Scale-eating specialists evolved adaptive feeding kinematics within a microendemic radiation of San Salvador Island pupfishes

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Abstract

Adaptation to novel ecological niches often includes shifts in behaviors, such as new foraging preferences or changes in kinematics. Investigating prey capture kinematics is an excellent way to understand behavioral mechanisms underlying the origins of novel trophic specialization, in which organisms begin to exploit novel resources. We investigated the contribution of kinematics to the origins of a novel ecological niche for scale-eating within a microendemic adaptive radiation of pupfishes on San Salvador Island, Bahamas. We compared the feeding kinematics of scale-eating, generalist, snail-eating, and F1 hybrid pupfishes while they consumed scales and shrimp in the lab and compared them to scale-eating kinematics observed in situ in the wild. We then connected variation in feeding kinematics to scale-biting performance by measuring the area removed per strike from standardized gelatin cubes. We found that scale-eating pupfish exhibited divergent feeding kinematics compared to all other groups and that these differences were consistent across food items. The peak gapes of scale-eaters were twice as large as all other groups, but their gape angles were simultaneously 32% smaller, in both laboratory and in situ wild observations. We also show that this kinematic combination of large peak gape and small gape angle resides on a performance optimum for scale-biting. Finally, F1 hybrid kinematics and performance were not additive, and were instead closer to the generalist pupfish. This suggests that impaired hybrid performance in the scale-eating niche may contribute to extrinsic postzygotic reproductive isolation between species. Ultimately, our results suggest that shifts in kinematics (i.e. peak gape and gape angle) are an adaptation to the novel niche of scale-eating and contribute to reproductive isolation between species.

Introduction

Determining how organisms use resources for the first time and occupy novel niches is an outstanding question in evolutionary ecology. Many changes accompany adaptation to a novel niche, and previous studies have identified that shifts in behaviors (Bowman and Billeb 1965; Tebbich et al. 2010; Curry and Anderson 2012), morphologies (Ferry-Graham et al. 2001; Ferry-Graham 2002; Hata et al. 2011; Davis et al. 2018), physiologies (Arias-Rodriguez et al. 2011; Tobler et al. 2015, 2018), and kinematics (Janovetz 2005; Patek et al. 2006; Cullen et al. 2013; McGee et al. 2013) can all facilitate this transition.

Shifts in kinematic traits—particularly those which affect prey capture and feeding—are especially promising, because they can provide biomechanical insights into the origins of novel trophic niches. For example, some species of trap-jaw ants have evolved specialized latch, spring, and trigger structures in their jaws which allow for rapid, power-amplified bites used to capture prey items unavailable to other ant species (Patek et al. 2006; Larabee and Suarez 2014); power-amplified jaws in Syngnathiform fishes have evolved two different latch mechanisms for specialization on evasive prey items (Longo et al. 2018); and the Pacific leaping blenny (*Alticus arnoldorum)* uniquely uses axial tail twisting to improve propulsion and stability for greater jumping performance and terrestrial prey capture (Hsieh 2010).

Differences in prey capture kinematics between species may also contribute to postzygotic extrinsic reproductive isolation by reducing hybrid foraging performance (Higham et al. 2016b), which may lead to speciation (Henning et al. 2017; Matthews and Albertson 2017). For example, McGee et al. (2015) measured prey capture kinematics and performance in two sunfish species (Centrarchidae) and their naturally occurring hybrids. Hybrid sunfish had an intermediate gape compared to parental types and initiated strikes from an intermediate distance.

However, the force exerted on prey items and the maximum escape force needed to evade attacks, estimated from the suction-induced flow field model (Holzman et al. 2012), was less than the intermediate performance expected. Hybrid Lake Victoria cichlids (produced by crossing *Haplochromis chilotes* (thick-lipped) and *Pundamilia nyererei* (thin-lipped) species) also exhibited lower foraging performance compared to parental species, most likely due to antagonistic pleiotropy and genetic correlations between head and lip morphology (Henning et al. 2017). Despite these findings, few studies investigate how hybrid kinematics affects the evolution of novelty or explicitly connect kinematics to performance consequences.

Investigating the kinematics of scale-eating is an excellent system for connecting a mechanistic understanding of feeding kinematics with adaptation to a novel trophic niche. Scale-eating (lepidophagy) is a novel behavioral trophic niche that is extremely rare which has independently evolved only 19 times in approximately 100 fish species out of over 35,000 (Sazima 1983; Martin and Wainwright 2013a; Kolmann et al. 2018). However, not much is known about its evolutionary origins or its kinematics. Current hypotheses for the origins of scale-eating vary, but they all take a strict behavior-first approach (Greenwood 1965; Sazima 1983; St. John et al. 2018). It is therefore plausible that kinematic variation may have also contributed to the origins of scale-eating.

Currently, only a few studies have investigated the feeding kinematics and performance of scale-eating fishes. Janovetz (2005) measured feeding kinematics of the wimple piranha (*Catoprion mento*) while consuming: 1) free floating scales, 2) whole fish, and 3) scales off the sides of fish, and found that scale-eating kinematics were divergent from those used in either suction-feeding or biting. Interestingly, scale-eating attacks produced gape angles that ranged from 30-100% larger than those produced from consuming free-floating scales or whole fish respectively— suggesting that a larger gape is necessary for scale-eating. Furthermore, this variation in gape angle across food items was documented within individuals indicating that scale-eating kinematics may be plastic (Janovetz 2005). The feeding kinematics of the Lake Tanganyikan scale-eating cichlid, *Perissodus microlepis*, have also been examined (Takeuchi et al. 2012; Takeuchi and Oda 2017); however, these studies primarily focused on how kinematics interacted with *P. microlepis*' antisymmetric mouth morphology and not on scale-eating kinematics *per se*. Nonetheless, these studies found a significant interaction between kinematic traits, behavior, and morphology as these fish were able to perform more successful scale-eating strikes using their dominant side (Takeuchi et al. 2012; Takeuchi and Oda 2017). A similar oral jaw antisymmetry and behavioral laterality was documented in a scale-eating characiform (*Exodon paradoxus;* Hata et al. 2011). While these studies provide valuable insights into scale-eating kinematics and performance, the lack of kinematic measurements in closely related non-scale-eating species or hybrids has so far limited further investigations of the origins of scale-eating.

There are four additional comparisons and measurements that would help shed light on the relationship between kinematic traits and occupation of a novel niche when investigating scale-eating. First, comparisons of scale-eating kinematics across scale-eating and closely related non-scale-eating outgroup species is necessary for investigating the origins of novelty. Without the comparative method it is impossible to determine which kinematic variables are unique or important for scale-eating. Second, very few kinematic studies investigate hybrid kinematics. Understanding hybrid kinematics, especially in the context of novelty, is informative because 1) impaired performance in hybrids is a form of extrinsic postzygotic isolation between species (McGee et al. 2015; Higham et al. 2016b) and 2) it can allow the decoupling of morphology, behavior, and kinematics making it easier to identify causative traits underlying performance (Holzman and Hulsey 2017). Third, very few studies investigate kinematics in the wild (but see: Keren et al. 2018; Whitford et al. 2019). While this is undoubtedly due to the difficulty of collecting these data in the field, it does introduce a level of uncertainty regarding whether kinematics observed in the lab are relevant to performance demands in nature (Shapiro et al. 2011; Stevens et al. 2011). Finally, few studies connect observed variation in kinematics to variation in whole organism feeding performance (but see: Svanbäck et al. 2003; Takeuchi et al. 2012; Whitford et al. 2019). Making this connection is important because it can identify kinematic traits associated with performance tasks relevant to evolutionary fitness rather than simply describing phenotypic variation in kinematic traits, most of which may not be relevant to performance or fitness (Hu et al. 2017).

The scale-eating pupfish (*Cyprinodon desquamator*) is an excellent organism to investigate the interaction of kinematics and novelty for several reasons. First, the scale-eating pupfish evolved within a recent sympatric radiation of pupfishes on San Salvador Island, Bahamas. This radiation is endemic to a few hypersaline lakes on the island (Martin and Wainwright 2013a), which were most likely dry during the last glacial maximum 10-15 kya (Hagey and Mylroie 1995). Second, the radiation provides closely related sister taxa for kinematic comparison. The radiation contains three species: 1) the scale-eating pupfish, 2) a generalist pupfish (*C. variegatus*), and 3) a snail-eating pupfish (*C. brontotheroides*). Phylogenetic evidence suggests that scale-eating pupfishes form a clade across all lakes where they are found on San Salvador and that this clade is sister to a clade containing generalists and snail-eaters (Martin and Feinstein 2014; Lencer et al. 2017). Generalists and snail-eaters, however, cluster together within lakes suggesting either extensive gene flow between species or multiple origins of snail-eating (Martin and Feinstein 2014; Martin 2016; Richards and Martin 2017). Third, all three pupfish species can be crossed in the lab to measure the kinematics and performance of hybrid phenotypes.

We investigated the interaction between kinematics and novelty in San Salvador generalist, snail-eating, and scale-eating pupfishes, and their F1 hybrids while performing high-speed strikes on three different food items (scales, frozen mysis shrimp, and gelatin cubes). We asked: 1) if scale-eating pupfish varied in their feeding kinematics compared to other groups, 2) if scale-eating strikes differed from strikes on frozen mysis shrimp, 3) if scale-eating strikes in the lab differed from scale-eating strikes in the wild, 4) whether variation in kinematics was associated with bite performance, and 5) if F1 hybrid feeding kinematics differed from parental species. Ultimately, we found that feeding kinematics of scale-eating pupfish resulted in bite sizes 50% larger than all other species, suggesting that scale-eater kinematics are a recent adaptation for scale-eating.

Methods

Collection and Husbandry

We used seine nets to collect generalist, snail-eating, and scale-eating pupfishes from Crescent Pond, Little Lake, and Osprey Lake on San Salvador Island, Bahamas in July, 2017 and March, 2018. Wild-caught fish were maintained in 37-75L mixed-sex stock tanks at a salinity of 5-10 ppt and temperature of 23-27°C. While in stock tanks, fish were fed a diet of bloodworms, mysis shrimp, and commercial pellet foods daily. In the lab, we crossed generalist and scale-eating pupfishes from both Little Lake and Crescent Pond to produce F1 hybrid offspring. Prior to filming, pupfish were isolated in a 2L tank to maintain individual IDs throughout the study.

Laboratory feeding kinematics

We recorded pupfishes feeding on three different food items: frozen mysis shrimp, scales, and standardized gelatin cubes (dimensions: 1.5 cm x5cm X 1.5 cm x 1.5 cm cube; Repashy Superfoods, Community Plus Omnivore Gel Premix; prepared following manufacturer's instructions). In the lab, fish freely consumed mysis shrimp (Hikari, Inc.), but we had to train all species to feed on scales from the sides of euthanized zebrafish (*Danio rerio*; stored frozen) and to feed from gelatin cubes (stored at 4℃). For training, we isolated each fish in a 2 liter plastic tank and presented a given food item (either euthanized zebrafish or gelatin cube) daily. If a pupfish began feeding on the item, it was left in the tank until the pupfish stopped feeding. If a pupfish did not begin feeding within one minute, the food item was removed from the tank. Any pupfish that did not feed received a supplemental feeding of commercial pellet food (New Life Spectrum Thera-A, medium sinking pellets). If an individual did not feed on a training item for more than two days, we reduced supplemental feedings to once every two days to ensure that the fish was sufficiently motivated. Once pupfish reliably began feeding on either scales or gelatin cubes, we proceeded to film their feeding behaviors according to the filming protocol below. Fish were never trained on more than one item at a time, and we instead ensured that all filming was completed for a single food item before proceeding to train for the next item.

For all three food items, we followed the same filming protocol and used the same equipment: 1) a Sony Cyber-shot DSC-RX10 III (480fps) or Sony Cyber-shot DSC-RX100 IV 20.1 MP (480fps) for high-speed video of foraging strikes and 2) a dimmable bi-color 480 LED light (Neewer) as an additional light source. Prior to filming, each pupfish—still in their 2 L tank—was placed in front of 0.5 cm grid paper. We placed our camera as close as possible to the tank while still keeping both the pupfish and food item in frame. We placed the additional LED light source approximately 0.3 m from the filming tank and allowed pupfish to acclimate to the lighting until they moved around their tank freely after approximately 5 minutes. Once a pupfish was acclimated we presented the pupfish with a given food item. For scale-eating we used forceps to hold a euthanized zebrafish horizontally in the water column and perpendicular to the front of an individual. For mysis shrimp and gelatin cubes, we dropped the food item a few inches in front of an individual. All videos were taken from a lateral perspective. Once filming for one food item was completed, the process was repeated until we filmed each individual consuming all three food items.

Scale-eating kinematics in the wild

We also filmed seven usable lateral scale-eating strikes by scale-eaters in the wild. It was not possible to film generalist or snail-eater scale-eating strikes, since these species do not naturally strike scales at a detectable frequency in nature (although scales have been found in the guts of both species at very low frequencies; Martin and Wainwright 2013). We used a Chronos camera (Kron Technologies, model 1.4, 16 GB memory, Color image sensor) with an f1.4 zoom lens in a custom underwater housing (Salty Surf, Inc. Krontech Chronos 1.4 housing with M80 flat port) to record natural high-speed videos of scale-eating in Crescent Pond, San Salvador Island (see Supplemental Video S1). We recorded videos while snorkeling along the shoreline in 0.3 – 0.5 m depth in March, 2018. We set the frame rate to the minimum of 1080 fps on the camera, however, our manual calibration of stopwatch videos filmed in the field indicated that the effective frame rate was only 270 fps.

Standardized reference objects for calibration (i.e. grid paper) were not present in the wild scale-eating videos and filming a reference object in a second video was a substantial obstacle given the constantly shifting distance between the camera and subject and the necessity of removing the camera from the underwater housing to store and reset after each video. Instead, we measured the diameter of 20 mermaid's cup macroalgae (*Acetabularia acetabulum*) from a photograph containing a ruler and estimated the mean cup diameter of adult cups (mean ± SE: 3.31 ± 2.86 mm). This macroalgae was present in all videos. We used this mean cup diameter to convert wild measurements in pixels to meters.

Kinematic analyses

We analyzed videos by converting them to image stacks using Adobe Media Encoder (Version 13.0.2). Image sequences were then imported into image processing software (FIJI) for analysis (Schindelin et al. 2012). To quantify feeding performance, we measured 8 kinematic trait metrics: peak jaw protrusion, time to peak jaw protrusion, peak gape, time to peak gape, gape angle at time of peak gape, starting distance from food item, time to impact, and ram speed (Table 1).

All time and distance metrics were measured from the start of a strike defined as when the lower jaw was opened to ~20% of peak gape, identified by visual inspection of each frame (following: Oufiero et al. 2012; Staab et al. 2012). In addition to our kinematic metrics, we also measured body length and lower jaw length (Table S1) using images from the video. We calibrated each video using the background grid for laboratory strikes and macroalgae for wild strikes. Kinematic profiles of a single representative scale-eating strike from each species are shown in Figure 1.

Figure 1.

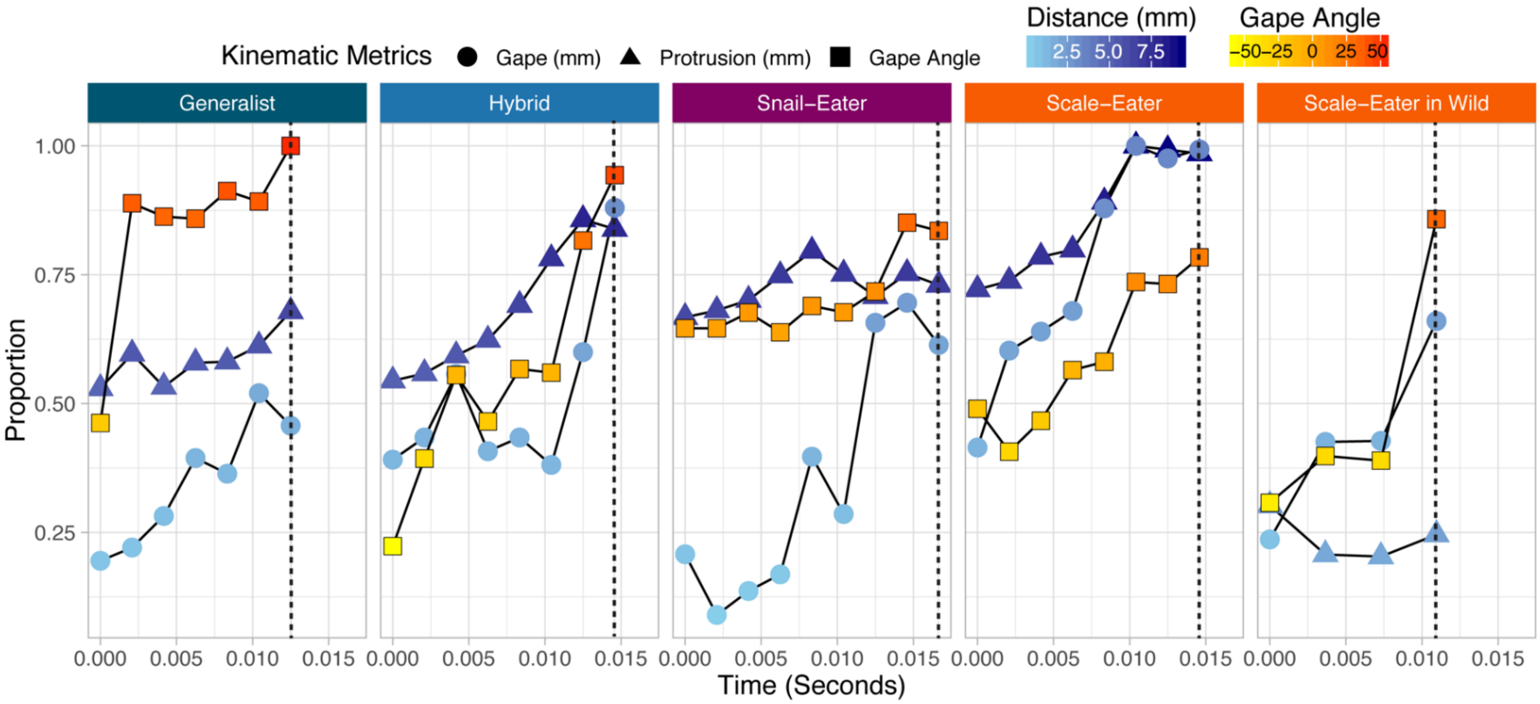

Figure 1. Kinematic profiles of pupfish species for scale-eating strikes observed in the lab and in situ in the wild. We produced kinematic profiles for a single representative strike for each group, and reported the proportional change in gape distance, jaw protrusion distance, and gape angle over the course of a single scale-eating strike. Time zero for each group represents the start of an attack (i.e. 20% of peak gape) and the dotted line indicates the time of impact with the prey. Point positions are relative to the highest value metric across groups (e.g. a value of 1 indicates the largest value of a given metric). Fill colors show raw values for each point.

Measuring bite performance

In order to connect variation in feeding kinematics to variation in bite size we recorded high-speed strikes on gelatin meal replacement for fish in the shape of a 1.5 x 1.5 x 1.5 cm cube. We filmed a feeding strike on a single cube and immediately removed the cube from the tank. The gel cube retains its shape in water and therefore allowed us to precisely photograph and measure the area removed by each bite. We used an Olympus Tough TG-5 camera to take photos of each lateral surface of the cube –ensuring that we had photographed the entire bite—and measured the total area removed from the cube (Figure 4).

Statistical analyses

Comparing strike kinematics

We collected and analyzed 101 feeding strikes from 31 individuals striking both shrimp and scales. (7 generalists; 7 snail-eaters; 9 scale-eaters; 8 F1 hybrids). We used linear mixed models in the lme4 package in R (Bates et al. 2014) and RStudio (R Core Team 2018) to determine if any of our kinematic metrics varied between species or food item. In each model we included: 1) the kinematic metric as the response variable, 2) species designation, food item, and their interaction as fixed effects, 3) individual fish IDs and population as random effects, and 3) log body size as a covariate (Table 3). Similarly, we used linear mixed models to determine if the feeding strikes of scale-eaters in the wild differed from those in the lab. We used each kinematic metric as the response variable, and modeled: 1) environment (e.g. lab or wild) as a fixed effect, 2) individual ID nested within environment as a random effect, and 3) log body length as a covariate (Table 4).

We also performed a linear discriminant analysis (LDA) on the combined shrimp and scales kinematic data to reduce dimensionality and identify which kinematic metrics contributed most to differences between species (Table 2, Figure 2A). We used a MANOVA and Wilks' ƛ to assess the significance of the LDA. Our MANOVA included 1) all 8 kinematic metrics as response variables, 2) species designation as a predictor variable, and 3) individual ID as a random effect.

Although we compared kinematic data across multiple species, very few genetic variants are fixed between species (<1,000 SNPs out of 12 million) and generalists and molluscivores cluster by lake rather than by species (McGirr and Martin 2017; Richards and Martin 2017).

Thus, is it appropriate to analyze species differences at these recent timescales as population-scale data using mixed model analyses of independent populations (e.g. Hatfield and Schluter 1999; McGee et al. 2013), rather than phylogenetic comparative methods.

Determining how kinematic variables affect bite performance

We used a linear mixed model to investigate if the area removed from a cube (mm^2) was associated with any of the kinematic variables measured during strikes by all three species and F1 hybrids. We first performed principle components analysis (PCA) of our 8 kinematic variables (Table S3) to obtain a set of orthogonal axes and subsequently used all eight PCs as fixed effects in our mixed model. We included 1) area removed from a cube (mm^2) as the response variable, 2) PC1-8 as fixed effects, 3) fish ID and species as random effects, and 4) log body length as a covariate (Table 5).

We also used generalized additive models (GAMs) using the mgcv package (Wood 2011) in R to further investigate how peak gape, peak protrusion, and gape angle affected bite size. We specifically chose these kinematic variables because our previous linear mixed model indicated that PC5 was a significant predictor variable of bite size which included major loadings of peak gape and peak protrusion (Table 5). Our first GAM included 1) area removed from a cube (mm^2) as the response variable and 2) a spline modeling the interaction between peak gape and peak protrusion as predictor. Since peak gape and peak protrusion are on the same relative scale (mm) we used an isotropic thin-plate smoothing spline for this model. In our second model, we included 1) area removed from a cube (mm^2) as the response variable and 2) peak gape, gape angle, and peak protrusion as predictor variables, and used univariate smoothing splines for each kinematic variable within the GAM.

Finally, we predicted the area removed per bite for each fish from their peak gape and gape angle kinematic measurements using a machine-learning algorithm from the caret package using a spline-based method (Kuhn 2008). Thus, for all scale-eating and shrimp-feeding strikes resulting in unknown bite sizes (unlike the gelatin cube strikes), we were able to build and tune a GAM model connecting our two kinematic variables of interest to the area removed from gelatin cubes (observed bite performance) in order to predict bite performance for all strikes in our dataset.

We built the model using 1) area removed from a cube (mm^2) as the response variable and 2) peak gape and gape angle as predictor variables. We trained the model using all strikes observed on gelatin cubes (31 strikes across all three species and F1 hybrids) and 10-fold cross-validations with three repeats as the resampling scheme. We tested the accuracy of this model by comparing fitted values from the model to observed values from the data set and found that our model was able to predict 68% of the variance in the dataset (df=1, F=63.84, P=8.2x10^{-9}, R^2=0.68). We then used this model to predict the bite area removed for each scale-eating and shrimp-eating strike based on the kinematic measurements alone. We used bootstrap resampling (20,000 iterations) to calculate mean bite size (predicted area removed) and 95% confidence intervals for each species.

Determining if hybrid kinematics match additive predictions

For peak gape, gape angle, and bite size, we calculated the predicted values of F1 scale-eater by generalist hybrids under the hypothesis that these kinematic traits would be additive and therefore intermediate between generalist and scale-eater values. We used a one sample *t*-test to test whether the observed values of peak gape, gape angle, and the predicted bite sizes for F1 hybrids deviated from additive predictions.

Results

Scale-eaters exhibited divergent feeding kinematics compared to other pupfishes

Scale-eaters exhibited divergent feeding kinematics, while consuming both shrimp and scales, compared to other groups (Figure 2A). A MANOVA supported the significance of this discriminant analysis and found species designation was a significant predictor of kinematics (Wilks' ƛ = 0.10; F = 2.9471; *df* = 3; *P*= 0.000394). Species significantly varied in their peak gape and gape angles during feeding strikes—regardless of the food item— in a linear mixed model controlling for individual ID and body length (Table 3). This pattern was driven by scale-eaters who had peak gapes that were twice as large as other species, but also had gape angles that were one third smaller than other species (Figure 2B-C).

Pupfish ram speed was 48% faster when consuming shrimp

Ram speed was the only kinematic variable that varied between food items, increasing by ~50% for shrimp strikes (0.098 m/s, CI: 0.082, 0.12) relative to scale-eating strikes (0.066 m/s, CI: 0.061, 0.070; Table 3; Figure S2).

347 **Figure 2.**

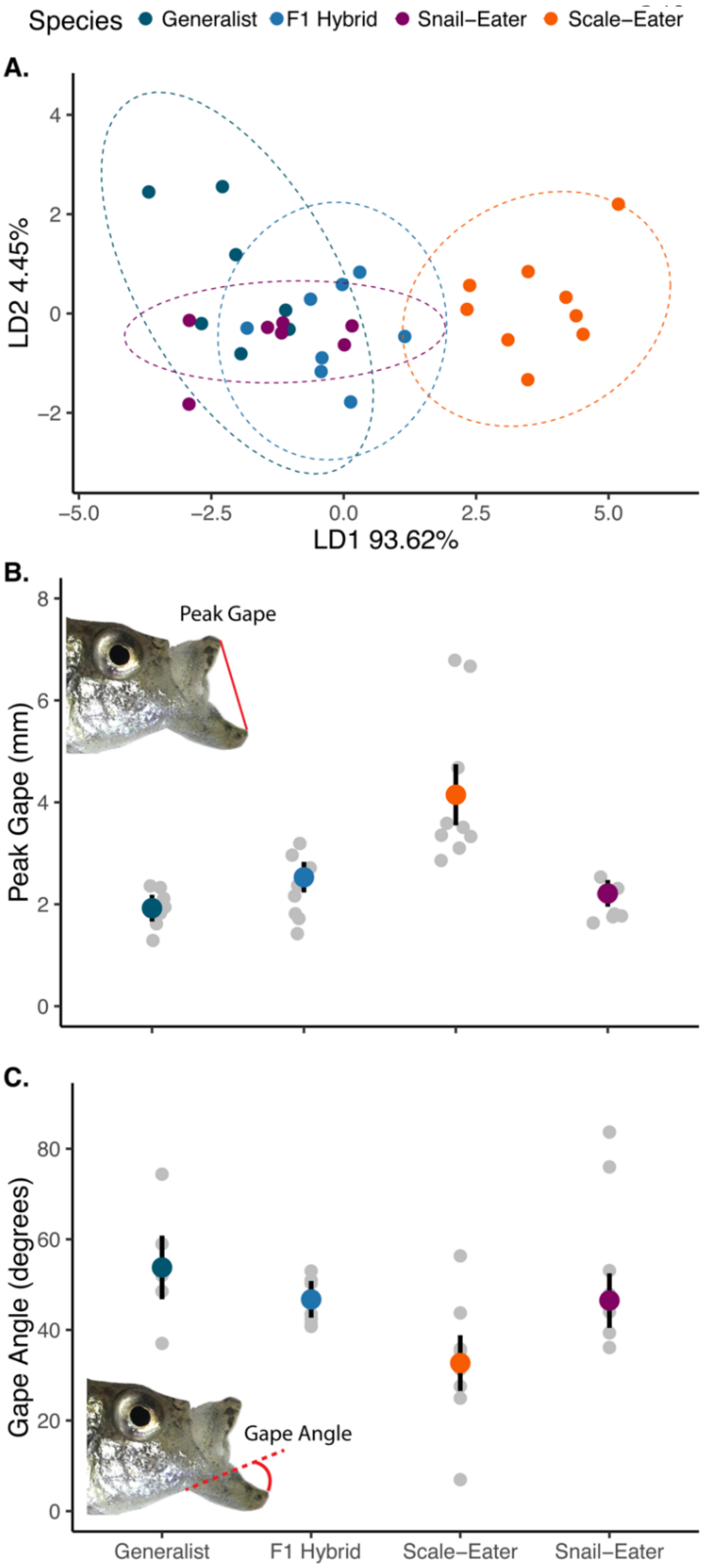

Figure 2. Divergent feeding kinematics in scale-eaters. A) Biplot of discriminant axes 1 (LD1) and 2 (LD2) describing overall kinematic differences among pupfish species (generalists, snail-eaters, scale-eaters, or F1 hybrids). Ellipses represent 95% CIs. B) Mean peak gape (mm) for each species with ± 95% CIs calculated via bootstrapping (20,000 iterations). C) Mean gape angle at peak gape (mm) for each species with ± 95% CIs calculated via bootstrapping (20,000 iterations).

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Scale-eating in the wild differed from scale-eating in the lab

Scale-eating attacks in the wild started from over twice as far away as those observed in the lab (*P* = 0.033; Table 4; Figure 3A) and ram speed was almost three times faster in the wild than in the lab (*P* = 0.00099; Table 4; Figure 1, 3B). However, the range of strike kinematics for both these variables overlapped between the lab and field, indicating substantial variation in scale-eating kinematics in both environments.

Figure 3.

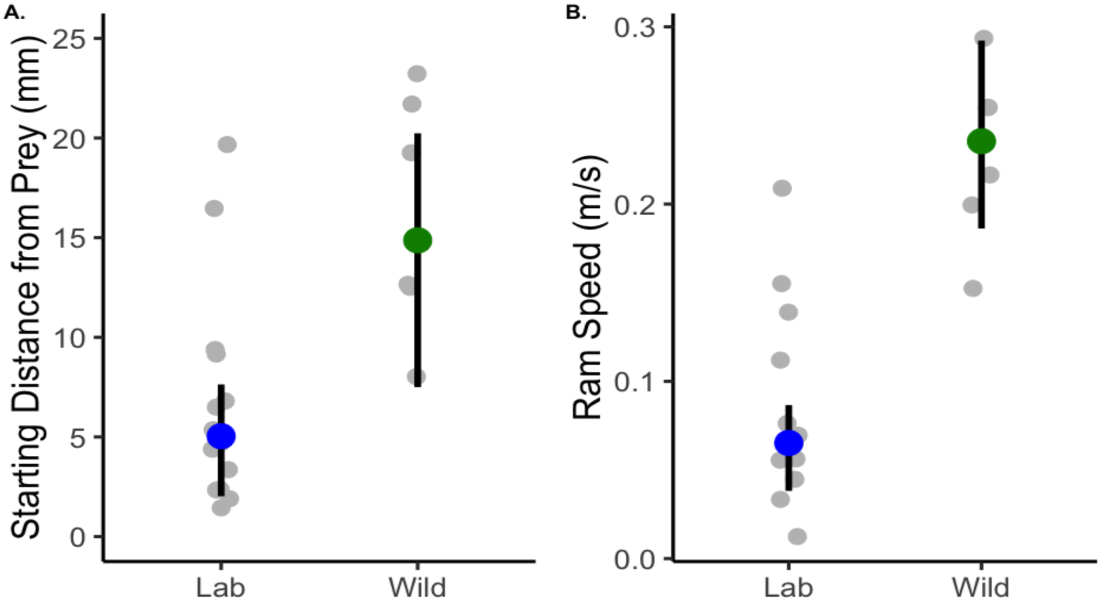

Figure 3. Wild scale-eating strikes are more than twice as fast and start three times farther away than lab strikes. a) Mean starting distance from prey defined as 20% of peak gape and b) mean ram speed (± 95% CIs; bootstrapping 20,000 iterations) for scale-eaters consuming scales in the lab and observed in situ in Crescent Pond, San Salvador Island, Bahamas.

Variation in feeding kinematics affected bite size performance

Two out of eight principal component axes of strike kinematics were significantly associated with the surface area removed from a gelatin cube per strike (Table 5). However, the significance of PC2 was entirely driven by a single outlier, and when removed, this axis was no longer significantly associated with bite size ($\chi^2 = 0.71$, *df*=1, *P* =0.40; Figure S3). PC5 remained significantly associated with bite size regardless of whether this outlier was included in the dataset. PC5 contained two major loadings (|loadings|>.4): peak gape and peak protrusion (Table S3), which loaded in opposite directions (Table 5, Figure 4).

Figure 4.

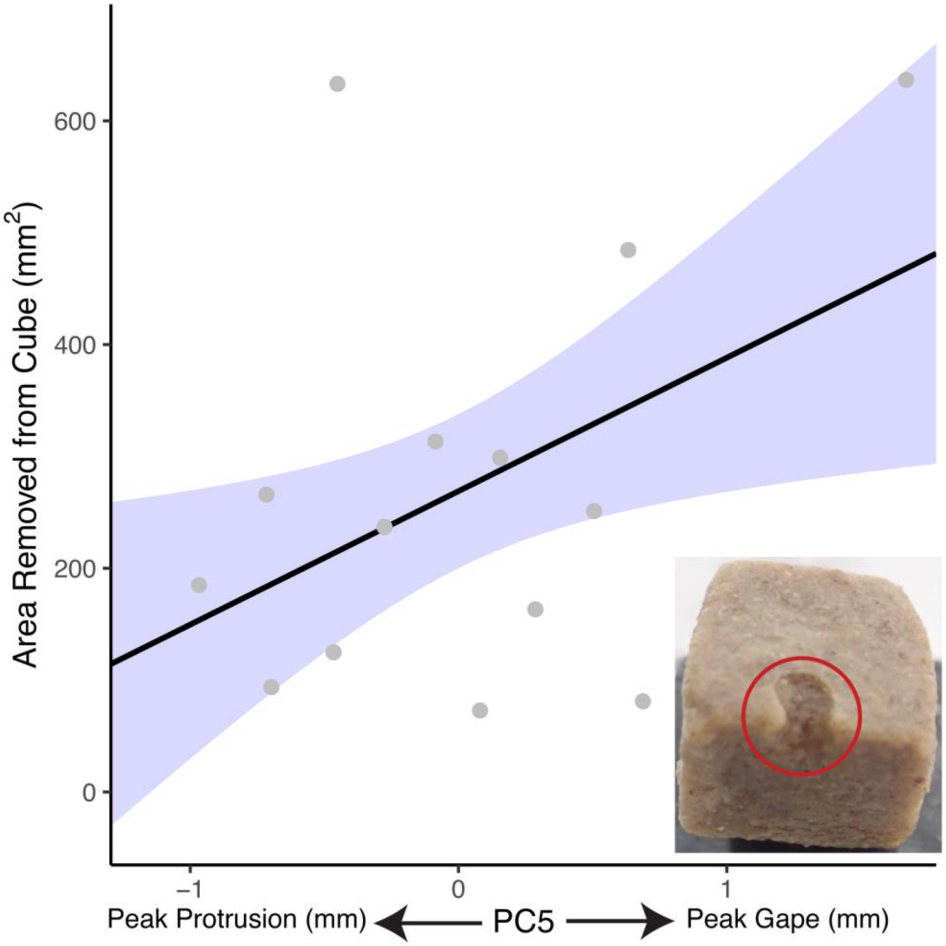

Figure 4. Peak gape and peak protrusion affect bite size. Graph illustrating the positive relationship between PC5 and the area removed from a gelatin cube (mm^2) from a single strike. The major loadings of PC5 are peak gape and peak protrusion. Grey dots represent individual fish, the line represents the fitted prediction of a linear model along with a 95% CI ribbon in blue.

Our GAMs also indicated that the interaction between peak gape and peak protrusion, modeled as a thin-plate spline, was significantly associated with bite size (*edf*=6.61, *Ref.df*=29, F= 0.59, *P*= 0.027), and explained 47.6% of the observed deviance in bite size (Figure 5A). Our second GAM, which modeled peak gape, gape angle, and peak protrusion as independent univariate smoothing splines, explained 69% of the deviance in bite size and indicated that increased peak gape (*edf*=4.96, F= 7.88, *P*= 6.22×10^{-5}) and reduced gape angle were significantly associated with increased bite size (*edf*=1, F= 4.72, *P*= 0.04). We also fit a model including gape angle and peak protrusion as linear fixed effects instead of splines; however, this model provided a poorer fit to the data (ΔAIC = 2.13). Taken together, our results suggest that a larger gape and smaller gape angle are associated with larger areas removed per bite, but that jaw protrusion distance has only a small effect (increases explained deviance by 0.9%; change in ΔAIC = 1.39) on bite size (Figure 5B-D).

Our machine learning model also predicted that scale-eater kinematics would result in bite sizes that are 65% larger than the predicted bites of the other species (Figure 6). Interestingly, estimates for F1 hybrid bite sizes were smaller than expected if kinematic metrics and bite performance are additive traits; however, this effect was only marginal (T-test, μ= 340.73, mean= 289.18, *P = 0.059*).

Figure 5.

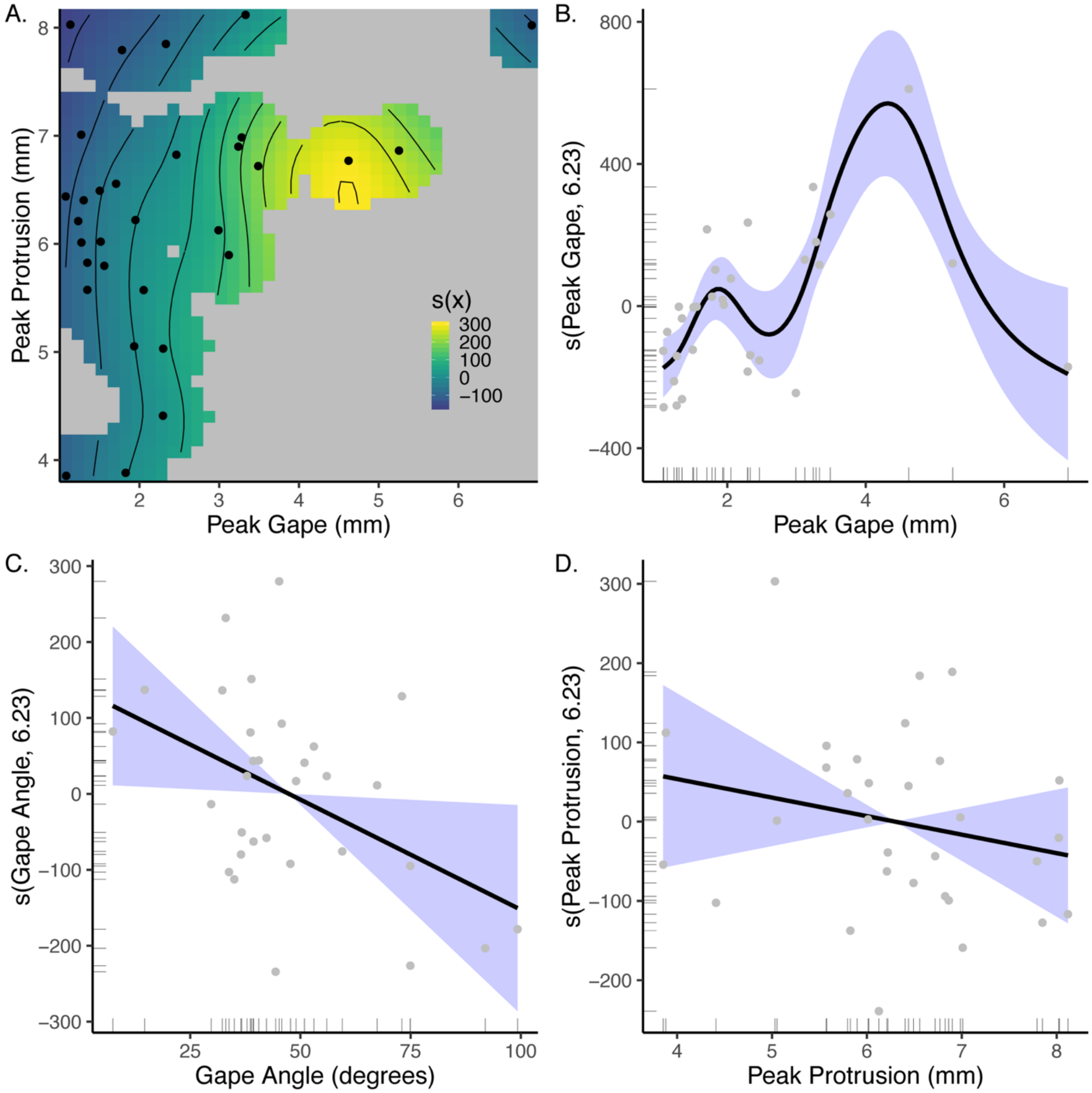

Figure 5. The interaction of peak gape and peak protrusion may result in a performance optimum for scale-biting. a) Visualization of a GAM model investigating how the thin-plate interaction between peak gape (mm) and peak protrusion (mm) affects bite size (area removed per strike from a gelatin cube). Yellow colors represent larger bites, while blues represent smaller bites. Grey areas represent bite sizes that cannot be predicted from the current data set. b) Visualizations of the smoothing splines for b) peak gape size, c) gape angle, and d) peak protrusion within the GAM model.

Figure 6.

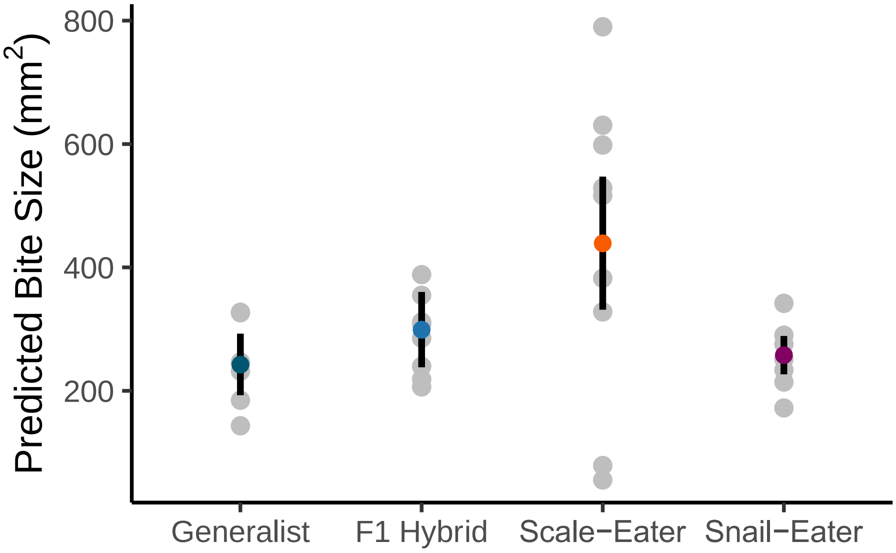

Figure 6. Scale-eaters have larger predicted bite sizes compared to other species. Predicted bite sizes for all strikes from each species using machine-learning optimization of GAM models. Grey points represent predicted bite sizes for individuals, color points represent means, and bars represent ± 95% CIs calculated via bootstrapping (20,000 iterations).

F1 hybrid kinematics are not additive and more closely resemble generalist kinematics

F1 hybrid feeding kinematics, across both food items, differed from scale-eater kinematics (TukeyHSD, $P = 4.59 \times 10^{-8}$), but were not significantly different from generalist kinematics (Tukey's HSD, $P = 0.21$). Mean hybrid peak gape was 58% smaller than scale-eater peak gape and 24% larger than generalist peak gape (Figure 2B). Similarly, mean hybrid gape angle was 56% larger than scale-eater peak gape angle, but was only 4.25% smaller than the mean generalist gape angle (Figure 2C). Hybrids failed to match additive predictions of intermediate kinematics for both peak gape (t-test, $\mu = 3.13$, mean= 2.25 mm, $P = 0.0032$) and gape angle (t-test, $\mu = 42.43$, mean= 48.37 degrees, $P = 0.044$).

Discussion

Scale-eating pupfish have divergent, but not plastic, feeding kinematics

Scale-eating pupfish exhibited peak gapes during scale-eating strikes that were twice as large and gape angles that were 32% smaller than other sympatric species within the San Salvador Island radiation. Similarly, Janovetz (2005) found that the peak gape angle of the scale-eating piranha *Cataprion mento* was 1.5 times larger during scale-eating strikes than while suction-feeding on whole fish. Unlike *C. mento*, however, pupfish feeding kinematics remained divergent between species, but consistent across prey items (Table 3). In fact, the only kinematic variable that varied between prey items was ram speed (Table 3, Figure S2). However, this may simply be due to the fact that shrimp were a moving target during feeding trials while scales were stationary on the side of a euthanized zebrafish. Alternatively, phenotypic plasticity due to rearing environment could produce a similar pattern where wild caught pupfish display plastic kinematic traits, while lab reared pupfish do not. However, we find this scenario unlikely as all fish were acclimated to the laboratory environment for several months before feeding trials began, and we did not observe any difference in kinematic traits between these two groups.

Is jaw morphology solely responsible for kinematic variation?

The kinematic variables that varied the most between scale-eating and non-scale-eating pupfishes were peak gape and gape angle—both related to the size of the oral jaws. Previous work has documented that the oral jaws of scale-eating pupfish are three times larger than their sister species (Holtmeier 2001; Martin and Wainwright 2013a; Martin 2016) and controlled by four moderate-effect QTL with all positive effects on jaw size, consistent with directional selection on this trait (Martin et al. 2017). It may be that increased oral jaw size is sufficient to create variation in feeding kinematics without an accompanying shift in behavior. Previous studies have documented how changes in morphology alone can alter feeding kinematics. For example, kinematic studies have found that the scaling of the lower jaw in bluegill (Wainwright and Shaw 1999) and body size in largemouth bass (*Micropterus salmoides;* Richard and Wainwright 1995) both significantly affected prey capture kinematics. Furthermore, Ferry-Graham et al. (2010) used the pike killifish (*Belonesox belizanus*) to show that simply doubling the length of the jaws significantly affected key kinematics variables such as peak gape size—even while keeping gape angle constant. Simply stated, the key innovation necessary for scale-eating may be an enlarged jaw. If this hypothesis were true, we would expect that peak gape would increase with jaw size, but that all other kinematics variables would remain constant across species. Our results reject this hypothesis. Instead, the gape angle of scale-eaters is 32% smaller than other groups resulting in a reduction in their potential peak gape size. This suggests that scale-eaters have evolved a smaller gape angle possibly to increase feeding performance (Figure 5 & 6). Furthermore, this reduced gape angle is consistent across food items and strikes, indicating that it is not a plastic trait relative to individual foraging context.

Scale-eating performance optimum

Scale-eaters may have reduced their gape angles relative to other species in order to remain on a performance optimum for scale-eating. Our models of bite performance supported this: peak gapes larger than approximately 4.5 mm counterintuitively resulted in smaller bite sizes (Figure 5A). A reduced angle in scale-eating pupfish results in a lower jaw that is essentially parallel with the ventral surface of its body—possibly resulting in greater stability for biting scales while retaining a large gape. This large gape and parallel jaw alignment may allow scale-eaters to attack prey from a roughly perpendicular angle (as frequently observed during field observations) —appearing to wrap their large lower jaw under prey items and subsequently scrape scales from their sides using their independently protrusible upper jaws (also observed in a scale-eating characin: Hata et al. 2011). Interestingly, perpendicular angles of attack and large gapes are associated with scraping in benthic feeding fish (Van Wassenbergh et al. 2008; O'Neill and Gibb 2013). In fact, one prominent hypothesis for the origins of scale-eating is that it arose from an algae-scraping ancestor (Sazima et al. 1983). One caveat for this hypothesis, however, is that our current performance estimates do not include all possible combinations of peak gape and gape angle. Future work should estimate performance across all combinations of peak gape and gape angle (e.g. Stayton 2019).

Kinematics in the wild differs from the lab

Scale-eating in the lab is decidedly different from in the wild. In the wild, scale-eaters must approach a prey pupfish while remaining unnoticed, attack them very quickly, hold on to the side of the pupfish as it attempts to escape, and finally rip off the desired scales and protein-rich mucus coat (Supplemental Video S1). In the lab, however, a scale-eating attack on euthanized immobilized prey involves none of these evasive prey aspects of an attack, which may affect kinematics. Despite these stark differences, many kinematic traits measured in both the lab and the wild were similar. Importantly, the two kinematics which varied the most between scale-eaters and other species in the lab, peak gape and gape angle, were consistent across environments (Table 4).

However, we did find that wild scale-eating strikes were faster than those in the lab and began from further away; however, the range of both these kinematics variables still overlapped with strikes recorded in the lab (Table 4, Figure 3). This may indicate that pupfish can vary their feeding kinematics based on evasive prey dynamics.

Non-additive F1 hybrid feeding kinematics may contribute to reproductive isolation of scale-eaters

Given that complex performance traits are most likely highly polygenic and thus may exhibit additive heritability on average, we expected F1 hybrids to exhibit intermediate kinematics and performance relative to both parental species. Instead, we found that F1 hybrid kinematics did not meet the predictions of additivity and that their kinematics and performance more closely resembled that of the generalist (Table 3; Figure 2 & 6), suggesting that F1 hybrids may have higher performance in a generalist trophic niche. Current evidence from field fitness experiments supports this idea. One field experiment in these lakes measured F2 hybrid fitness in the wild and found high mortality and low growth rates for hybrids most closely resembling the scale-eating phenotype (Martin and Wainwright 2013b). Furthermore, for the few hybrids resembling scale-eaters which did survive, only 36% had recently consumed any scales compared to 92% of wild-caught scale-eaters (Martin and Wainwright 2013a,b). Impaired hybrid performance in the scale-eating niche may contribute to extrinsic postzygotic isolation between species (McGhee et al. 2007; McGee et al. 2013; Higham et al. 2016). Reproductive isolation may also evolve more quickly in species that occupy a more distant fitness peak with a larger fitness valley such as the scale-eating pupfish due to stronger selection against hybrids and reinforced pre-mating isolation (Martin and Feinstein 2014). Thus impaired hybrid scale-eating performance could also contribute to increased diversification rates through the mechanism of a wider fitness valley.

Low hybrid performance may also be due to their morphological differences from purebred scale-eaters and generalists. As mentioned above, it is possible that a shift in morphology – such as enlarged oral jaws in scale-eaters—may be sufficient to change kinematic profiles alone. F1 hybrid kinematics clearly differed from scale-eater kinematics, but their jaw lengths were also significantly smaller than the jaws of scale-eaters (Tukey's HSD, $P = 0.018$). Furthermore, previous work has shown that hybrid pupfish offspring (produced from generalist x scale-eater crosses) tend to develop along a more similar trajectory to their maternal parent (Holtmeier 2001). This could indicate that hybrid pupfish with scale-eating mothers are more likely to develop jaws resembling a purebred scale-eater, but may also retain their generalist-like kinematics. The resulting mismatch between morphology, kinematic traits, and ecological niche may be driving low hybrid survival in the scale-eating niche and contributing to reproductive isolation between generalist and scale-eating pupfish species.

Conclusion

In conclusion, this study suggests that shifts in kinematic traits may have preceded or facilitated the origin of scale-eating in *Cyprinodon* pupfishes. Scale-eating pupfish exhibited peak gapes that were twice as large as other pupfish species, but simultaneously had gape angles that were significantly smaller. Surprisingly, we found that this unique combination of scale-eater kinematics may reside on a performance optima, as large peak gapes and small gape angles result in larger bite sizes. Impaired F1 hybrid kinematics and performance in the scale-eating niche also suggests that kinematic traits contribute to reproductive isolation of the scale-eating pupfish and the evolution of novelty. Future work should investigate if other performance optima exist on the kinematic landscape and whether F2 hybrid fitness in the wild is due to a mismatch between morphology and feeding kinematics.

Authors' Contributions

MES and CHM conceptualized the project, MES collected data and performed all analyses, MES wrote the manuscript, MES and CHM revised drafts, and CHM funded the study.

Data Accessibility

All raw data will be deposited in the Dryad Digital Repository.

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Table 1. Description of the 8 kinematic metrics and 3 morphological metrics measured from high-speed videos of feeding strikes.

Metric	Measurement
Peak Protrusion (mm)	The distance (mm) from the center of the eye to the anterior tip of the premaxilla.
Time to Peak Protrusion (s)	Time (s) from the start of an attack (20% of peak gape) to peak protrusion.
Peak Gape	The distance (mm) from the anterior tip of the premaxilla to the anterior tip of the dentary.
Time to Peak Gape (s)	Time (s) from the start of an attack to peak gape.
Gape Angle (degrees)	180° minus the angle produced at peak gape between the lower jaw, the quadrate-articular joint, and the ventral surface of the fish beneath the suspensorium (Fig. 2).
Time to Impact (seconds)	Time (s) from the start of an attack to first contact of oral jaws with the prey item.
Starting Distance from prey (mm)	The distance (mm) from the center of the orbit at the start of an attack to the center of the orbit at impact with prey item.
Ram speed (m/s)	Starting distance from prey (m) / time to impact (s)
Body length (mm)	The length (mm) from the anterior tip of the dentary with mouth closed to the posterior end of the hypural plate at the midpoint insertion of the caudal fin.
Lower jaw length (mm)	The length (mm) from the anterior tip of the dentary to the quadrate-articular joint (jaw joint).

Table 2. Results of a linear discriminant analysis for kinematic variables between strikes on shrimp and scales.

Kinematic Metrics	**LD1**	**LD2**	**LD3**
Peak Protrusion (mm)	0.23	-0.44	0.14
Peak Gape (mm)	1.33	0.47	-0.83
Gape Angle (degrees)	0.08	0.00	0.02
Time to Peak Protrusion (s)	37.30	-12.88	9.90
Time to Peak Gape (s)	-5.10	-14.42	49.24
Time to Impact (s)	9.85	35.43	0.18
Starting Distance from prey (mm)	-614.27	28.85	-545.38
Ram speed (m/s)	25.12	14.23	45.50
Proportion of Trace	0.94	0.05	0.02

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Table 3. Results of linear mixed models investigating if strike kinematic variables vary between 1) species (generalists, snail-eaters, scale-eaters, or hybrids), 2) food item (shrimp or scales), or 3) the interaction between the two. Significant predictors are indicted in bold.

Response	**Predictors**	χ^2	***df***	***P***
Peak Protrusion (mm)	Species	4.5	3	0.21
	Food Item	1.2	1	0.27
	log(Body Length)	3	1	0.085
	Species:Food Item	2.3	3	0.51
Peak Gape (mm)	**Species**	**23**	**3**	**$3.4\text{x}10^{-5}$**
	Food Item	1.1	1	0.3
	log(Body Length)	1.3	1	0.26
	Species:Food Item	0.83	3	0.84
Peak gape angle (degrees)	**Species**	**18**	**3**	**0.00037**
	Food Item	0.016	1	0.9
	log(Body Length)	3.4	1	0.066
	Species:Food Item	3.6	3	0.3
Time to Peak Protrusion (s)	Species	4.1	3	0.25
	Food Item	1.1	1	0.3
	log(Body Length)	1.2	1	0.27
	Species:Food Item	3.3	3	0.34
Time to Peak Gape (s)	Species	2.5	3	0.48
	Food Item	0.87	1	0.35
	log(Body Length)	2	1	0.16
	Species:Food Item	1.6	3	0.65
Time to Impact (s)	Species	3.4	3	0.33
	Food Item	2.1	1	0.14
	log(Body Length)	1.5	1	0.21
	Species:Food Item	4.5	3	0.21
Starting Distance from prey (mm)	Species	2.8	3	0.42
	Food Item	0.032	1	0.86
	log(Body Length)	2.1	1	0.15
	Species:Food Item	0.35	3	0.95
Ram speed (m/s)	Species	3.7	3	0.3
	Food Item	**4**	**1**	**0.047**

log(Body Length)	1.2	1	0.27
Species:Food Item	2.2	3	0.54

Table 4. Results of linear mixed models investigating if scale-eating kinematics vary between lab trials and wild strikes observed in situ in Crescent Pond, San Salvador Island, Bahamas. Significant predictors are indicted in bold.

Response	**Predictors**	**χ^2**	***df***	***P***
Peak Protrusion (mm)				
	Environment	1.5	1	0.22
	log(Body Length)	5.9	1	0.016
Peak Gape (mm)				
	Environment	0.0018	1	0.97
	log(Body Length)	2.6	1	0.11
Gape Angle (degrees)				
	Environment	0.0027	1	0.96
	log(Body Length)	3.1	1	0.078
Time to Peak Protrusion (s)				
	Environment	0.017	1	0.9
	log(Body Length)	1.7	1	0.19
Time to Peak Gape (s)				
	Environment	0.27	1	0.6
	log(Body Length)	3.7	1	0.053
Time to Impact (s)				
	Environment	0.98	1	0.32
	log(Body Length)	2	1	0.16
Starting Distance from prey (mm)				
	Environment	**4.5**	**1**	**0.033**
	log(Body Length)	0.9	1	0.34
Ram speed (m/s)				
	Environment	**11**	**1**	**0.00099**
	log(Body Length)	0.1	1	0.75

Table 5. Results of linear mixed models investigating if the area removed from a cube by a single bite was affected by any of the PCs from our PCA analysis. Significant predictors are

Response	Predictor	χ^2	df	*P*
Area Removed from Cube (mm^2)	PC1	1.60	1	0.21
	PC2	**5.49**	**1**	**0.02**
	PC3	0.11	1	0.74
	PC4	0.29	1	0.59
	PC5	**8.80**	**1**	**0.003**
	PC6	0.46	1	0.50
	PC7	1.86	1	0.17
	PC8	0.00	1	1.00
	log(Body Length)	2.21	1	0.14

Supplemental Material

Table S1. Jaw length varies between species. Results of linear mixed model investigating if any morphological traits varied between species. Significant predictors are indicated in bold.

Response	**Predictors**	χ^2	***df***	***P***
Log Body Length				
	Species	6.36	3	0.095
Log Jaw Length				
	Species	**45.87**	**3**	$\mathbf{6.039 \times 10^{-10}}$

Table S2. PCA Loadings describing kinematic variation in pupfish while feeding on shrimp and scales. Major loadings (|loading|> 0.4) are indicated in bold.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Peak Protrusion (mm)	0.376	0.142	-0.063	**-0.449**	**0.786**	-0.015	0.077	-0.095
Peak Gape (mm)	0.368	-0.036	-0.175	**-0.669**	**-0.575**	0.131	0.188	0.056
Gape Angle (degrees)	0.08	-0.277	**-0.925**	0.224	0.069	-0.077	-0.015	0.021
Time to Peak Protrusion (s)	**0.419**	-0.188	0.19	0.192	-0.096	**-0.784**	0.287	-0.1
Time to Peak Gape (s)	**0.434**	-0.249	0.123	0.035	-0.063	0.044	**-0.845**	-0.118
Time to Impact (s)	**0.412**	-0.23	0.137	0.39	0.012	0.588	0.391	-0.328
Starting Distance from prey (mm)	**0.407**	0.39	-0.002	0.292	-0.005	0.099	0.004	**0.766**
Ram speed (m/s)	0.135	**0.774**	-0.199	0.159	-0.184	-0.071	-0.099	**-0.518**
Standard Deviation	2.096	1.19	0.987	0.734	0.621	0.427	0.311	0.123
Proportion σ2	0.549	0.177	0.122	0.067	0.048	0.023	0.012	0.002
Cumulative Proportion σ2	0.549	0.726	0.848	0.915	0.963	0.986	0.998	1

Table S3. PCA Loadings describing kinematic variation in pupfish while feeding on fish cubes. Major loadings for PC2 and PC5 (|loading|> 0.4) are indicated in bold.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Peak Protrusion (mm)	-0.04	-0.16	0.72	0.43	**-0.51**	0.12	-0.04	0.00
Peak Gape (mm)	-0.30	-0.18	0.55	-0.09	**0.72**	-0.20	0.08	0.01
Gape Angle (degrees)	0.00	0.33	0.36	-0.82	-0.28	0.02	-0.08	0.02
Time to Peak Protrusion (s)	-0.55	0.06	-0.08	-0.03	0.05	0.80	0.21	-0.05
Time to Peak Gape (s)	-0.56	0.08	-0.12	0.08	-0.08	-0.19	-0.79	0.00
Time to Impact (s)	-0.53	0.15	-0.12	0.04	-0.29	-0.50	0.57	0.15
Starting Distance from prey (mm)	-0.12	**-0.62**	-0.10	-0.25	-0.18	-0.10	0.05	-0.69
Ram speed (m/s)	-0.04	**-0.64**	-0.10	-0.24	-0.12	0.06	-0.05	0.71
Standard Deviation	1.73	1.50	1.13	0.90	0.71	0.38	0.18	0.05
Proportion σ2	0.37	0.28	0.16	0.10	0.06	0.02	0.00	0.00
Cumulative Proportion σ2	0.37	0.65	0.81	0.91	0.98	1.00	1.00	1.00

Figure S1. Visualization of PC1 and PC2 describing kinematic variation in pupfish while feeding on shrimp and scales. Dots represent individuals, dotted lines represent 95% confidence intervals around species.

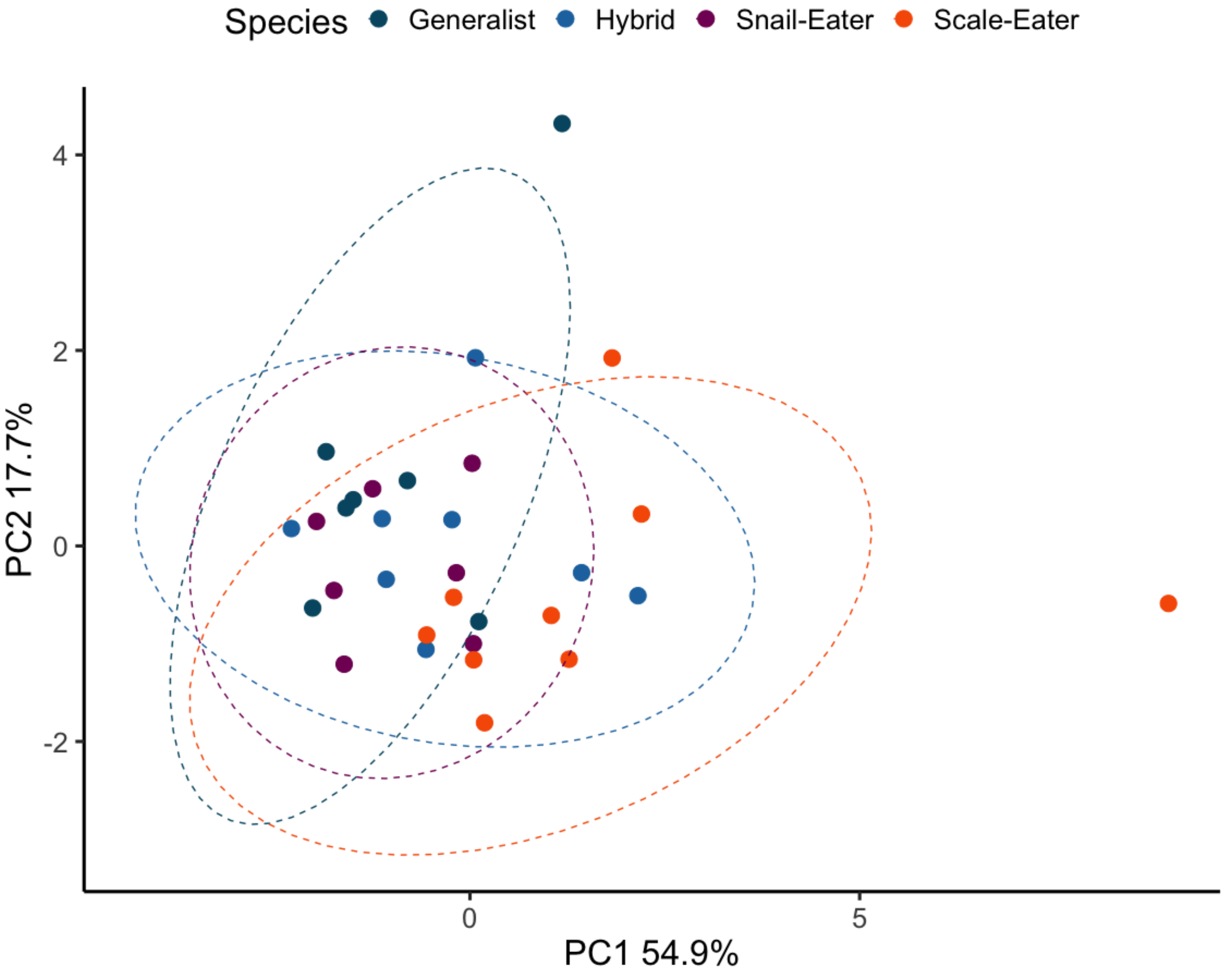


Figure S2. Pupfishes approach shrimp more quickly than they do scales. Colored points represent mean ram speed (m/s) and 95% confidence intervals when consuming shrimp versus scales for all species.

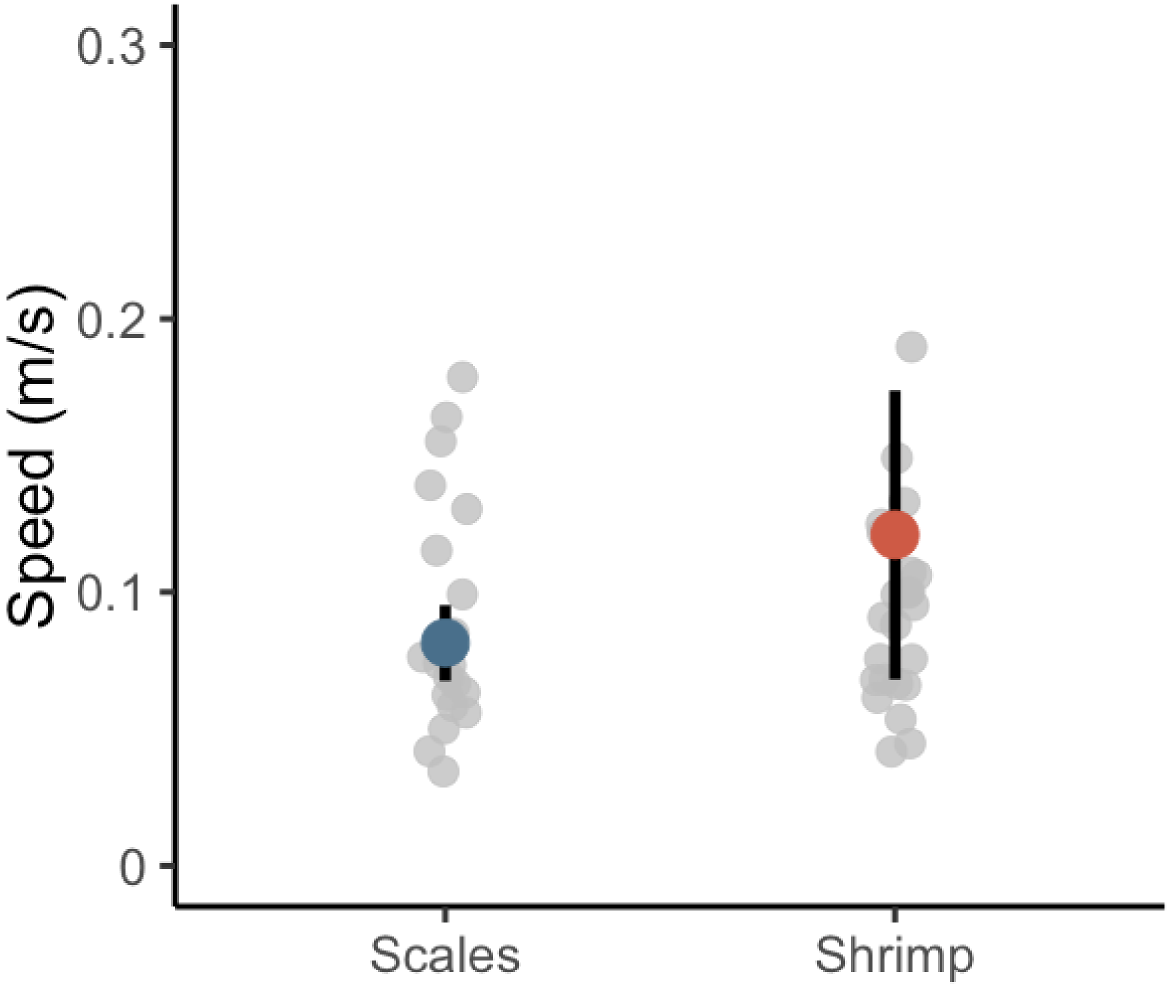
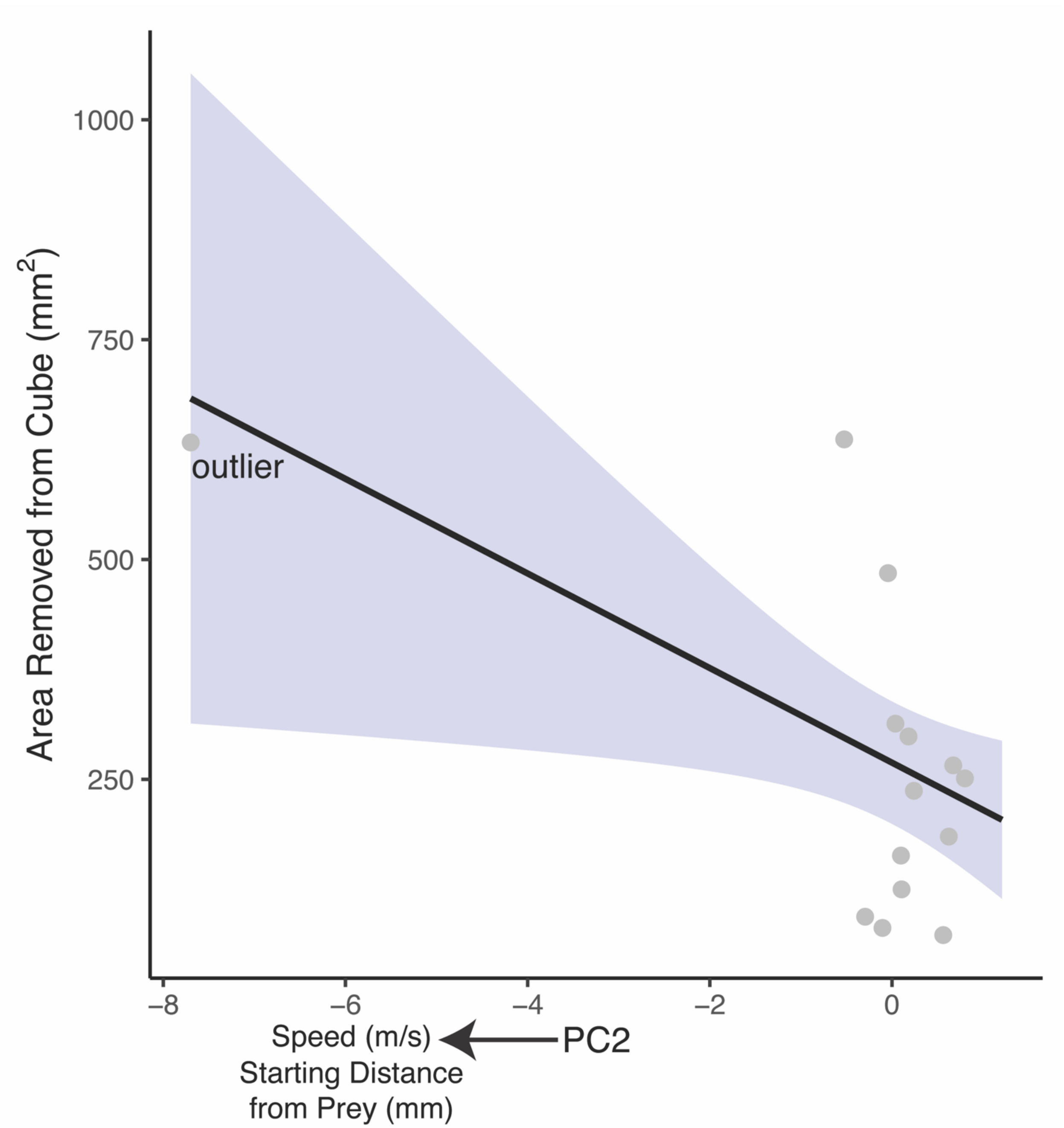


Figure S3. Ram speed and starting distance from prey affects bite size. Graph illustrating the negative relationship between PC2 and the area removed from a fish cube (mm^2) from a single bite. The major loadings of PC2 are ram speed and starting distance from a prey item. Grey dots represent individual fish, the line represents the fitted prediction of a linear model along with a standard error ribbon in blue.



Supplemental Video 1
Scale-eating pupfish attacks a generalist pupfish in the wild.