspects of the morphologies in question that could recursively inform behavior.

While the relative contribution of different plasticities remains in question, the evolutionary basis of overall differences between species may become clearer if we look beyond the laboratory back toward the wild evolutionary landscape occupied by each organism. From this perspective, it seems differences in localization of plasticity might be due to distinctions in canalization of phenotypes between the lineages, because selection has favored the conservation of specific developmental pathways within the disparate phylogenies (Siegal & Bergman 2002). For instance, evolution has seemingly

reduced the zebrafish's craniofacial lability in response to mechanotransduction. Given that the laboratory and wild environment of this small fish tends to be slow moving pools of water with little to no rocky substrate, we might understand why (Engeszer/*et al.* 2007). Such environments present little opportunity for excessive mechanical input, and requires a small body due to low fluid volume of habitats (reducing both nutritional availability and physical space). After generations, the *D. rerio*'s ability to accommodate for these stresses is reduced. *M. zebra*, conversely, evolved in a highly dynamic, deep-water rift lake environment with great a disparity in exploitable ecologies (Ribbink/*et al.* 1983). Because this represents a recent environmental factor in their phylogeny, it follows this species' gross morphology would be more plastic, as this would enable greater degrees of alternate habitat exploitation during colonization. An interesting topic of future investigation would be to determine whether plasticity in *M. zebra* evolved within this lineage of Malawi cichlid, or represents the retention of an ancestral feature.

Despite some degree of disparity in level of manifestation between species, it seems reasonable to consider the deformation visible here as accommodations for the applied experimental feed regimes. Accommodations, theoretically, represent flexibilities an organism exercises in the face of environmental challenges. Characterized initially as an organism's ability to utilize developmental plasticity to overcome pathologies or unique morphology, we can also extend accommodations to include wild-type organisms experiencing novel inputs as well. Generally, if a novel behavior or morphology not only accommodates for the challenges of a new environment, but also improves fecundity despite it, then the ability to develop the trait more efficiently, the plasticity itself, should propagate through the population in question (West Eberhard 2005). Given the

differences due to treatment suggested here, it seems possible such a scheme would be corroborated by phylogenetic evidence, or perhaps multi-generational studies in invertebrates with shorter generation times.

From what can be interpreted here, it seems the plasticities of zebrafish and cichlids do bear some comparability in the laboratory setting. While the individual response of each species remains disparate at the level of craniofacial versus pharyngeal jaw shape, there is concrete evidence the restructuring of bones occurs in each fish in response to treatment. Based upon this we may hypothesize that the responses bear homology at the genetic level and thus share phylogenetic history, as the bones in question are subject to long-conserved mechanically sensitive osteogenic pathways (Witten & Hall 2015).

This too requires further exploration to confirm, but the results presented here do establish suggestions on which tissues may be ripe for molecular analyses of transcriptional similarity. Already, there is some evidence that signaling in the *Hedgehog* pathway precedes divergent phenotypes in the cichlid feeding apparatus (Hu & Albertson 2014) and may play a role in mechanically induced morphological plasticity (Navon 2019). Additionally, they provide evidence that alternate trophic regime studies may be augmented via the addition of a third feeding parameter in hard pellet feeding. Statistical differences were found for both species subjected to this treatment, suggesting additional exploration of methodological permutations may allow for a more nuanced picture of the landscape for teleost craniofacial plasticity. The novel three-pronged feeding approach may be of even further benefit in interspecies studies such as the previously discussed, as differences between species manifested at alternate levels of morphological organization,



one of which (the pharyngeal jaw) was expected to be particularly stimulated via pellet feeding treatments. That being said, based upon the methods of other studies on ceratobranchial plasticity, it may be prudent to also explore alternate analyses of similar data, as some of this disparity might be due to the measurement employed, especially considering confirmation of plasticity in each species via new metrics would only stand to improve the case for regularly including pellet treatments in the experimental model. In addition, it may be useful to fine-tune food-type or delivery methods, as the pharyngeal jaw apparatus, especially in cichlids, may require food which induces more forceful processing than what was used here to induce a plastic response. Overall and in spite of whatever methodological challenge, the takeaway of this study represents a good prognosis for the analytical power of alternative trophic niche studies, past and future.

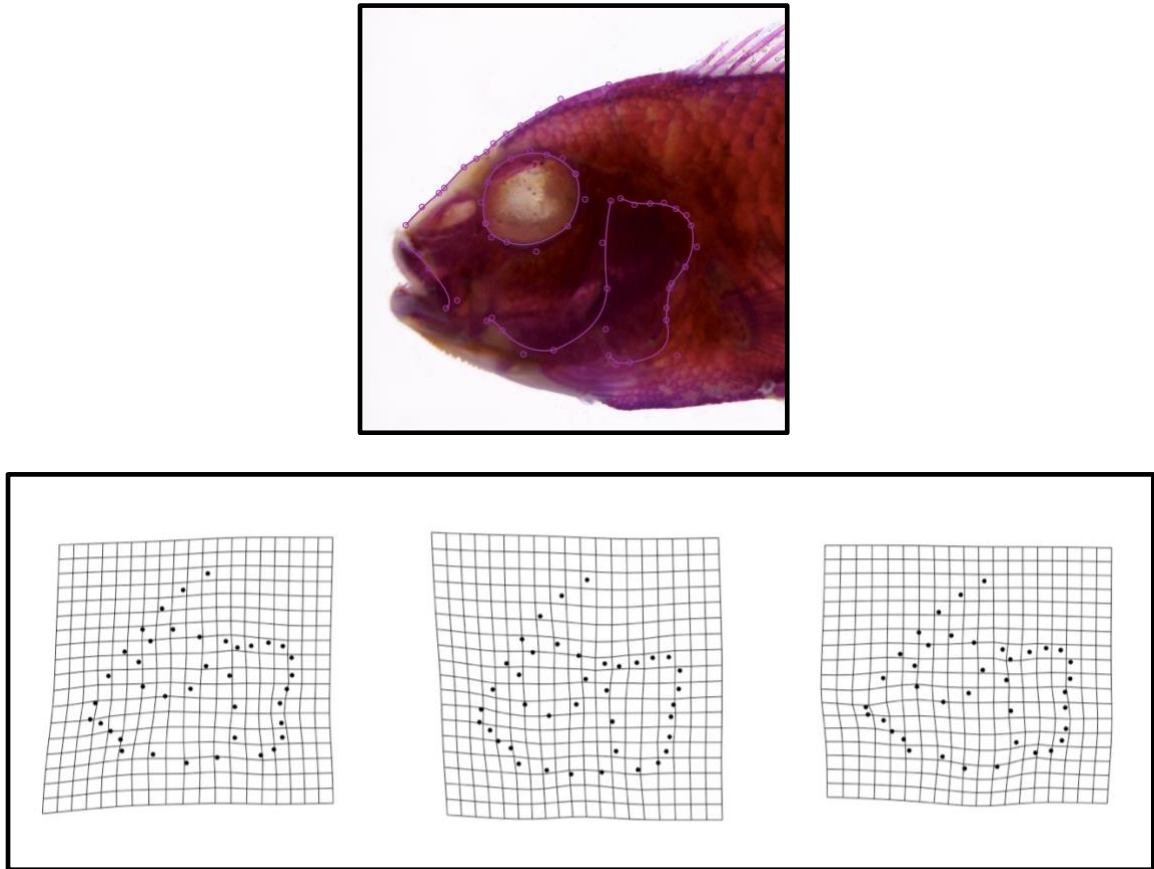
## CHAPTER 5

### CONCLUSIONS

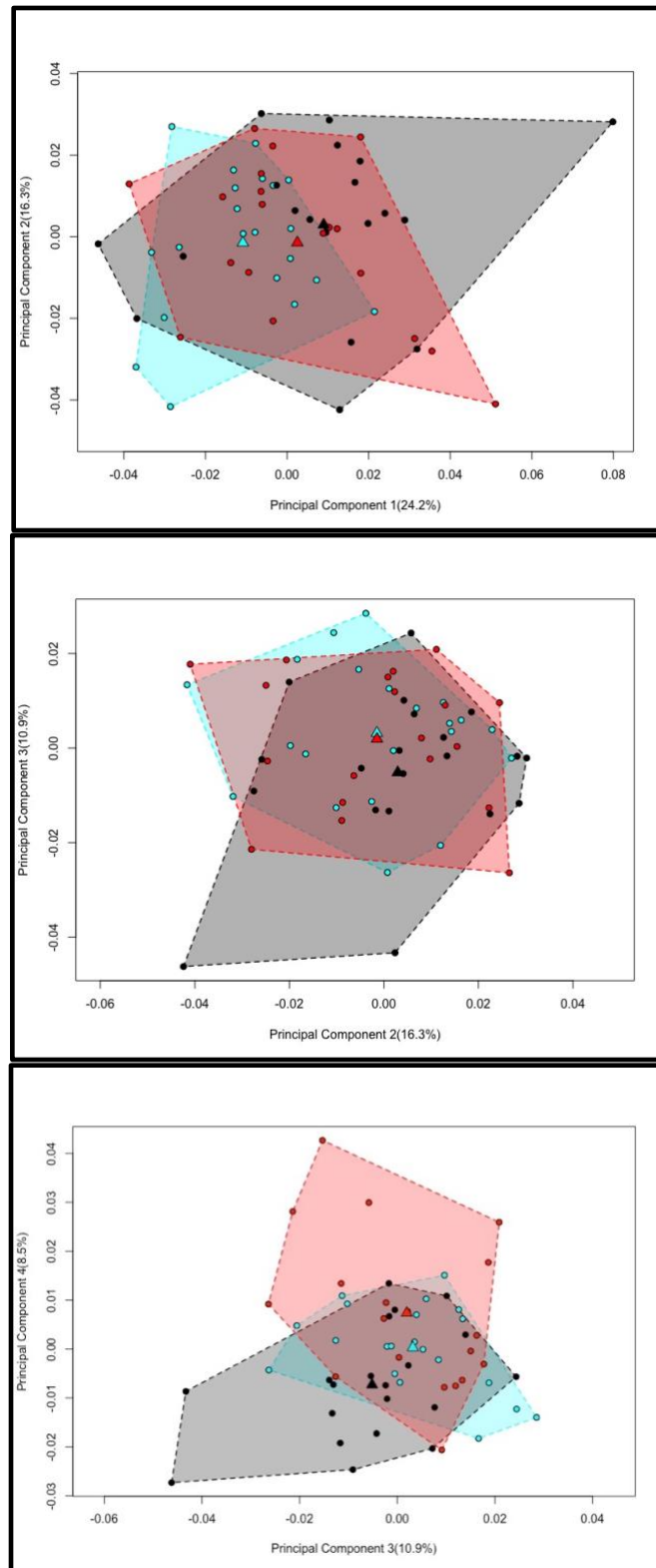
Both *D. rerio* and *M. zebra* are capable of mounting morphological/behavioral plastic responses in the face of novel feeding environments, though each is different and can largely be accounted for once form and function relationships are considered. Broadly, this means they do bear comparison in laboratory settings when keeping disparity of canalized traits of feeding apparatuses in mind. Additionally, the results of the previously discussed research show promise for both the application of three-part alternative trophic niche experimentation and the possibility of deep genetic homologies for craniofacial restructuring in fish, but confirmation of these notions would undoubtedly require further examination of the subject (for example, in Navon 2019). Molecular analyses and alternate measures of physical properties are especially recommended for future study.

APPENDIX: TABLES AND FIGURES

Lateral Data Tables and Figures



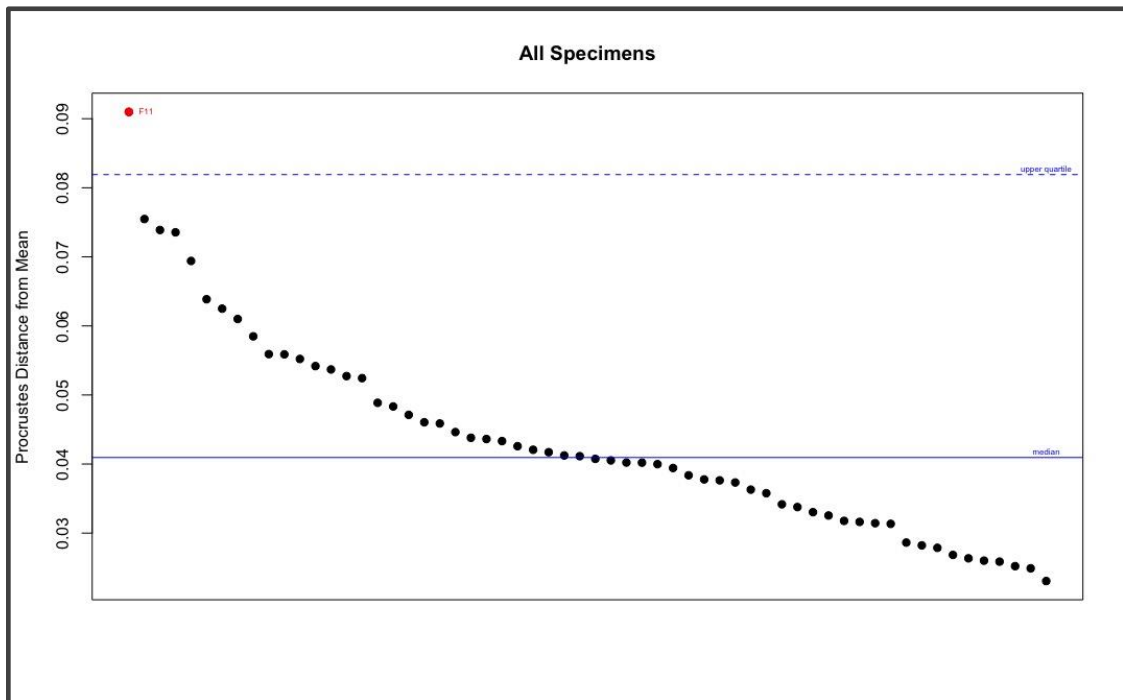
**Figure 1: *M. zebra* lateral morphometric mean shape per treatment. Each panel represents deformation from standard mean of all fish regardless of treatment. Pictured left to right, benthic treatment, pelagic treatment, and pellet treatment groups.**



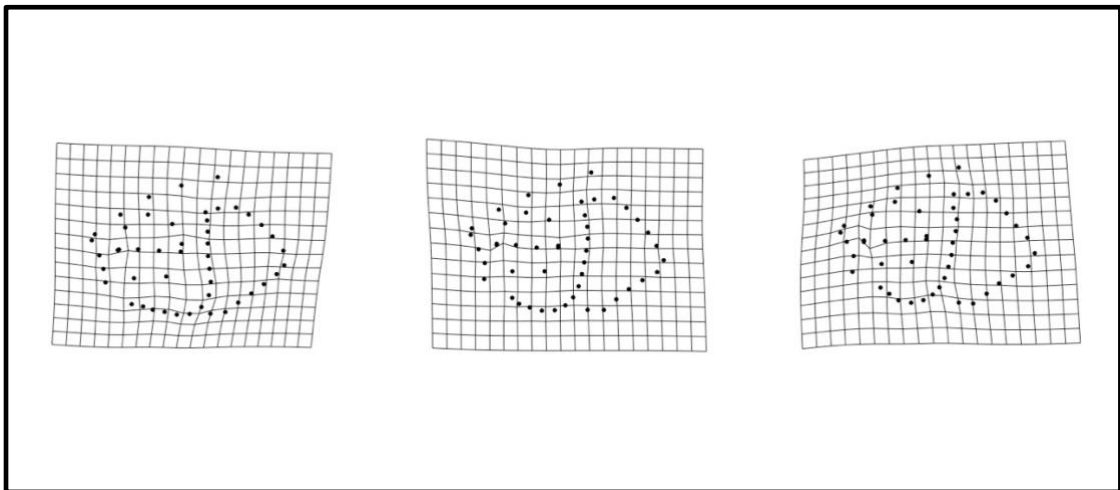
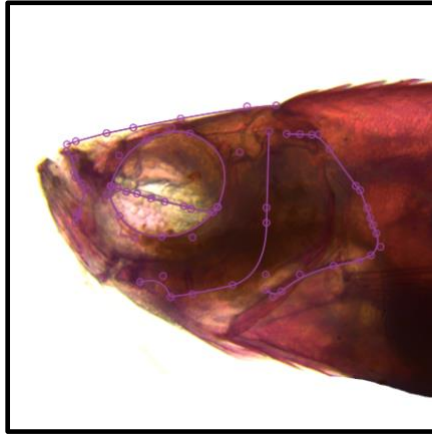
**Figure 2: Polygon plots of Principal Component axes 1-4 for *M. zebra* lateral morphometric data. Benthic treatments are colored in cyan, pelagic in black, pellet in red.**

<b>Advanced Procrustes D Linear Model ANOVA (By Treatment)</b>							
	<b>DF</b>	<b>RSS</b>	<b>SS</b>	<b>R-squared</b>	<b>F-value</b>	<b>Z-score</b>	<b>P-value</b>
	56	0.11638	0.0100000	0.079195	2.4082	3.0577	0.0015
<b>P-values/Effect Sizes (b/t Treatments)</b>				<b>Morphological Disparity P-values/Abs. Diff.</b>			
	<b>Benthic</b>	<b>Pelagic</b>	<b>Pellet</b>		<b>Benthic</b>	<b>Pelagic</b>	<b>Pellet</b>
<b>Benthic</b>	1/0	3.92545	1.958990	<b>Benthic</b>	1/0	0.0008	0.0006
<b>Pelagic</b>	0.0010	1/0	2.050589	<b>Pelagic</b>	0.0442	1/0	0.00022
<b>Pellet</b>	0.0384	0.0357	1/0	<b>Pellet</b>	0.1455	0.6088	1/0
<b>ANOVA of PC Axes</b>				<b>ANOVA Treat. Effect vs Exp. Effect vs Size</b>			
	<b>Mean Sq.</b>	<b>F-value</b>	<b>P-value</b>		<b>F-score</b>	<b>Z-score</b>	<b>P-value</b>
<b>1</b>	0.00142	3.0239	0.03704	<b>Log(CS)</b>	20.3731	6.5294	0.0001
<b>2</b>	0.000112	0.3099	0.8182	<b>Treatment</b>	2.5370	4.1680	0.0001
<b>3</b>	0.00036	1.5799	0.2047	<b>Experiment</b>	1.5265	3.2623	0.0002
<b>4</b>	0.00070	4.5772	0.00617				
<b>5</b>	0.00025	1.7746	0.1625				
<b>6</b>	0.00041	4.4528	0.00710				

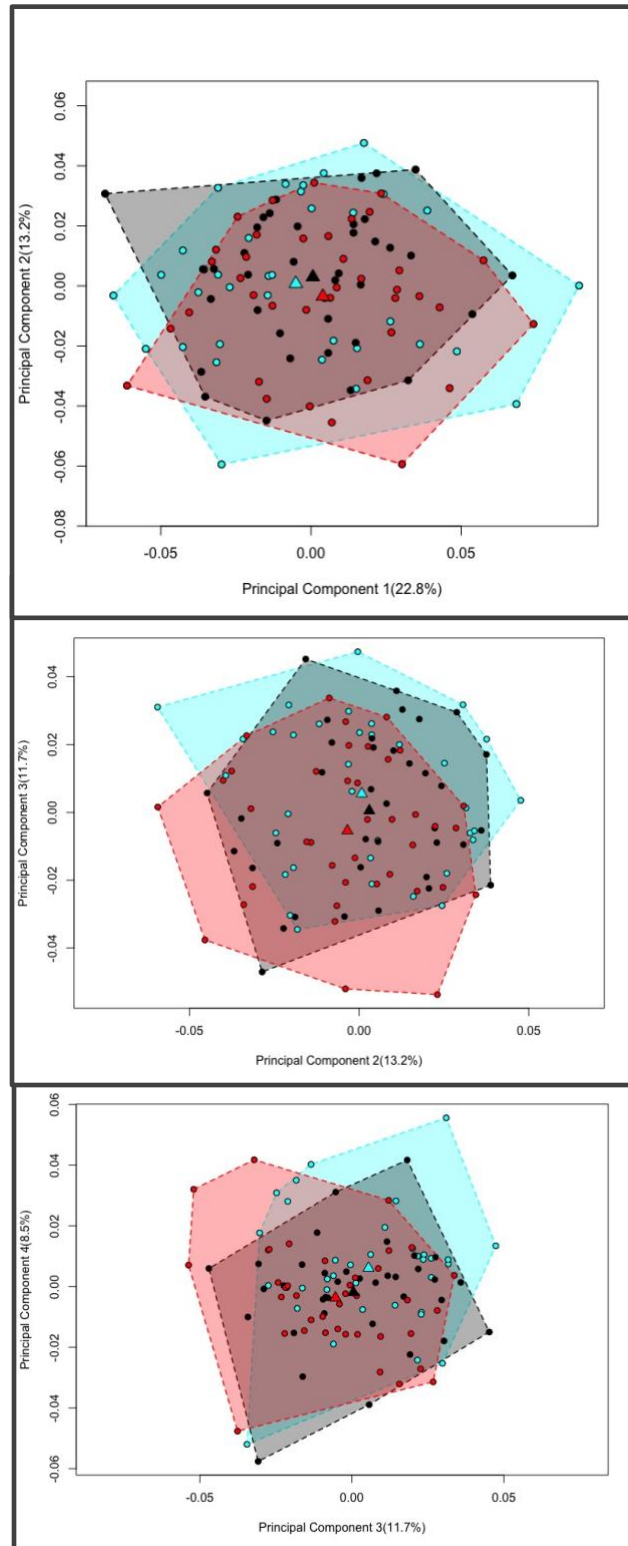
**Table 1: Numerical returns for all statistical testing on *M. zebra* lateral morphometric data. Divided tables are labelled in the respective order they are split.**



**Fig. 3: Results of an outlier test on *M. zebra* lateral morphometric data. The proximity of the lone outlier to the threshold led to its retention in the data set.**



**Figure 4: *D. rerio* lateral morphometric mean shape per treatment. Each panel represents deformation from standard mean of all fish regardless of treatment. Pictured left to right, benthic treatment, pelagic treatment, and pellet treatment groups.**

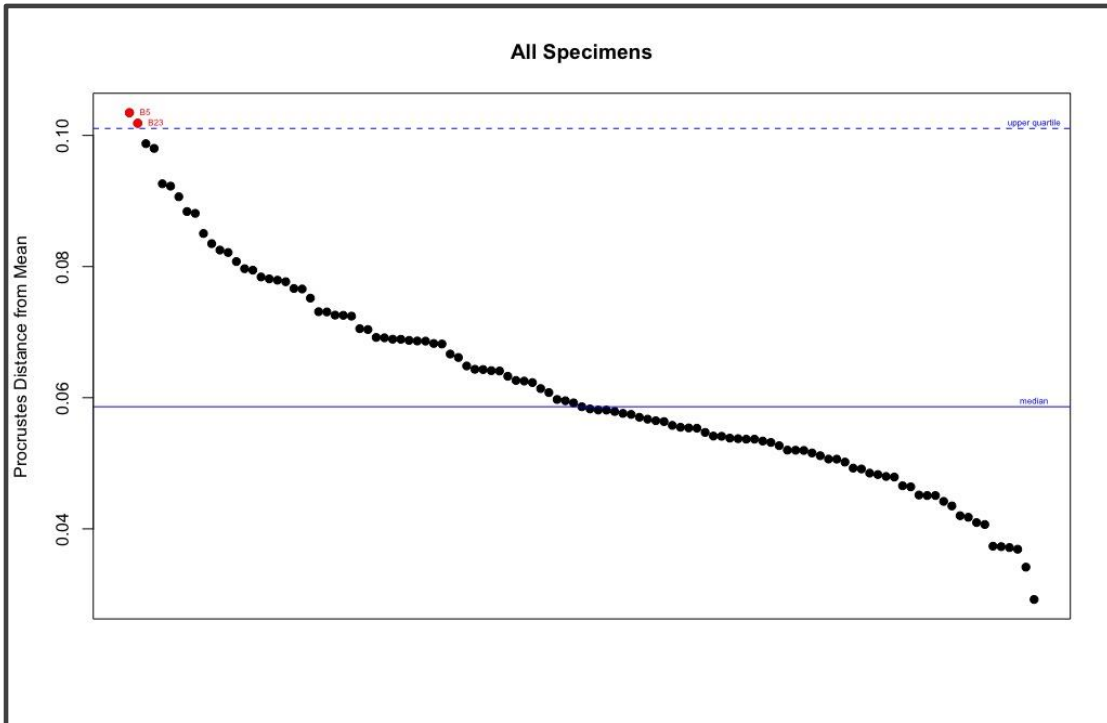


**Figure 5: Polygon plots of Principal Component axes 1-4 for *D. rerio* lateral morphometric data. Benthic treatments are colored in cyan, pelagic in black, pellet in red.**



<b>Advanced Procrustes D Linear Model ANOVA (By Treatment)</b>							
	<b>DF</b>	<b>RSS</b>	<b>SS</b>	<b>R-squared</b>	<b>F-value</b>	<b>Z-score</b>	<b>P-value</b>
	107	0.44139	0.01226	0.027026	1.486	1.4602	0.0712
<b>P-values/Effect Sizes (b/t Treatments)</b>				<b>Morphological Disparity P-values/Abs. Diff.</b>			
	<b>Benthic</b>	<b>Pelagic</b>	<b>Pellet</b>		<b>Benthic</b>	<b>Pelagic</b>	<b>Pellet</b>
<b>Benthic</b>	1/0	0.862593	2.646113	<b>Benthic</b>	1/0	0.00077	0.00092
<b>Pelagic</b>	0.1840	1/0	-0.29114	<b>Pelagic</b>	0.1151	1/0	0.00015
<b>Pellet</b>	0.0138	0.5738	1/0	<b>Pellet</b>	0.0524	0.7485	1/0
<b>ANOVA of PC Axes</b>				<b>No Testing for Experimental Effects in Danio</b>			
		<b>F-value</b>	<b>P-value</b>				
	0.00076						
<b>1</b>	0.00041	0.8021	0.4511				
<b>2</b>	0.00046	0.7487	0.4754				
<b>3</b>	0.00095	2.3367	0.1015				
<b>4</b>	0.00061	2.8971	0.0595				
<b>5</b>	0.00018	2.7495	0.0684				
<b>6</b>	0.00018	0.8921	0.4128				

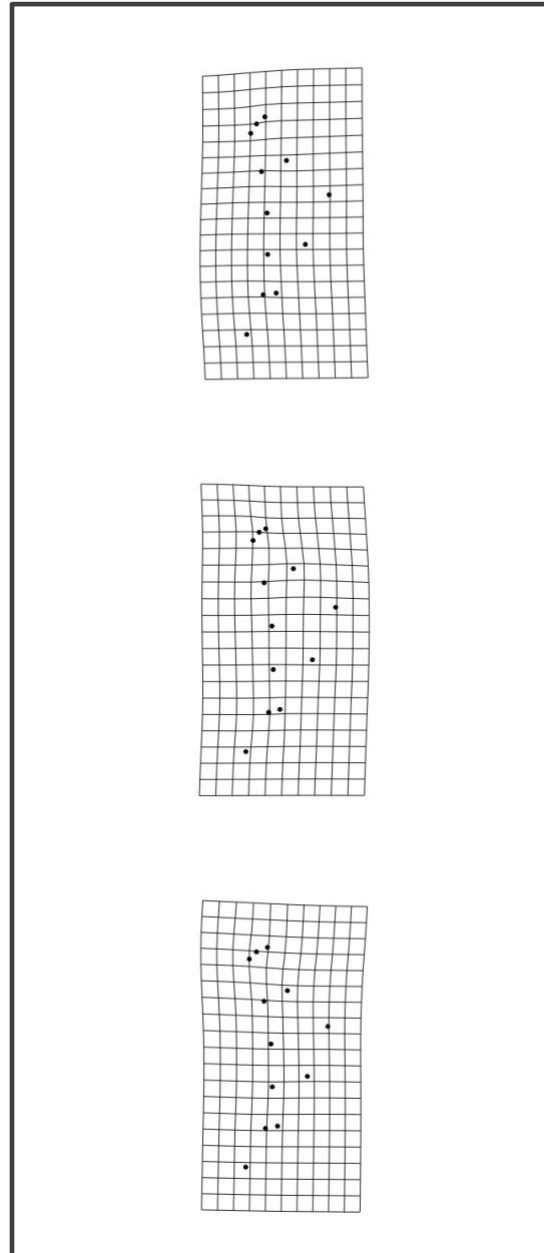
**Table 2: Numerical returns for all statistical testing on *D. rerio* lateral morphometric data. Divided tables are labelled in the respective order they are split.**

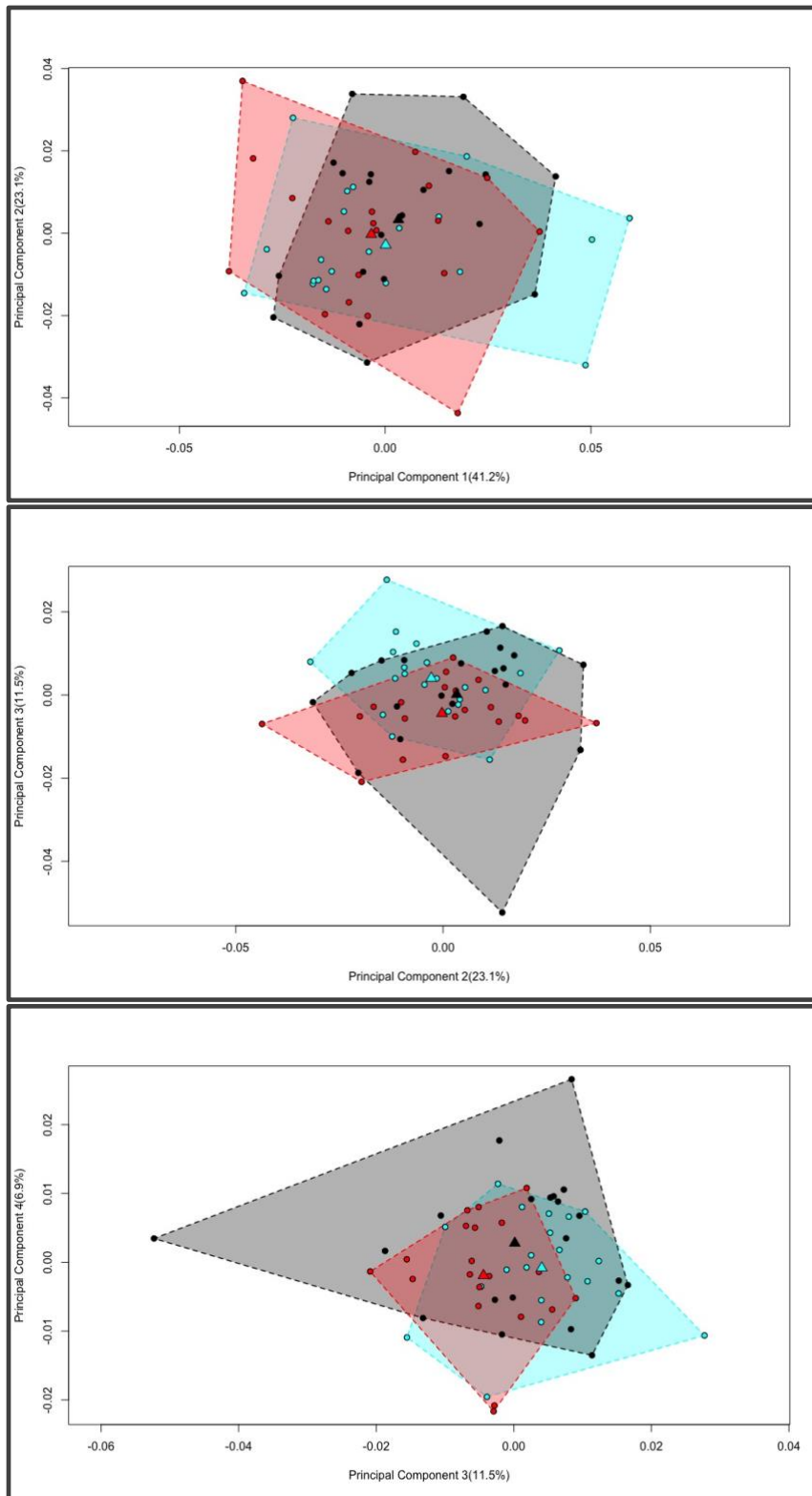


**Figure 6: Results of an outlier test on *D. rerio* lateral morphometric data. The proximity of the outliers to the threshold led to their retention in the data set.**

## Pharyngeal Jaw Data Tables and Figures

**Figure 7: *M. zebra* pharyngeal jaw morphometric mean shape per treatment. Each panel represents deformation from standard mean of all fish regardless of treatment. Pictured top to bottom: benthic treatment, pelagic treatment, and pellet treatment groups.**

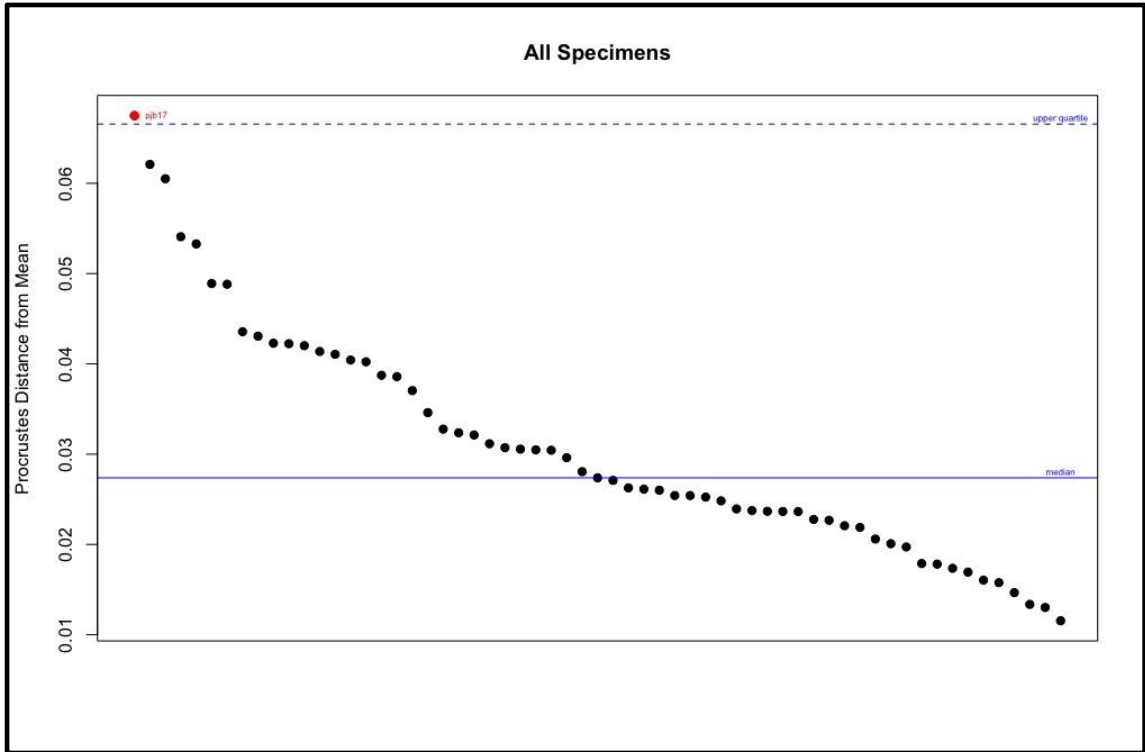




**Figure 8: Polygon plots of Principal Component axes 1-4 for *D. rerio* pharyngeal jaw morphometric data. Benthic treatments are colored in cyan, pelagic in black, pellet in red.**

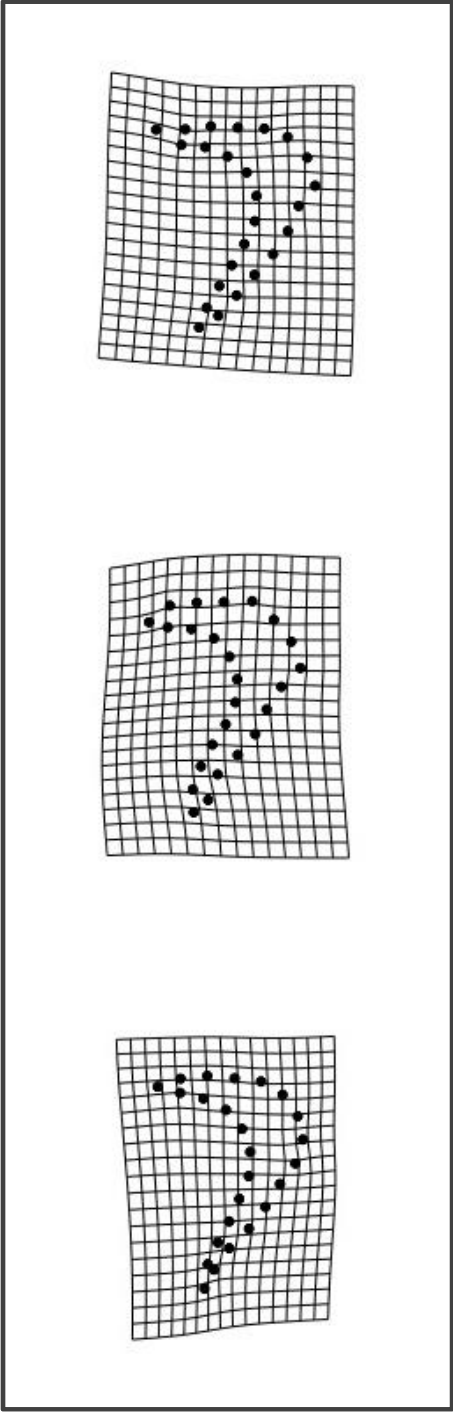
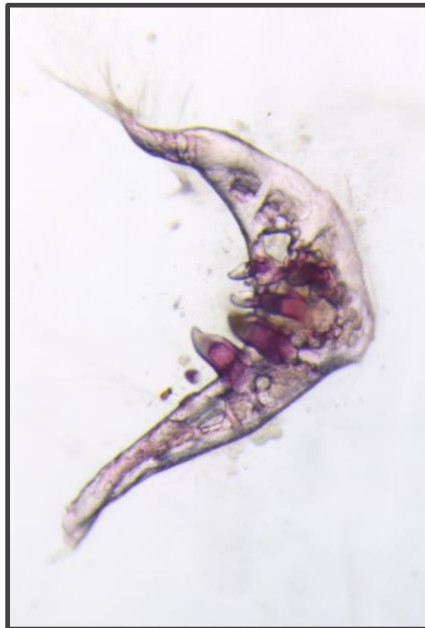
<b>Advanced Procrustes D Linear Model ANOVA (By Treatment)</b>							
	<b>DF</b>	<b>RSS</b>	<b>SS</b>	<b>R-squared</b>	<b>F-value</b>	<b>Z-score</b>	<b>P-value</b>
	57	0.06571	0.002260	0.03325	0.9802	1.4732	0.4471
<b>P-values/Effect Sizes (b/t Treatments)</b>				<b>Morphological Disparity P-values/Abs. Diff.</b>			
	<b>Benthic</b>	<b>Pelagic</b>	<b>Pellet</b>		<b>Benthic</b>	<b>Pelagic</b>	<b>Pellet</b>
<b>Benthic</b>	1/0	0.01001	0.01072	<b>Benthic</b>	1/0	0.00003	0.00023
<b>Pelagic</b>	0.4528	1/0	0.01098	<b>Pelagic</b>	0.8993	1/0	0.00020
<b>Pellet</b>	0.3799	0.3523	1/0	<b>Pellet</b>	0.4323	0.5185	1/0
<b>ANOVA of PC Axes</b>				<b>ANOVA Treat. Effect vs Exp. Effect vs Size</b>			
	<b>Mean Sq.</b>	<b>F-value</b>	<b>P-value</b>		<b>F-score</b>	<b>Z-score</b>	<b>P-value</b>
<b>1</b>	0.00022	0.4582	0.6347	<b>Log(CS)</b>	1.4893	0.91384	0.1834
<b>2</b>	0.00019	0.7355	0.4837	<b>Treatment</b>	1.0589	0.35966	0.3636
<b>3</b>	0.00037	3.0019	0.0575	<b>Experiment</b>	0.6994	-0.1616	0.5735
<b>4</b>	0.00012	1.5711	0.2165				
<b>5</b>	0.00021	0.3730	0.6903				
<b>6</b>	0.00080	2.0235	0.1414				

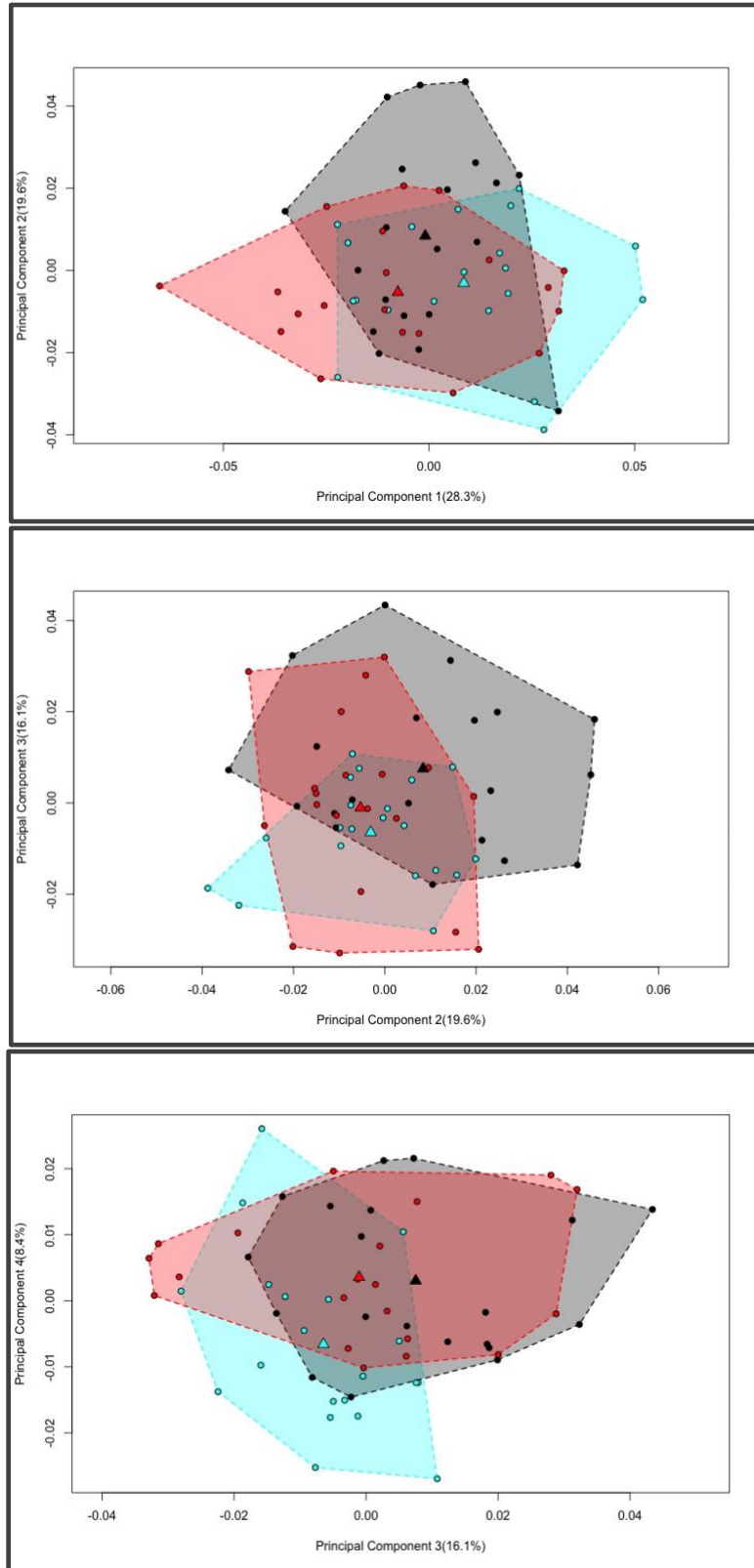
**Table 3: Numerical returns for all statistical testing on *M. zebra* pharyngeal jaw morphometric data. Divided tables are labelled in the respective order they are split.**



**Figure 9: Results of an outlier test on *M. zebra* pharyngeal jaw morphometric data. The proximity of the outlier to the threshold led to its retention in the data set.**

**Figure 10: *M. zebra* pharyngeal jaw morphometric mean shape per treatment. Each panel represents deformation from standard mean of all fish regardless of treatment. Pictured top to bottom: benthic treatment, pelagic treatment, and pellet treatment groups.**



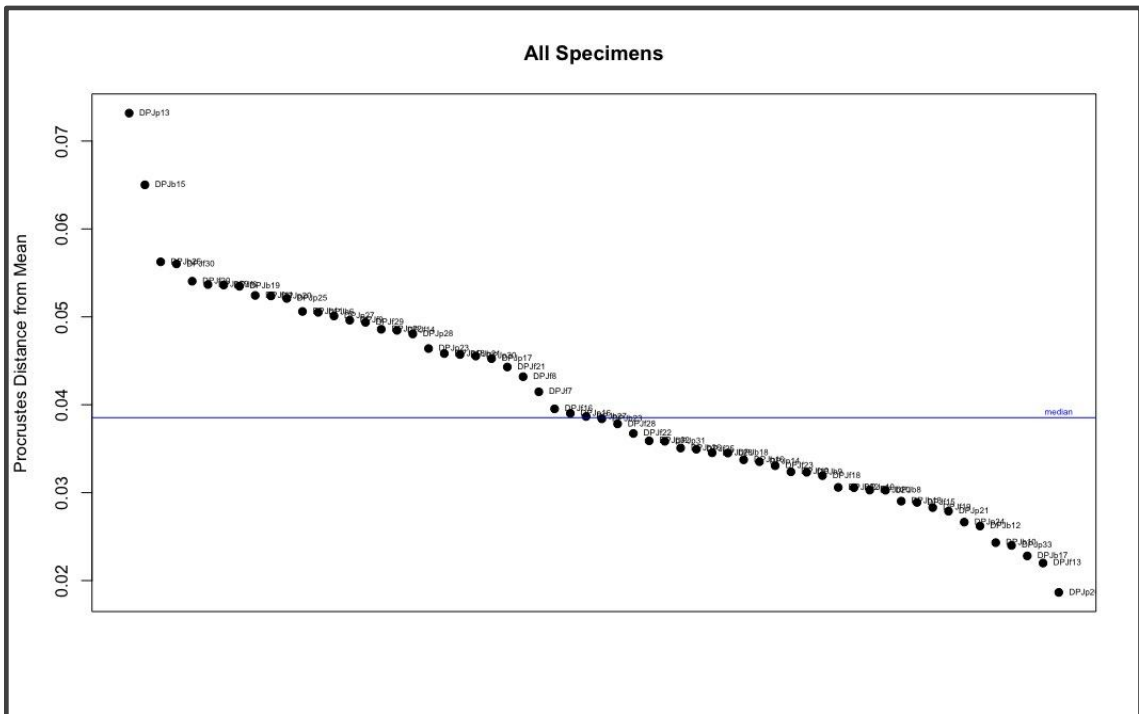


**Figure 11: Polygon plots of Principal Component axes 1-4 for *D. rerio* pharyngeal jaw morphometric data. Benthic treatments are colored in cyan, pelagic in black, pellet in red.**



<b>Advanced Procrustes D Linear Model ANOVA (By Treatment)</b>							
	<b>DF</b>	<b>RSS</b>	<b>SS</b>	<b>R<sub>2</sub></b>	<b>F-value</b>	<b>Z-score</b>	<b>P-value</b>
	56	0.0953 4	0.009421 8	0.089929	2.7668	2.9005	0.0013
<b>P-values/Effect Sizes (b/t Treatments)</b>				<b>Morphological Disparity P-values/Abs. Diff.</b>			
	<b>Benthic</b>	<b>Pelagic</b>	<b>Pellet</b>		<b>Benthic</b>	<b>Pelagic</b>	<b>Pellet</b>
<b>Benthic</b>	1/0	3.3210 4	2.49836	<b>Benthic</b>	1/0	0.0002 0	0.0003 4
<b>Pelagic</b>	0.0041	1/0	2.12813	<b>Pelagic</b>	0.4524	1/0	0.0001 3
<b>Pellet</b>	0.0156	0.0293	1/0	<b>Pellet</b>	0.1993	0.6050	1/0
<b>ANOVA of PC Axes</b>				<b>No Testing for Experimental Effects in Danio</b>			
	<b>Mean Sq.</b>	<b>F-value</b>	<b>P-value</b>				
<b>1</b>	0.00072	2.7301	0.07374				
<b>2</b>	0.00039	3.357	0.04185				
<b>3</b>	0.00042	3.7989	0.02826				
<b>4</b>	0.00020	4.9886	0.01008				
<b>5</b>	0.00017	0.9843	0.3799				
<b>6</b>	0.00076	1.4329	0.2471				

**Table 4: Numerical returns for all statistical testing on *D. rerio* pharyngeal jaw morphometric data. Divided tables are labelled in the respective order they are split.**



**Figure 12: Results of an outlier test on *M. zebra* pharyngeal jaw morphometric data. There were no statistical outliers detected.**

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