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Evolution of the boxfish carapace: functional consequences of shape

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Evolution of the boxfish carapace: 
functional consequences of shape 

A thesis submitted in partial satisfaction 
of the requirements for the degree of 
Master of Science in Biology 

by 

Tina Ashley Marcroft 

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ABSTRACT OF THE THESIS

Evolution of the boxfish carapace:

functional consequences of shape

by

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Master of Science in Biology

University of California, Los Angeles, 2015

Professor Michael Edward Alfaro, Chair

Boxfishes are a group of heavily armored Tetraodontiform fishes that are highly variable in shape. Disparification of shape could be driven by a simple performance trade-off between its two hypothesized primary functions: protection from predation and maneuverability. Alternatively, disparification could be driven by many-to-one mapping of shape to performance, where a relaxation in morphological constraint where many of morphologies have the same performance. We tested this by isolating the major features of the boxfish carapace shape and tested for their correlation to performance, as well as for a negative correlation between performances. We found that some features were correlated but very weakly, and that the two performances did trade-off but also weakly. This weak correlation primarily suggests that many-to-one mapping of shape to performance is driving disparification, which was unobserved in continuous 3D shape systems until this study.
The thesis of Tina Ashley Marcroft is approved.

Blaire Van Valkenburgh

David K. Jacobs

Michael Edward Alfaro, Committee Chair

University of California, Los Angeles

2015
I dedicate this thesis to Carrie Umetsu, Joseph Aprill, Mai Nguyen, Princess Gilbert, Francisca Wufu, Deb Pires, Jonathan Chang, Herbert Icasiano, and many others, without whose unwavering emotional and professional support I would not have completed this text.

Thank you!
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Introduction

Macroevolutionary biologists seek to describe and explain conspicuous patterns of disparity and diversity. Clades with incredible amounts of disparity, such as Galapagos’ finches and the Hawaiian Silverswords (Lack 1947; Grant 1986; Carr 1985), are frequently best explained by an adaptive radiation or speciation due to sexual selection (Carr and Kyhos 1981; Bowman 1961; Streelman et al. 2002). Trait variation in the clades that have experienced the former are frequently described as adaptive, fitting particular ecological niches (Schluter 2000). Those traits are of particular interest as they suggest mechanisms by which diversification and disparification can occur. Traits that map to performance in a one-to-one manner have been extensively studied, and frequently suggest there a trade-off in competing performances (e.g. Ghalambor, Reznick, and Walker 2004; Hoey, Bellwood, and Barnett 2012; Toro, Herrel, and Irschick 2004), where increased performance in one ability corresponds to decreased performance in another. While counterintuitive, this constraint can result in high disparification if selection is strong (Holzman et al. 2011; Holzman et al. 2012). However, great disparification can also occur due to the accumulation of non-adaptive changes that do not affect performance. Alfaro et al. suggest that immense amounts of disparification can result from decoupled mapping of complex traits to performance, called many-to-one mapping (2004; Wainwright et al. 2005). Many distinct arrangements of the 4-bar structure in Labrids, for example, can have the same biting performance (Alfaro, Bolnick, and Wainwright 2004). Additionally, many non-adaptive differences, as well as differences that are adaptive for performance unrelated to the one being studied, can accumulate in lineages that have been
independent of other lineages for long periods of time (Garland, Harvey, and Ives 1992; Wainwright 2007). Correlative studies would be unable to detect this mapping, as they assume no redundancy of performance; complex morphologies made of many parts tend to have multiple morphologies that correspond to the same performance (Collar and Wainwright 2006; Wainwright 2007; Holzman et al. 2011; Garland and Huey 1987). Functional properties arising from complex traits have not been as extensively studied, despite presumably being very common among organisms (Wainwright 2007).

Boxfishes are 36 species of Tetraodontiform fishes, consisting of the families Ostraciidae and Aracaniiidae. The ostraciids are generally reef-associated and located in most tropical habitats (Froese and Pauly 2014). The aracanids are temperate and are frequently described as “deep-water” despite extremely limited information about their ecology (e.g. Froese and Pauly 2014). Most aracanids live in areas with cooler rocky reefs, such as the coasts of Japan and South Australia (Matsuura 2008). Boxfishes are characterized by a hard carapace made of fused modified scales called scutes (Tyler 1980). Each scute is divided by a jagged suture, much like sutures between dermal bones in mammals (Besseau and Bouligand 1998). The scutes together form one continuous surface with openings for the mouth, eyes, gills and fins. Aracanid

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1 Ecological information of aracanids is limited to a few field texts and reports (e.g. Matsuura 2008).
2 The original definition of scute is restricted to a particular type and shape of osteoderm found in tetrapods (Blows 2001; Owen 1863), but much of the literature calls boxfish scales scutes, despite not being as first described.
carapaces are incompletely fused on the ventral and caudal sides and are hypothesized to represent the ancestral condition of the boxfishes (Fujii and Uyeno 1979).

The function of the carapace is assumed to be like that of other armoring systems. Its most probable primary purpose is protection from predation as in Testudines, Dasypus or Gastropods. While no one has directly tested these morphologies’ effectiveness via actual predator handling, except in gastropods (DeWitt, Robinson, and Wilson 2000; Johannesson 1986) and stickleback fishes (Reimchen 1992; Reimchen 2000), mechanical studies of some of these organisms suggest they perform similarly and are probably convergent (Chen et al. 2011). The boxfishes arose ~63 Ma (Santini et al. 2013; Alfaro, Santini, and Brock 2007), at a time when the oceans were either filled with many large durophagous predators such as the plesiosaur, or being filled with new large predators after the K-T extinction event. Different predators may have helped select for different extremes in diversity of boxfish shape, as they have in sticklebacks (Reimchen 1980). Additionally, depending on the precise age of the boxfishes and Tetraodontiformes\(^3\), predators of the Mesozoic Marine Revolution (Vermeij 1977) could have played a role in the carapaces’ evolution, though it is important to note that the boxfishes are not sessile or as limited in movement as the invertebrates discussed. However, Bartol et al. suggest the carapace serves another function: hydrodynamic stability (2002). The edges of the carapace, called keels, produce vortices that stabilize the fish (Bartol et

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\(^3\) Tetraodontiform fishes have all manner of bizarre defenses and include the pufferfishes, ocean sunfishes and triggerfishes. Brainerd and Patek suggest these defenses could have evolved because Tetraodontiform fishes have fewer vertebrae and are unable to perform a c-start, a predation escape behavior (Brainerd and Patek 1998).
al. 2002; Bartol et al. 2003; Bartol et al. 2005; Bartol et al. 2008). Bartol et al. suggest stability might be beneficial in turbulent aquatic environments, such as around a coral reef (2003). Other hypothesized functions of fish armoring include mineral storage, protection from male-male competition, and increased maneuverability (see discussion for details).

Boxfish carapaces come in a large variety of shapes similar to cubes, ellipsoids, triangular prisms and hexagonal prisms (Figure 1). Generally, ellipsoids and hexagonal prisms are found in the aracanids, while cubes and triangular prisms are found in the ostracids. These carapaces are of particular interest because they are continuous complex 3D structures, which have not been well investigated in the context of many-to-one mapping. Most complex traits that have been investigated are multi-part structures that interact mechanically with one another. No continuous 3D surfaces have been examined in this way, even though all features of these shapes could potentially influence how it handles stress and how it moves given that the entire surface is in contact with water. Explaining the diversity in this group can also suggest general mechanisms of trait evolution that lead to variation in comparable complex 3D surfaces such as antlers, turtle carapaces (Stayton 2011), and lock-and-key reproductive structures such as cerci (McPeek, Shen, and Farid 2009). Additionally, explaining the role that each function plays in shaping the evolution of armor could suggest the ecology of ancient armored fishes.

Ostracoderms and placoderms are famous for being very heavily armored. Little is known about their armor’s function, though it has been hypothesized to resist predation (Mark-Kurik 1992; Janvier 1996) and increase maneuverability or stability (Botella and Fariña 2008). Finally, trade-
offs or competing functions of armoring systems have been rarely tested except in Gastropods (DeWitt, Robinson, and Wilson 2000) and Testudines (Stayton 2011).

Competing functions may have selected for the large range of morphologies. As predation and need for adequate locomotion, either to forage or escape predation, are likely to be strong selective pressures, both could play a role in boxfish disparification. What features of the carapace explain most of the variation in boxfish shape? What is the nature of the relationship between carapace shape and these functions? Are particular shapes more stable than others? Do particular shapes distribute stress more, implying they can resist biting forces? Do these functions trade-off? Answering these questions will suggest if the carapace comprises a simple one-to-one or a complex many-to-one trait system, and if one or both patterns can explain the great disparification in carapace shape.

The first step is to identify the major axes of variation. This will elucidate what features explain most of the variation in carapace shape. The second is to determine the relationship between morphology and performance. If the carapace behaves like a simple trait system, we expect the largest axes of variation to predict each of the performances. We also expect there is some relationship between the two performances if morphology does significantly predict performance. Armoring has been shown to decrease fast-start swimming performance (Bergstrom 2002) so having a strong carapace may make a carapace less stable or less maneuverable. This trade-off could be driving the disparification of the boxfishes. We expect
these correlations to be strong if this is true. Additionally, boxfishes that perform a particular 
way should converge onto the same area of the morphospace as presumably one shape should 
result in one performance profile.

Alternatively, the continuous structure of the carapace may allow for many shapes to confer 
the same function. In other words, the 3D surface of the carapace behaves like a complex trait 
where multiple morphologies can result in the same performance. In this case, we expect to 
find little or no correlation between axes of shape variation and function, as shapes with the 
same function would obscure any correlation by receiving similar performance values. We also 
expect there to be little or no relationship between the two functions. Finally, qualitatively 
speaking, clustering of species on the morphospace will follow areas of the same function but 
the space in which that function can occur will be wide, i.e. many morphologies of boxfish 
shape can have approximately the same performance.

Finally, we can make qualitative observations of how particular groups diversified and their 
relationship to shape. Perhaps one group’s traits map many-to-one but another’s traits do not, 
suggesting other variables are at play. Perhaps there are temporal differences in how these 
traits mapped, suggesting that there were different selective pressures at different times in the 
clade’s history that resulted in different patterns among groups or ancestral reconstructions. If 
the carapace acts a many-to-one system, we expect to see diversification into areas of the 
morphospace of the same approximate function but each species shape is quite different. If the
carapace acts as a simple system, we expect to see species converging onto one morphology that confers only one performance.

**Methods**

*Data acquisition*

Our sample comprised 92 boxfish specimens representing 33 of 36 extant species. All specimens were preserved in ethanol or isopropanol and were at least 5 cm long to reduce the impact of juvenile allometry on our dataset. A list of museum specimen catalog numbers is available upon request. We recorded each specimen’s standard length using a digital caliper or ScanStudio’s measurement feature.

Each specimen was scanned twice: one 360° set of 8-13 vertical scans (tail up, head down), and one bracket set of scans to capture the head. Individual scans were either automatically aligned or manually aligned in ScanStudio (NextEngine, Santa Monica, CA) using corners of scutes, dots and other obvious coloration as landmarks. After fusing, models were cleaned in both ScanStudio and Geomagic (Geomagic, Research Triangle Park, NC). Artifacts and features difficult to scan such as fins, eyes and spines were flattened or removed. The bases of the pectoral, anal and dorsal fins were retained for use as landmarks in SPHARM. Final cleaned models were decimated to 20,000 triangular elements. We also realigned the model axes for use in the computational fluid dynamics (CFD) analysis in Geomagic (see below).
Shape analysis

We used a spherical harmonic analysis as implemented in SPHARM Ver. 1.2 (Shen, Farid, and McPeek 2009) to quantify shape variation in our boxfish models. Spherical harmonics are a modified Fourier analysis applied to complex three-dimensional shapes. We chose spherical harmonics over traditional geometric morphometric approaches because this approach allowed us to comprehensively describe variation in the three-dimensional shape of the boxfish carapace without need for a large number of homologous landmarks (Shen, Farid, and McPeek 2009). SPHARM is also capable of describing complex concavities and convexities unlike previous methods (Shen, Farid, and McPeek 2009). Additionally, SPHARM helps aligns models such that tempo of shape evolution can be observed by suggesting what portions of the carapace that have not been landmarked are homologous (Shen, Farid, and McPeek 2009). We performed this analysis on one specimen from each species that was largest and the most free of defects. Possible defects arose from damage to the original specimen and scanning issues.

Prior to spherical harmonic analysis, we placed eight homologous landmarks (Table 1) on each boxfish model to facilitate specimen alignment using AMIRA (“Amira”). Specimens were resized to unit centroid size to ensure our coefficients only documented differences in shape and registered (translated, rotated and aligned) using a generalized Procrustes procedure (Shen, Farid, and McPeek 2009). Smoothing parameters were set to the default value of 18 for both
the template and normal SPHARM smoothing steps. Higher parameter values, such as 18, captured a lot of detail but no surface texture.

After obtaining spherical harmonic coefficients, we performed a principal components analysis (PCA) in SPHARM to reduce the dimensionality of the morphological data and extract major axes of shape variation. To visualize overall shape change, we extracted eigenvalue models at -3, -2, -1, 0, 1, 2 and 3 standard deviations along each principal component (PC) axis. We modified SPHARM scripts available from the (McPeek) to obtain 121 additional grid models, i.e. shapes from parts the PC1 v. PC2 morphospace that may or may not represent any one species.

**Carapace strength**

To assess the relationship between carapace shape and strength, we performed a Finite Element Analysis (FEA) on the spherical harmonic models of 25 boxfish species. To control for effects of size and carapace thickness, we created a series of boxfish finite element (FE) models with the same surface area, thickness and volume, using the FE scaling protocol outlined by Dumont et al. (2009). We first imported the spherical harmonic models into Geomagic Studio and refined them to 10,000 triangular elements. Next, models were exported to Strand7 FE software (“Strand 7 FE Software”) where they were scaled to the same relative surface area (1.87762*10^4 mm^2) and set to a plate element thickness of 1 mm. While the materials properties of the horn of one species of boxfish has been examined (Yang et al. 2014), the
boxfish carapace has not yet been examined. Arrangements of fibers in the horn may not be the same as those in scales and fiber arrangement affects the materials properties (Yang et al. 2014). Additionally, this information was not available at the time of analysis. We therefore assigned all elements the material properties of the bichir pelvis (Young’s modulus, \( \varepsilon = 17.3 \); Poisson’s ratio, \( \nu = 0.3 \); (Erickson, Catanese, and Keaveny 2002)). Although not appropriate for measuring absolute performance, our use of common scaling protocols and homogenous material properties ensures that we have generated a series of models that, all else being equal, should exhibit equal stress for a given amount of force applied in the same loading scheme. Thus, any variation among models in stress values resulting from a given, identical load should result from difference in carapace shape only (Dumont, Grosse, and Slater 2009).

We simulated two different loading scenarios that boxfishes might experience when being bitten by a predator. For each scenario, a total load of 240N was used. Loading scheme 1 (Figure 2) simulated a dorsoventrally directed bite. We applied three ventrally directed loads across the central dorsal surface. Each load comprised 20 elements, with a normal pressure of 4N applied to each element. We applied three restraints in a triangular formation on the ventral surface. Loading scheme 2 simulated a mediolaterally directed bite. Restraints and forces were placed on the models as in the above loading scheme but at a 90° angle, such that they were to the left and right of the boxfish. The points were not always exactly in the same plane as some species, such as the *Lactoria* spp., are prism shaped and thus had sloping sides. Restraints were applied to the right side.
All models were subject to a linear static FE analysis. We subsequently extracted average plate von Mises stress for each model. Von Mises stress is a good predictor of failure in materials that undergo ductile failure, such as bone (Nalla, Kinney, and Ritchie 2003).

To construct accurate heat maps of mean stress and investigate the performance of models in areas of the morphospace uninhabited by extant species, we repeated the procedures described above with the 121 theoretical grid models spanning the morphospace derived from PCs 1 and 2.

**Hydrodynamic performance**

To assess the hydrodynamic performance of the different carapace shapes of 30 species of Ostraciidae and Aracanidae, we performed computational fluid dynamics (CFD) simulations. First, we aligned the 3D laser scans with a reference frame (*X* = mediolateral axis, *Y* = dorsoventral axis, *Z* = anterior-posterior axis). The origin of the coordinate system was placed at the volumes’ center. Next, we corrected for size by scaling all models to a volume of 150 cm³. The oriented and scaled models were imported into Ansys TGrid (“Ansys TGrid”) and inserted into a flow domain, and meshed.
We generated a flow domain that was 1.98 m x 0.84 m x 0.84 m box in TGrid with the boxfish placed in the middle. The distance from the boxfish to the velocity inlet was 0.396 m, or 20% of the length of the box flow domain. A refinement zone of 0.4 m was placed around the boxfish. Tetrahedra here grew slowly (by a growth factor of 1.1). 0.32 mm triangles were meshed onto the surface of the boxfish while the outer boundary was meshed with 20 mm triangles. About 10 million cells comprised the finite-volume mesh. A mesh convergence study of *Lactophrys triqueter* showed that further refinement (to 20 million cells, triangle size 0.2 mm) only resulted in a difference of about 1% for drag and lift force. This suggests that the 10 million cell mesh was the better choice, especially given that the latter would have doubled computation time.

The square face of the flow domain in front of the boxfish was designated as a velocity inlet with a flow speed of 0.44 m s\(^{-1}\), estimated by Bartol et al. (2003) as representative of fast swimming in relatively large boxfish individuals. Incoming flow is assumed to contain no turbulent energy when we use turbulence models (see below). A no-slip boundary condition was applied to the boxfish surface. The rest of the faces of the flow domain were designated as pressure outlets with no gauge pressure, as the faces are too distant from the boxfish to encounter pressure disturbances.

These meshes were analyzed in the computational fluid dynamics (CFD) solver Ansys Fluent 12.1 (“Ansys Fluent”). The fluid was modeled as 20°C seawater with a density of 1024.75 kg m\(^{-3}\)
and a dynamic viscosity of 1.08 Pa s (Kaye and Laby 1995). A validation test was performed for 
*L. triqueter*, as force balance measurements are available (Bartol et al. 2003). A standard 
laminar flow model was compared to a model accounting for turbulence. A model with 
enhanced wall treatment, the least computationally demanding model, was chosen as it is 
recommended by the Ansys Fluent manual for low Reynolds number flows, and predicts drag 
better. Size requirements for the first cell adjacent to the wall for this model were fulfilled. 
When the Reynolds number is approximately 50 000, the boxfish’s wake is turbulent, but the 
boundary layer might be laminar. This depends on the texture of the model and the intensity of 
turbulence. The validation test showed that our model outperformed the laminar flow model, 
and agrees with empirical measurements of *L. triqueter*. Therefore, our Fleunt data were 
collected with the turbulence model where after 3000 iterations (10 hours of calculation) 
convergence was reached.

To standardize the 0° pitching angle orientation for each model, after the first round of CFD 
simulations, models showing less than 5% of lift force relative to drag were kept after 
performing one CFD simulation. If none were found, we adjusted the model’s orientation and 
reperformed the simulation. Models with the lowest drag generate the least resistance during 
straight swimming. We also simulated swimming at 20° (nose-up) angle of attack. Such 
orientations can occur when turning up to negotiate obstacles. In that case, high lift force 
(which would allow the boxfish to gain height when rotating nose up) and high L/D (to reduce 
the relative loss in forward velocity during swimming over an obstacle; a measure of how
efficient the lift is generated) should increase the swimming performance in complex 3D habitats such as coral reefs (Bartol et al. 2003).

**Comparative analyses of performances and shape**

Comparative analyses were performed on a subset of boxfishes we tested, 24, that are represented by the phylogeny hypothesized by Santini et al. (Santini et al. 2013). To test if morphology predicted performance, and if any of the performance variables traded-off with one another, we performed a phylogenetic generalized least squares analysis (PGLS) using R (R Development Core Team 2008) on each of the largest morphological axes of variation against major hydrodynamic coefficients and mean stress values (Table 2; 3). To test whether there was a trade-off or not, we performed PGLS on each performance variable against each other (Table 2; 3). PGLS was used because it removes relatedness, and by extension time, from the dataset; smaller time since divergence make traits appear correlated when they actually are not (CITATION?). We assumed a Brownian model of trait evolution; preliminary tests for rate shifts in trait evolution using R package Auteur yielded no significant shifts and model fitting using R package Geiger could not differentiate between Brownian Motion, Ornstein-Uhlenbeck, and Accelerating-Decelerating models of trait evolution.

Heat maps and phylomorphospaces were generated using R (R Development Core Team 2008). Performance values for theoretical shapes (i.e. in the morphospace but not represented by an
extrapolated from the specimen values for the CFD using a Lagrangian interpolation. We were unable to calculate each theoretical model individually as each CFD analysis requires at least a day of calculation and a single plot requires processing at least 121 models. In contrast, performances of the theoretical models for the FEA heat map were directly calculated using the methods outlined previously.

Results

Shape analysis and phylogenetic implications

To isolate the axes of variation that contribute to most of the overall variation in shape, we performed a PCA of 765 SPHARM coefficients per model. We considered the three major axes of shape variation in the boxfish carapace (Figure 3). The first axis explained about 30.7% of the variation. This axis described varying degrees of presences/absences of the dorsal and ventral keels. The second axis explained about 25.2% of the variation. This axis described the presence/absence of lower mediolateral keels. The last axis explained about 13.2% of the variation and described variation between square and triangular transverse sections—4 main keels oriented to 3 main keels. All other PC axes each described less than 5% of the variation in boxfish carapace shape and were not examined.
Using the phylogeny hypothesized by Santini et al. (Santini et al. 2013), we mapped the boxfish phylogeny and reconstructed ancestral states on the reoriented shape space (PCA). The resulting phylomorphospaces show clear distinctions in inhabitance of morphospace between most of the aracanids and ostracids, with members of the *Ostracion* genus clustering tightly together (Figure 4-6).

The PC2 v. PC3 phylomorphospace (Figure 6), and to a lesser extent PC1 v. PC3 phylomorphospace (Figure 5), has a conspicuously empty zone between the two families of boxfishes. This could be explained by the group not being old enough to have evolved this shape or those shapes being non-adaptive and subject to functional constraint. It is not likely due to sampling error as the theoretical shapes populating that area appear as flattened prisms with extremely deep depression on the ventral side and do not resemble any known boxfish species, extant or not (Figure 3). Such a depression might also interfere with the placement of the viscera.

**Shape as a predictor of hydrodynamic performance and strength**

To test whether variation in morphology explained performance, and suggest if the major features relationship to performance is many-to-one or one-to-one, we tested for correlation between PCs and mean stress or hydrodynamic performance using a PGLS analysis. The reoriented PCA SPHARM coefficients did significantly correlate with some aspects of
performance (Table 2; Figure 7; 8). They were weakly positively correlated with at least 90% confidence. PC2, which describes mediolateral keel presence, does predict coefficient of lift ($C_L$) at a pitch of 20°, lift/drag ratio ($L/D$) at a pitch of 20°, and mean stress when forces were applied dorsoventrally with 95% confidence. This suggests that shapes with wider lateral keels, such as the prism-like *Tetrosomus* spp., predicted increased $C_L$ and $L/D$. This also suggests prism shapes distribute stress better when bitten mediolaterally but worse when bitten dorsoventrally. PC2 is also correlated with mean stress when forces were applied mediolaterally with 90% confidence. PC3, which describes differences in overall shape (triangular prism to cuboid), correlates with coefficient of drag ($C_D$) and $C_L$ but not the combined coefficient. Mean stress of when forces were applied both mediolaterally and dorsoventrally, did indeed significantly negatively correlate (at least 95% confidence) with $C_L$ and drag at both 0° and 20° (Table 2; Figure 8), despite having corrected for shared evolutionary history. Note that species with prism or box-like shapes have $C_L > 0$ (Table 2). For reference, a Cessna 172 at approximately 20° pitch has a $C_L$ of about 1.25 and they produce a maximum $C_L$ of 1.6 at about 15 pitch (Scott 2004).

**Trade-offs between maneuverability and strength**

To test for a relationship between the two performances and also the strength of correlation, we performed a PGLS analysis on mean stress versus hydrodynamic performance. This can tell us if a traditional one-to-one trade-off system, and/or relaxation of constraint due to many-to-one mapping of morphology to performance, is driving disparification. We expect to see a
strong correlation in the former case and weak or no correlation in the latter case. The only significant trade-offs of 95% confidence were between $C_D$ at a pitch of $20^\circ$ with both stress when applied dorsoventrally and mediolaterally (Table 3). This result is suggestive of some sort of relationship between the two functions; those high stress boxfish, such as the *Aracana* spp. when bitten mediolaterally, tend to have much slimmer silhouettes which we know to be lower drag as they resemble the common fusiform shape of fishes. The other performances are significant at lower confidence intervals, 90% and 85%, and are very weakly negatively correlated (Table 3). Many of these specific performances are significant and near significant and they share a negative relationship, suggest some sort of general trade-off between mean stress and hydrodynamic performance. However, the pattern is inconsistent, suggesting a weak relationship, and thus could be an indicator of many-to-one mapping of the boxfish carapace.

**The evolution of shape and function**

To examine if boxfishes have been diversifying like a many-to-one system of trait mapping or not, we generated phylomorphospaces with contour heat maps of performance. We expect to see disparification and diversification within functional groups but each species may still vary in trait, i.e. not be in the exact same part of the morphospace. Our heat maps qualitatively suggest that recent clades of boxfishes are diversifying along isoclines, while the ancestral reconstructions—presumably indicative of older lineages—tend to cross multiple isoclines. In other words, recent clades tend to spread along areas of the morphospace with approximately the same performance value. Older splits span several performances, either because they only
diversified across multiple performances or because other lineages went extinct. This is especially apparent in the case of PC1 and PC2 compared to mean stress of the carapaces when forces were applied dorsoventrally (Figure 10a). This suggests that there is indeed some many-to-one mapping occurring but early splits may have been more restricted in their diversification.

Discussion

**Shape diversity of the boxfish carapace**

The largest variation in carapace shape potentially coincide with two things, ancestry and performance of potential functions of the carapace. PC1 describes difference between those that have significant ventral and dorsal keels and those that do not. The only boxfishes that have both ventral and dorsal keels are the ellipsoid aracanids such as *Aracana, Anoplocapros*, superficially suggesting there is indeed a difference due to family membership. There is some overlap of the genera in morphospace, however, as there are ostraciids with dorsal keels: the prism shaped boxfishes such as *Tetrosomus* or *Anoplocapros*. Possibly, presence and absence of keels is also functional, so we tested whether this axis, or any of the other axes, were significant predictors of performance. This would also suggest if the majority of the incredible disparification of the boxfishes were driven by a trade-off.
We first needed to confirm if most of the variation in boxfish shape were due to the keels. We expected that the axes of highest variation showed differing arrangements of keels, assuming the carapace has one or more functions, and we did find that this was the case (Figure 3). PCs 1 and 2 show differences keel presence, with PC1 showing dorsoventral keel expansion and PC2 mediolateral keel expansion. Keels can generate vortices to stabilize the boxfishes (Bartol et al. 2002; 2003; 2005; 2008). Keels are also much larger structures than other potential features, such as scale texture, and thus are much more likely to make a difference in swimming ability at high Reynold’s numbers. Additionally, the types of keels used are likely to make a difference. Keels that are oriented dorsoventrally are likely to generate lift or stabilize in the yawing direction (nose tilting left-right), while keels that are oriented mediolaterally are likely to generate lift or stabilize in the pitching direction (nose tilting up-down). Boxfishes that require movement or stabilization in particular directions due to ecology, might show an overrepresentation of one of or both of those arrangements. Interestingly, the last PC3 suggests a potential difference in the generic “up and down” and “left and right” arrangement of a keels. The axis suggests a change of position as the prism represents one extreme, while the cube represents the other, essentially suggesting variation between sets of keels that are diagonal to one another.

Connections between body shape and hydrodynamic performance

The only major axis of variation that correlated with any aspect of hydrodynamic performance was PC2, which described the presence and absence of lateral keels. PC2 was significantly
correlated with $C_L$ at 20° pitch and coefficient of lift/drag at 20° pitch (Table 3; Figure 7). This is suggestive of a hydrodynamic function for the keels that is different than what was suggested by Bartol et al., i.e. that the boxfish carapace acts like a delta wing, shedding vortices which stabilize the fish in turbulent waters (2002; 2003; 2005; 2008). Boxfishes do eat sessile invertebrates and are sometimes considered reef-associated (Randall 1972; 1967; Randall and Hartman 1968; Moyer and Sano 1987; Matsuura 2008; 2014), so feeding on still food items in such a turbulent area might necessitate greater stability, as unexpected flow velocities would require active correction, and thus, energy (Bartol et al. 2003). However, turning with this strategy would prove difficult, requiring extra energy.

While we found $C_D$ similar to those obtained by Bartol et al., our CFD simulation results in this study and our previous study (Van Wassenbergh et al. 2015) show comparatively large $C_L$ and L/D when pitching, suggesting some boxfishes, ostraciids in particular, are actually unstable and maneuverable, rather than stable. A higher $C_L$ suggests that they are generating upward forces (and thus, pitching motion) and a higher L/D suggests more efficient lift generation given the amount of drag their shape confers. This is consistent with ecological data suggesting many boxfishes are actually not closely tied to the reef and frequently venture to sand flats and seagrass beds to forage (Randall 1967; Matsuura 2008) where waters are less turbulent (Castro and Huber 2003).
Potential reasons why they might prefer maneuverability in the pitching direction could include predation, sexual selection, or feeding. Our results corroborate a comparative study of mosquitofish where populations experiencing high predation populations had body shapes that enhanced unsteady swimming, i.e. that were highly maneuverable (Langerhans 2009). Some boxfish species, those scoring relatively high $C_L$, perform elaborate spawning ascents up the water column that require pitching (Sancho 1998; Moyer 1984; Ricco’s World 2011; BBC Earth 2015). Some males rotate upward to show their ventral side before fighting (Moyer 1984). Losing males rapidly ascend the water column (Moyer 1984). Some Atlantic boxfishes forage for worms by blowing sand while pitched downwards towards the substrate (Randall 1967; Oceano VideoSub 2014; Blogie Robillo 2012).

Does the fact that boxy and triangular ostraciids are more maneuverable (higher $C_L$ and L/D) mean that ellipsoid aracanids less maneuverable? Not necessarily. They may not be maneuverable in the way we originally thought. Just as lateral keels generates lift, or forces upward, when pitched, wouldn’t dorsoventral keels potentially generate lift, forces left or right, when yawed? The aracanids could be maneuverable when yawing but not pitching. Laterally compressed fish body, similar to the aracanids, produce lateral forces necessary to make tight turns (Parson, Fish, and Nicastro 2011). There is video of an aracanid being chased by a white-ear damselfish. The ellipsoid aracanid making incredibly tight rotational turns after the white-ear darts at it (bowlofpetunias1 2010). The boxfish, though surprisingly fast for its shape and size (Hove et al. 2001), does not try to out-swim it, rather it quickly makes small turns and the
white-ear is unable to turn quickly enough to strike the fish. The white-ear misses and makes a wide turn to strike again. The boxfish seems to be using its left-right maneuverability to avoid strikes and perhaps tire out the damselfish. Unfortunately, in this study, we did not measure the forces that could arise when boxfish yaw, but is a promising area of future investigation in boxfish carapace morphology.

It is important to note that boxfishes might not utilize the full performance capability of the carapace (in our paper; i.e. maneuverability, possibly low drag), as many animals are almost never observed performing optimally in situ (Husak 2006; Husak and Fox 2006; Irschick et al. 2005). Additionally, they may perform alternative behaviors in particular situations (Helmuth, Kingsolver, and Carrington 2005); boxfishes may use their toxin, for example, to escape predation rather than rely on their maneuverability, perhaps if the attacker is too large. However, there may also be issues with this strategy as pahutoxin/ostraciitoxin varies in efficacy and concentration released by species (Goldberg, Duffield, and Barrow 1988).

**Connections between carapace strength and body shape**

Though it seems obvious that armor plates or shells can protect an organism, few have directly tested effect of having different armoring systems on their interaction with predators, except in sticklebacks and mollusks. The plates and spines of the stickleback have been shown to decrease handling time, injuries by some predators and decrease estimated predation
successes (Reimchen 1992; Reimchen 1994; Reimchen 2000). Size of aperture, thickness of shell and shape of shell have been also shown to decrease handling time and successful predation attempts (DeWitt, Robinson, and Wilson 2000; Johannesson 1986).

The amount of indirect evidence suggesting armor protects organisms from predators is fair. Some thicker scales have been empirically shown to resist penetration (Bruet et al. 2008; Meyers et al. 2012) and the form of different scale arrangements have been modeled (Browning, Ortiz, and Boyce 2013; Vernerey and Barthelat 2010) showing that certain configurations are better at reducing shear deformation and back deflection which can result in tissue damage. Bichir scales have been shown to resist biting forces from piranha teeth (Meyers et al. 2012). Our study falls into this category, suggesting that morphology of shell shape does relate to armoring performance.

Ideally, we would observe predation events directly but we do not. Boxfish predation has rarely been observed. All that is available on the internet are two pictures of the gut contents of tuna that contain boxfishes (Mjohns 2014)\textsuperscript{4}, a video of scorpionfish attempting to eat a juvenile boxfish (bowlofpetunias1 2010) and a video of an aracanid boxfish desperately evading the attacks of a white-ear damselfish (Weird Underwater World 2011). Very few records of boxfish

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\textsuperscript{4} One of these pictures I obtained c. 2011 from a fishing forum. The website has since disappeared. As I do not have permission to reproduce it, I describe it here. It was a photograph of a gutted fish containing hundreds of larval \textit{Lactoria diaphana}. The owner said it was a tuna fished from the Pacific Ocean.
contained in gut contents exist, and when they do, they are a tiny portion of the animals’ diet (Rose and Hassler 1974; Oxenford and Hunte 1999; Manooch III, Mason, and Nelson 1984; Randall 1967). This dearth of data could be due to a lack of systematic investigation into boxfish predation as predation events are relatively rare to be witnessed or because adults are not predated upon frequently. If the latter were true, one might assume that predation might not be a huge factor in the selection of the boxfish carapace, at least in more recent times. Randall observes many of them frequently appear on sand flats and seagrass beds, away from the reef, suggesting they may not experience as much predation as other fishes (1967). Even if true, predation on heavily armored organisms may significantly impact their fitness, as in turtles (Heithaus et al. 2008). I have personally observed a potential case. A colleague kept a small shark and a boxfish in the same tank and the shark eventually bit off all the boxfish’s fins until it died. As they do not undulate their bodies to propel themselves (Sfakiotakis, Lane, and Davies 1999), absence of one or more fins would presumably have a larger effect on boxfish locomotion than other fishes. Some heavily armored organisms still exhibit anti-predator behavior despite being protected more than others (Dewitt, Sih, and Hucko 1999) which could suggest that predators still pose some threat.

Our study suggests that there is some relationship between particular shapes and resistance to biting forces. PC2, lateral keel appearance, does correlate to stress when being bit dorsoventrally. Shapes with more lateral keels tend to have higher average stress when being bit than those with no lateral keels. Those latter shapes tend to be more ellipsoid and thus have
more material between each force. We would expect them to perform that way, as the classic example of bone loaded in one direction more than another appears elliptical. What is unclear, however, is if there is a similar distinction in shape in PC1, but they were not significantly correlated. This could be due to sampling error or to many-to-one mapping of shape to function. Support for the latter is that while PC2 does correlate significantly, is weak.

**Trade-offs between the two performances**

As these performances represent our two main hypothesized functions, maneuverability and protection from predation, we expect to see them to trade-off very weakly or not at all, if the carapace is a trait (or set of traits) that behaves in a many-to-one manner. If multiple species’ different morphologies have the same performance value, then any correlation between two performances should be masked and that is indeed what we see (Table 2; 3). The disparification that leads to this weak correlation might have arisen only recently, if we look at the heat map and phylomorphospace (see below), as different genera evolved within the same functional isocline yet explored different carapace morphologies. Additionally, as these two functions are at even higher levels than the base morphologies that presumably comprise them, there is less chance any trade-off signal will be detected, especially since it is likely that the carapace behaves as a many-to-one system of trait mapping.
Evolution of the boxfish carapace, functional clustering, and sources of many-to-one mapping

The phylomorphospace showed significant clustering of shape between the two families (Figures 4-6). Aracanids inhabit the morphospace of taller thinner shapes. They also include shapes that have hexagonal silhouettes such as the Kentrocapros spp. Some of those species occasionally encroach on ostraciid morphospace (of those PC axes presented) (Figure 4). This is expected as variation tends to decrease as a time since divergence decreases (Garland, Harvey, and Ives 1992; Wainwright 2007). The two families cluster together as those of the same group tend to resemble each other. This suggests that generally each family innovated on particular shapes and generally varied around those initial shapes with the few overlapping exceptions of the Kentrocapros spp., Lactoria spp., and Anoplocapros spp., consistent with most organisms' traits (Figure 4).

The heat map and phylomorphospace is even more illuminating. While functional isoclines are somewhat arbitrary divisions of functional space, they can give us an idea of how each group of species is evolving in a functional space. In one particular case, some groups of boxfishes, especially those of the same genus, tend to be diversifying within isoclines (Figure 9c; 9d; 10a) suggesting that each genus's carapace has the same potential function but are somewhat different morphologically. This is consistent with the many-to-one pattern of trait evolution where multiple morphologies can explain the same function and such a pattern allows for diversification without disruption of function (Alfaro, Bolnick, and Wainwright 2004;
Wainwright et al. 2005). This is also consistent with our results that show weak or no correlations between morphology and performance and the two performances.

There could be multiple reasons for this diversification. The first is that species within each genus, which represent recent speciation, are evolving different morphologies due to necessity—perhaps they require a slightly different shape that would affect some other function we have not yet addressed. This is unlikely as the differences between each species are not large enough to affect any strength or locomotive performance, save perhaps drag. As boxfish shapes have higher \( C_D \) than the common fusiform shapes of most fishes (Van Wassenbergh et al. 2015), we would most likely see convergence onto some shape features that reduce drag, however we see a range of shapes with a range of \( C_D \). What is more likely is that this suggests disparification due to either released selection or simply age of the clade as older groups tend to accumulate more differences, adaptive or not. It is possible that both are occurring. If the first hypothesis is true, and there is no selection on the carapace for a particular function, we should see no pattern (circular arrangement) of species on the morphospace. However, we do see the species following isoclines suggesting the second hypothesis. There is probably still enough selection to preserve the function and shapes of those species but they are still allowed to evolve within that functional group.

Another interesting thing to note is that the ancestral reconstructions of boxfish shapes tend to cross isoclines. Considered with the previous idea, this may suggest that the boxfishes initially
experienced enough selection to disparify quickly across isoclines, à la a traditional simple mechanical system exhibiting a trade-off, and then recently exhibited disparification within genera, and by extension functional groups, as in many-to-one systems. All of this should be taken as speculation, however, as we have not rigorously tested these ideas and are making qualitative observations. If supported, however, these would pose a novel pattern of trait disparification not previously observed, where timing and variable constraint plays a role.

Sources of compensation and other potential functions of the carapace

Though the variation in carapace shape could be explained by a lack of predation in certain circumstances or a need for a different mode of locomotion, organisms may compensate for these performances in another manner, either through another trait (Wainwright 2007; Alfaro, Bolnick, and Wainwright 2004) or through behavior (Lima 1998). For example snails with weaker shells forage less when predators were visible than snails with stronger shells (Bourdeau 2013; Mowles, Rundle, and Cotton 2011), similarly smaller snails tended to avoid predators more than large ones (Dewitt, Sih, and Hucko 1999). However, these types of behaviors can also be used to boost performance; snails with smaller shell openings, which are presumably more protected, tend to show more hiding and less foraging behavior (Dewitt, Sih, and Hucko 1999). In fishes, only the latter pattern has been shown; populations of stickleback with less armor tend to hide less than those that were more heavily armored, suggesting higher predation in the armored populations (Spence et al. 2013). Compensatory behaviors or traits may lessen the effect of a trait, in this case carapace shape, on ultimate performance, such as
predator avoidance, and mask correlations of those traits with ecology (Wainwright 2007; Holzman et al. 2011).

Currey suggests armoring serves one or two main predation-related functions. First, armoring always to resist knocks and perforation into the flesh of the animal, such as being bit or struck by a predator. Second, which may not always be necessary, is to preserve shape (Currey 2010). The human skull, for example, requires being stiff rather than elastic, because any deformation after a blow might damage the soft brain (Currey 2010). As our analysis examined how shape and properties might contribute to the above function, it left the second function unstudied. It is possible that in some cases, stiffness might be preferred as elasticity might affect some forms of locomotion. Elasticity of the carapace might affect the efficiency with which it moves by having to expend more energy, however it may decrease development time as heavily armored fishes tend to develop slower in low calcium environments (Spence et al. 2012).

Elasticity might also be a source of compensation. The lack of correlation of shape to performance might be due to variation in elasticity, and thus due to variation in materials properties. While some have dismissed addressing diversity in materials properties as mammals do not differ in bone materials properties (Erickson, Catanese, and Keaveny 2002), there are indeed other traits that do vary. Orb web spider silk display a wide amount of variation in strength and elasticity (Blackledge and Hayashi 2006; Swanson et al. 2006). Ankylasauruses, dinosaurs with all manner of different kinds of defensive osteoderms, have very variable
configurations of collagen in their scutes (Scheyer and Sander 2004) and may have varied in materials properties as a result. Different proportions of hydroxyapatite and collagen can affect materials properties of bone (Erickson, Catanese, and Keaveny 2002) and, by extension, scales, as they contain the same materials. Additionally different collagen orientations can affect materials properties (Meyers et al. 2008).

Boxfishes may indeed vary in materials properties. I observed that different species of boxfishes tend to vary in hardness, where some of the *Ostracion* spp. are seemingly rock hard and impenetrable even when stabbed with a scalpel, and others, such as the *Lactoria* spp., have carapaces that deform under moderate pressure using bare hands. The latter fishes’ carapaces also are much more translucent suggesting they are less mineralized, and thus, less stiff. Materials properties of hard fish structures have not been thoroughly addressed (Erickson, Catanese, and Keaveny 2002) until recently (see Currey 2010 for review). Assessing whether variation in boxfish carapace materials properties could reveal if such diversity exists in the fishes as well as provide a vertebrate example of properties variation.

There are many potential ultimate explanations for variation in Young’s modulus. One is calcium storage. As the carapace is quite large, it could act as a reservoir for minerals. Giles hypothesized that calcium explained the variation in stickleback armoring (Giles 1983), but several studies suggest both calcium and predation play a role (Spence et al. 2013; Michael A Bell et al. 1993). However, these studies were on freshwater stickleback populations who live in
areas that vary greatly in calcium concentration (Spence et al. 2013). Calcium is relatively abundant (Castro and Huber 2003), so this explanation is not likely to explain the disparity of boxfish carapace shape. Additionally, having such a large bulky set of armor to retain calcium seems unlikely given its cost in terms of restricting some locomotion and maneuverability. Lack of calcium availability actually impairs armored stickleback growth (Spence et al. 2012), and there is no evidence to suggest that boxfish suffer from any growth issues in different locations, which would suggest variation calcium concentration in the ocean. However, given the ubiquity of shelled organisms in the ocean, this is unlikely. It is more probable that the boxfishes evolved such a presumably costly structure because Tetraodontiformes in general had fewer vertebrae and were already highly restricted in movement (Brainerd and Patek 1998). Subsequent demineralization could have been due to different buoyancy requirements, as in stickleback (Myhre and Klepaker 2009).

Deterring the attachment of surface parasites could also be a function of the boxfish carapace. Applegate suggests that placoid scales function to prevent parasite attachments (1967); similarly, hardened carapaces could potentially prevent parasites from anchoring into or feeding off the flesh of boxfishes. However, as in sharks (Raschi and Tabit 1992), there is no evidence to support this hypothesis.

Sexual selection may have also helped shape the evolution of the boxfish carapace. Differential materials properties could affect how sound is transmitted through the carapace and the
surrounding water. Some boxfish species communicate to during spawning using a buzzing noise (Chanet, Guintard, and Lecointre 2014; Lobel 1996). They either communicate with females or aggressively towards males (Lobel 1996). Additionally, evolution of carapace shape and materials properties could also have been affected by male-male competition. Rarely, male boxfishes ram each other (Lobel 1996; Zippin 2014), presumably to compete for females as some species are haremic (Moyer 1979; Moyer 1984).

**Other future directions**

A comprehensive, comparative study of boxfish development has not been attempted save for a few species (Leis and Moyer 1985), probably because boxfishes are notoriously difficult to breed and grow in tanks (Foster and Smith 2015). However, recent developments in boxfish breeding in the private sector are promising (Baensch 2004). We would like to examine shape in juveniles as armoring might presently be more important in the early life of boxfishes as there are more observed examples of larval predation. The larvae’s carapaces look quite different than the adults’ (Leis and Moyer 1985) and thus could perform differently. However, we might observe more predation in larvae and juveniles simply because the armor is less effective at that stage.

This study’s results are intriguing but more research is required on the subject. Sampling of aracanids was low, due especially to the lack of tissues available to improve the boxfish
phylogeny. Though there were more aracanid specimens available for morphological examination, the number per species was quite low in comparison to ostraciids, most likely because the rarer species are fished from deeper water than most reef or rocky reef associated boxfish. Inclusion of more aracanids would yield more interesting and comprehensive results as there are some extreme shapes, i.e. *Polyplacapros* and *Capropygia/Caprichthys* spp. that are currently unexplained.
Figures

Table 1. Ordering and locations of landmarks used on boxfishes.

<table>
<thead>
<tr>
<th>Order number</th>
<th>Placement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Left pectoral fin base; posterior-most point</td>
</tr>
<tr>
<td>2</td>
<td>Center of left eye</td>
</tr>
<tr>
<td>3</td>
<td>Center of upper lip of mouth</td>
</tr>
<tr>
<td>4</td>
<td>Center of right eye</td>
</tr>
<tr>
<td>5</td>
<td>Right pectoral fin base; posterior-most point</td>
</tr>
<tr>
<td>6</td>
<td>Dorsal fin base; anterior-most point</td>
</tr>
<tr>
<td>7</td>
<td>Dorsal fin base; posterior-most point</td>
</tr>
<tr>
<td>8</td>
<td>Anal fin base; anterior-most point</td>
</tr>
</tbody>
</table>

Table 2. PGLS results from testing if morphology correlates to performance. Principal components 1-3 (PC1-3) were tested for correlation to coefficients of drag ($C_D$), coefficients of lift ($C_L$), lift/drag ratio (L/D), at a pitch of 0° and 20°. Each PC was tested for correlation to mean stress when forces were placed dorsoventrally (DV) or mediolaterally (ML). Variable 1 was the predictor. Significant results are highlighted in yellow. Approximate percent confidence is listed if correlation was significant.

<table>
<thead>
<tr>
<th>Variable 1</th>
<th>Variable 2</th>
<th>p-value</th>
<th>Correlation (r)</th>
<th>Slope</th>
<th>Significant?</th>
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<tr>
<td>PC1</td>
<td>$C_D$ at p 0°</td>
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<td>0.062026</td>
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<tr>
<td>PC1</td>
<td>$C_D$ at p 20°</td>
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<tr>
<td>PC1</td>
<td>$C_L$ at p 20°</td>
<td>0.104867</td>
<td>0.062026</td>
<td>-10.809</td>
<td>No</td>
</tr>
<tr>
<td>PC1</td>
<td>L/D at p 20°</td>
<td>0.256897</td>
<td>0.062026</td>
<td>-44.7827</td>
<td>No</td>
</tr>
<tr>
<td>PC2</td>
<td>$C_D$ at p 0°</td>
<td>0.668669</td>
<td>0.006543</td>
<td>-0.88521</td>
<td>No</td>
</tr>
<tr>
<td>PC2</td>
<td>$C_D$ at p 20°</td>
<td>0.477631</td>
<td>0.006543</td>
<td>-5.32823</td>
<td>No</td>
</tr>
</tbody>
</table>
Table 3. PGLS results from testing if hydrodynamic performance correlates with mean stress.

Coefficients of drag ($C_D$), coefficients of lift ($C_L$), lift/drag ratio ($L/D$), at a pitch of 0° and 20° were tested for a correlation with mean stress when forces were applied dorsoventrally (DV) and mediolaterally (ML). Significant results are highlighted in yellow. Approximate percent confidence is listed if correlation was significant.

<table>
<thead>
<tr>
<th>Variable 1</th>
<th>Variable 2</th>
<th>p-value</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_D$ at p 0°</td>
<td>Mean stress DV</td>
<td>0.1101</td>
<td>No</td>
</tr>
<tr>
<td>$C_D$ at p 0°</td>
<td>Mean stress ML</td>
<td>0.0652</td>
<td>90%</td>
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<tr>
<td>$C_D$ at p 20°</td>
<td>Mean stress DV</td>
<td>0.0241</td>
<td>95%</td>
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<td>$C_D$ at p 20°</td>
<td>Mean stress ML</td>
<td>0.0059</td>
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<td>Mean stress DV</td>
<td>0.071</td>
<td>90%</td>
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<td>Mean stress ML</td>
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<td>No</td>
</tr>
<tr>
<td>L/D at p 20°</td>
<td>Mean stress DV</td>
<td>0.1407</td>
<td>No</td>
</tr>
<tr>
<td>L/D at p 20°</td>
<td>Mean stress ML</td>
<td>0.0937</td>
<td>90%</td>
</tr>
</tbody>
</table>
Figure 1. Diversity in boxfish shape for most genera of both boxfish families. Each drawing is a transverse plane view of the boxfish to show the diversity in shape, especially keel shape.
**Figure 2.** Example loading scheme with forces applied dorsoventrally. The caudal portion of the boxfish model is on the left side of this diagram. The top figure is left lateral view of the boxfish and the bottom figure is a left ventral view. Forces were applied dorsally (arrows) and restraints ventrally (crosses). The points indicate where the dorsal forces were applied as viewed from the ventral side comparison to the restraints.
Figure 3. Range of shapes that each principal component (PC) describes. PC1 explained 30.7% of the variation (top row), PC2 explained 25.2% of the variation (middle row), and PC3 explained 13.2% of the variation (bottom row). Each column refers to the number of standard deviations away the models is from the mean model and the middle column represents the mean.
Figure 4. Phylomorphospace of principal components (PCs) 1 and 2 using a time-calibrated phylogeny published by Santini et al. (2013). Each point represents a species. Shapes of the points indicate genera. Aracanid genera are denoted by filled symbols (Anoplocapros, Aracana, Capropygia and Kentrocapros) and ostraciid genera are denoted by lined symbols. Grey points indicate ancestral reconstructions of boxfish shape along each PC, assuming Brownian Motion model of trait evolution.
**Figure 5.** Phylomorphospace of principal components (PCs) 1 and 3 using a time-calibrated phylogeny published by Santini et al. (2013). Each point represents a species. Shapes of the points indicate genera. Aracanid genera are denoted by filled symbols (*Anoplocapros*, *Aracana*, *Capropygia* and *Kentrocapros*) and ostraciid genera are denoted by lined symbols. Grey points indicate ancestral reconstructions of boxfish shape along each PC, assuming Brownian Motion model of trait evolution.
Figure 6. Phylomorphospace of principal components (PCs) 2 and 3 using a time-calibrated phylogeny published by Santini et al. (2013). Each point represents a species. Shapes of the points indicate genera. Aracanid genera are denoted by filled symbols (Anoplocapros, Aracana, Capropygia and Kentrocapros) and ostraciid genera are denoted by lined symbols. Grey points indicate ancestral reconstructions of boxfish shape along each PC, assuming Brownian Motion model of trait evolution.
Figure 7. Scatterplots of PC2 versus \( C_L \) and L/D at 20° pitch. They were significantly correlated with 95% confidence. The regression line is shown. Each point represents one species.

![Scatterplots of PC2 versus C_L and L/D at 20° pitch](image)

Figure 8. Scatterplots of PC2 versus mean stress when forces were applied dorsoventrally and mediolaterally. They were significantly correlated with 90% and 95% confidence respectively. The regression line is shown. Each point represents one species.

![Scatterplots of PC2 versus mean stress](image)
**Figure 9.** Heat map of hydrodynamic performance overlaid with a phylomorphospace of PCs 1 and 2. Each point represents a species and each type of symbol represents a genus (see legend of Figures 3-5). Each smaller point represents ancestral reconstructions of boxfish shape. The white circle indicates the ancestral reconstruction of the lineage at the root of the tree. (a) Heat map of $C_D$ at a pitch of 0°. Warmer colors indicate higher drag. (b) Heat map of $C_D$ at a pitch of 20°. Warmer colors indicate higher drag. (c) Heat map of $C_L$ at a pitch of 20°. Warmer colors indicate more lift (up-force). $CL = 0$, light blue, indicates no lift. Coolest colors indicate negative lift (down-force). (d) Heat map of L/D at a pitch of 20°. Warmer colors indicate higher efficiency.
**Figure 10.** Heat map of mean stress overlaid with a phylomorphospace of PCs 1 and 2. Each point represents a species and each type of symbol represents a genus (see legend of Figures 3-5). Each smaller point represents ancestral reconstructions of boxfish shape. The white circle indicates the ancestral reconstruction of the lineage at the root of the tree. (a) Heat map of mean stress when forces were applied dorsoventrally. Warmer colors indicate higher stress. (b) Heat map of mean stress when forces were applied mediolaterally. Warmer colors indicate higher stress.
References


“Amira.” FEI Corporation.

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