ORIGINAL ARTICLE

doi:10.1111/evo.13

SSE
SOCIETY for the STUDY of EVOLUTION

EVOLUTION
INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

Feeding ecology underlies the evolution of cichlid jaw mobility

Christopher M. Martinez,[1,2] Matthew D. McGee,[3] Samuel R. Borstein,[4] and Peter C. Wainwright[1]

[1] *Department of Evolution and Ecology, University of California, Davis, California 95616*

[2] *E-mail: cmimartinez@ucdavis.edu*

[3] *School of Biological Sciences, Monash University, 3800 Victoria, Australia*

[4] *Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, Tennessee 37996*

Received January 31, 2018
Accepted May 21, 2018

The fish feeding apparatus is among the most diverse functional systems in vertebrates. While morphological and mechanical variations of feeding systems are well studied, we know far less about the diversity of the motions that they produce. We explored patterns of feeding movements in African cichlids from Lakes Malawi and Tanganyika, asking whether the degree of kinesis is associated with dietary habits of species. We used geometric morphometrics to measure feeding kinesis as trajectories of shape change, based on 326 high-speed videos in 56 species. Cranial morphology was significantly related to feeding movements, both of which were distributed along a dietary axis associated with prey evasiveness. Small-mouthed cichlids that feed by scraping algae and detritus from rocks had low kinesis strikes, while large-mouthed species that eat large, evasive prey (fishes and shrimps) generated the greatest kinesis. Despite having higher overall kinesis, comparisons of trajectory shape (linearity) revealed that cichlids that eat mobile prey also displayed more kinematically conserved, or efficient, feeding motions. Our work indicates that prey evasiveness is strongly related to the evolution of cichlid jaw mobility, suggesting that this same relationship may explain the origins and diversity of highly kinetic jaws that characterize the super-radiation of spiny-rayed fishes.

KEY WORDS: Cichlid, diet, geometric morphometrics, kinematics, motion trajectory.

Modern fishes occupy many of the trophic niches available in aquatic habitats, owing their extraordinary range in diets to a complex feeding apparatus that has displayed a high level of evolutionary lability (Lauder 1982). Moreover, patterns of morphological and functional diversity that we observe are closely linked to the type of prey consumed. For example, jaw function is often associated with prey mobility, where fishes that eat slow-moving or hard prey evolve feeding mechanisms that transmit more force through jaw levers and those that eat evasive prey transmit more motion (Westneat 2004; Wainwright et al. 2004; Cooper and Westneat 2009). It is also common in predators of evasive prey to incorporate body movement and/or increased jaw protrusion, known as ram, into the feeding motion in order to quickly reduce their distance from the prey (Longo et al. 2016). Species that rely heavily on ram feeding typically have large gapes that allow them to engulf prey by drawing large volumes of water into their mouths (Wainwright et al. 2007; Oufiero et al. 2012). Additionally, the level of prey mobility that a species is adapted to feeding upon has important implications for its feeding success and behavior across different prey types (Vinyard 1980; Drenner et al. 1982; McComas and Drenner 1982; Vinyard 1982; Meyer 1987; Coughlin and Strickler 1990; Nemeth 1997). This study focuses on a diverse assemblage of teleost fishes, the East African rift lake cichlids, to draw connections between the diversity of the feeding apparatus, its capacity to generate kinesis, and primary diet habits.

Kinematics research provides an explicit link between biological motions and the morphological features that generate them. Over the past three decades, methods for visualizing and tracking the morphological changes associated with motions have improved substantially (Ferry-Graham and Lauder 2001; Brainerd et al. 2010). However, the treatment of morphological data itself has not experienced equivalent levels of innovation. Standard procedure in many kinematic studies is to measure temporal changes for multiple disparate morphological features during an activity (e.g., Liem 1979; Lauder 1980; Wainwright et al. 1991; Wilga and Motta 2000; Higham et al. 2017). While appropriate for assessing relative contributions of different components of the feeding apparatus, this approach is limited in its ability to treat this complex structure as the integrated system that it is. We address this issue by incorporating the manifold movements that occur across the feeding apparatus during prey capture into a single object, a trajectory of shape change.

In geometric morphometrics (GM), shapes are defined by a set of homologous landmarks, and after scaling and alignment via generalized Procrustes analysis, each shape comprises a single point in a multidimensional morphospace. When landmarks are assigned at multiple stages during a motion, the resulting shapes (in this study, sampled at equivalent intervals in time) trace an ordered series of points, or trajectory, through morphospace (Adams and Cerney 2007). These trajectories contain important information about motions and, while no previous studies have applied these methods across large numbers of species, they show promise for evaluating kinesis in a comparative framework. We focused on two motion traits that are linked to features of trajectories. First, the magnitude of kinesis is a measure of the total amount of shape change that the system has undergone during a motion and is the sum of pairwise Procrustes distances between successive trajectory shapes (Fig. 1). This approach has been used to estimate phenotypic trajectory lengths in different contexts, including morphological evolution (Adams and Collyer 2009; Collyer and Adams 2013), development (Powder et al. 2015), and biomechanics (Adams and Cerney 2007). For fishes feeding at maximum effort, this metric provides a measure of the relative capacity of the feeding apparatus to generate kinesis.

The second trait, kinematic efficiency, is a novel performance metric based on the relative linearity of shape trajectories. As this concept is new, both to kinematics and GM studies, we introduce some background and rationale. The shortest distance between two shapes is a linear trajectory through morphospace (Fig. 1), along which the manner of shape change remains invariable. Along a linear trajectory, each landmark maintains a constant rate and direction of translation. In contrast, nonlinearity exists when more than one type of shape change exists within the same motion, resulting in an alteration of trajectory course through morphospace. Nonlinearity exists when (1) one or more landmarks exhibit a shift in their direction of translation, (2) landmarks display temporal variation in their rate of translation, or (3) a combination of these two situations. In fishes, kinematic efficiency captures the fact that activation of different components of the feeding apparatus is often temporally offset. While a level of temporal discordance is thought to be necessary for suction feeding, like the generation of an anterior-to-posterior wave of buccal expansion (Lauder 1980; Bishop et al. 2008), too much will result in a strike where transitions between disparate components of motion become less streamlined and additional kinesis is used to achieve the same task. Consequently, we define kinematically efficient motions as those that are more linear in morphospace, thereby conserving the amount of kinesis produced to achieve full gape.

Among fishes, cichlids (Teleostei: Cichlidae) are well known for their success at modifying a suction-based craniofacial theme to invade a diversity of trophic niches (Stiassny 1981; Winemiller et al. 1995; Muschick et al. 2012). Their value as a model group for understanding adaptive radiations is widely appreciated (Genner and Turner 2005; Seehausen 2006; Muschick et al. 2014), with multiple lineages displaying recent and rapid bouts of diversification (Burress and Tan 2017). In the present work, we focus on the morphologically and trophically diverse cichlids of the East African rift lakes, Tanganyika and Malawi. We evaluated feeding kinesis in 56 species (Table S1) that range in diets from sessile (algae) to highly mobile prey (shrimps and fishes). In a previous study of these same species, evolutionary transitions between biting and suction feeding were linked, specifically, to the degree of upper jaw mobility (McGee et al. 2016). While dissociation of the upper jaw (maxilla and premaxilla) represents a major functional innovation in fishes, its kinematic output, taken in isolation, can be an inconsistent indicator of feeding ecology. For instance, reduced jaw protrusion is shared in some species of herbivorous and piscivorous cichlids (McGee et al. 2016), superficially suggesting kinematic convergence despite highly divergent feeding systems. Here, we extend the study of McGee et al. (2016), increasing the sampling of feeding sequences as well as the morphological scale of kinematic evaluation to incorporate components of feeding motions beyond upper jaw kinesis (i.e., lower jaw rotation, cranial elevation, and hyoid depression). Coupled with the analysis of geometric trajectories, described above, this approach allowed us to address novel questions centered on the evolution and diversity of feeding motions in cichlids, both in extent and pattern, and their relationships to an axis of prey evasiveness. We predicted that cichlids adapted to eating larger, more mobile prey would generate greater kinesis, potentially relating to an increase in gape size and the extent of volumetric expansion of the buccal cavity, two key components of suction feeding (Wainwright et al. 2015). In addition, we expected that these species would have more kinematically efficient feeding motions (i.e., more linear shape trajectories) as a compensatory mechanism for possessing highly kinetic feeding systems. This would allow cichlids to conserve additional kinesis arising from temporally discordant activation of the various components of the feeding apparatus.

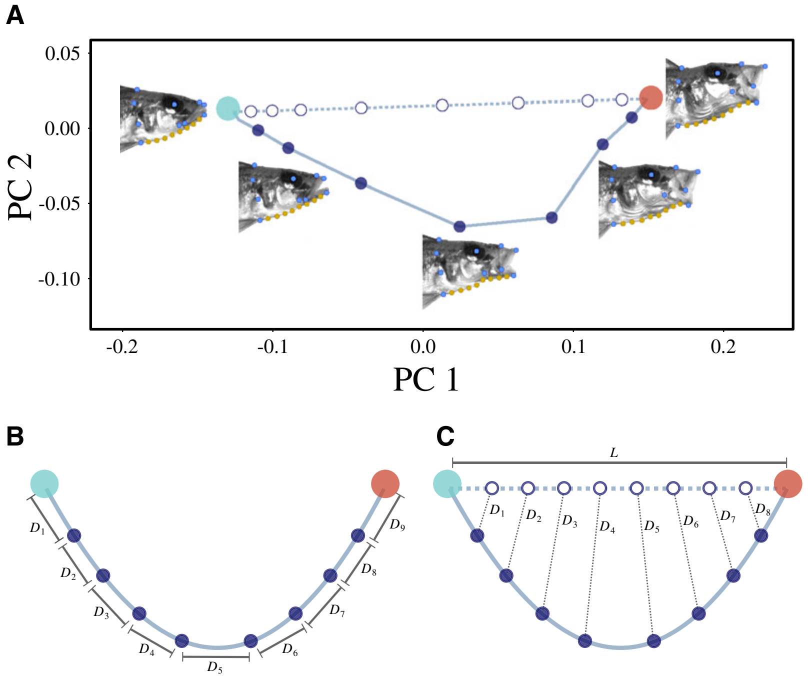

Figure 1. (A) Principle components 1 and 2 for a single motion trajectory (solid blue line) in the species *Lamprologus lemairii*. A linear baseline between start and end shapes is also plotted (blue dotted line). Selected video frames are shown with landmarks (light blue dots) and sliding semilandmarks (yellow dots). (B) Kinesis is calculated as the sum of Procrustes distances, *D*, between successive motion shapes. (C) Kinematic efficiency (trajectory linearity) is evaluated by a set of eight Procrustes distances between matching linear and nonlinear trajectories (thin dotted lines), each standardized by the linear distance between start and end shapes, *L*.

Materials and Methods

FEEDING VIDEOS

We used 326 high-speed videos of suction feeding strikes in 87 individuals, from 56 species of cichlid (Table S1). Approximately 30% of the videos were used in a previous study (McGee et al. 2016). The remaining videos largely represented within-individual replication of feeding sequences that were not previously evaluated. We included species from lakes Malawi and Tanganyika, with the highest taxon sampling from the tribes Haplochromini and Lamprologini (Fig. 2). Videos were filmed at 2000 frames per second from a lateral view. Great care was taken to elicit high-effort suction strikes to standardize feeding motions and minimize variation relative to the range of motions, or feeding repertoire (*sensu* Liem 1980), that the fish is capable of producing.

Given the trophic diversity of rift lake cichlids, our comparisons included species with disparate feeding behaviors in nature, like those that feed from the water column (e.g., *Haplotaxodon microlepis*), sift sand from the benthos (e.g., *Ectodus descampsii*), or scrape algae from rocks (e.g., *Eretmodus cyanostictus*). However, the common (and ancestral) feeding mode retained in all species was suction. To accommodate the diversity of feeding mechanisms evaluated in this study and also control for diet, we used moderately evasive prey that could be captured by suction in all species. As a result, fishes were fed either mosquito larvae (*Culex pipiens* donated from cultures at Sacramento-Yolo Mosquito and Vector Control District) or an aquarium strain of *Daphnia magna*. Out of 326 total feeding strikes, 17 included *D. magna* as prey. Of these, most feeding events (11) were such that the same individual or conspecifics were also filmed feeding on mosquito larvae. In these cases, there was no evidence of an influence of prey type on kinematics. The remaining strikes (6) come from two species that were only filmed feeding on *D. magna* (*Pseudotropheus crabro* and *Neolamprologus helianthus*), but produced similar levels of kinesis to closely related taxa within the same dietary guild. While prey type often has important implications for the kinematic response of a predator (Vinyard 1980; Vinyard 1982), the inclusion of a small percentage of strikes on *D. magna* did not appear to be a source of bias in this study.

We generated a new kinematic dataset from feeding videos, choosing 10 frames from each to represent shape change during prey capture. The starting point was the first frame in which the mouth began to open for a strike. In some individuals, the mouth was held slightly open upon approach to the prey and the start frame was chosen when the feeding motion began in earnest. The final frame was the point at which the feeding apparatus reached peak expansion (i.e., full gape). Eight additional frames were

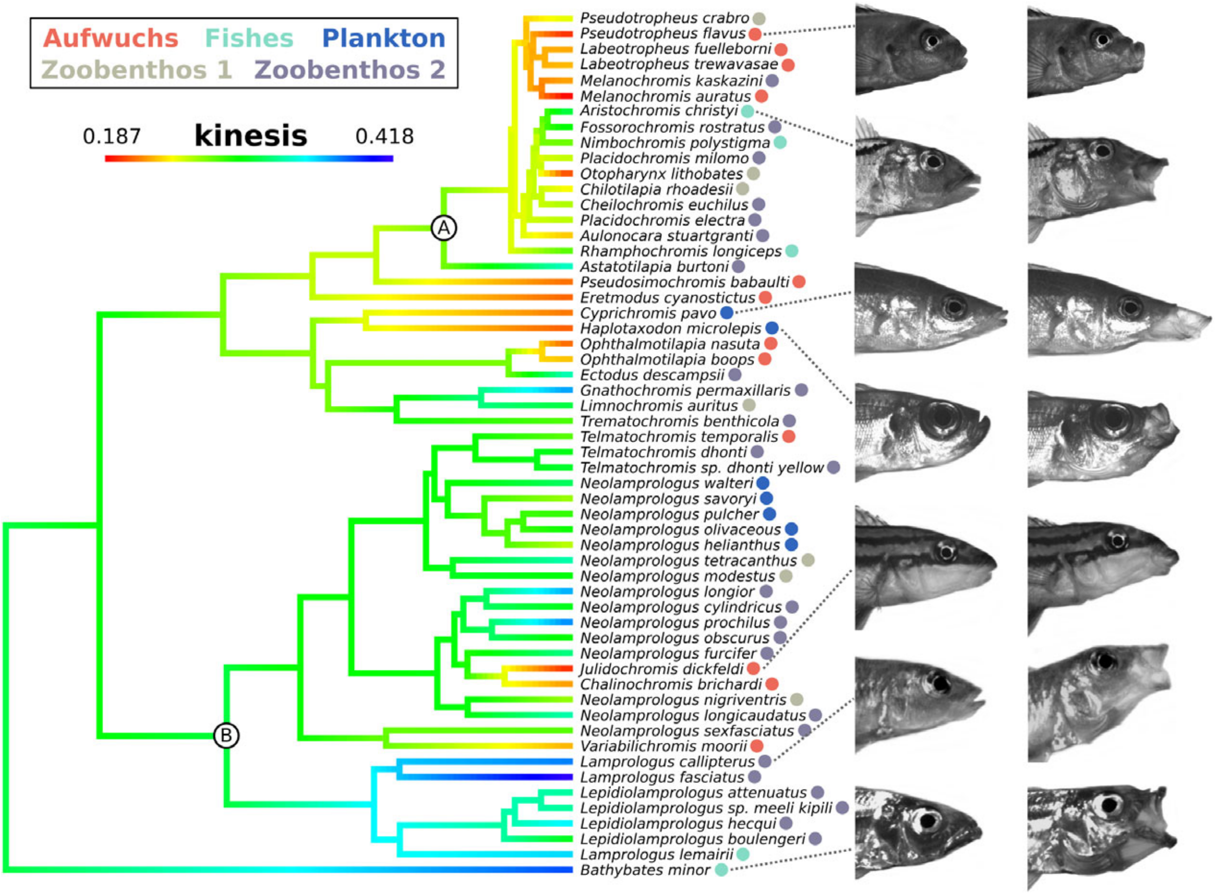

Figure 2. Maximum likelihood (ML) phylogeny of African rift lake cichlids compared in this study, with the most heavily sampled tribes labeled, "A" Haplochromini and "B" Lamprologini. The magnitude of kinesis is mapped onto branches, based on ML ancestral reconstruction and assigned feeding categories are shown as colored dots. Video frames from the start and end of strikes are shown for selected species to represent cranial morphologies associated with kinematic diversity.

chosen at approximately equivalent time steps between start and end points.

MOTION ANALYSES

We used geometric morphometrics to describe cichlid feeding motions. For each strike, selected frames were digitized in TpsDig2 (Rohlf 2015), with 10 fixed landmarks and eight sliding semilandmarks along the ventral margin of the head (Fig. 1A). Landmark selection was designed to capture several known components of teleost feeding kinesis, including maxillary rotation, premaxillary protrusion, hyoid depression, lower jaw rotation, and cranial elevation (Fig. S1). Shape alignment and scaling was achieved with generalized Procrustes analysis in the "geomorph" package (Adams and Otarola-Castillo 2013) in R (R Core Team 2015). Once aligned, successive shapes comprising a feeding event traced out a trajectory in morphospace (Fig. 1A). The diversity of motion trajectories was visualized by plotting axes generated from a principle component analysis (PCA) of aligned shape coordinates.

KINEMATIC VARIABLES

Two quantitative traits were calculated from shape trajectories, each representing a different form of variation in feeding motions. Both traits are based on Procrustes distance, a metric used to calculate distances between shapes in morphospace (Zelditch et al. 2004). First we estimated kinesis, which is the total length of a shape trajectory or the magnitude shape change that the feeding apparatus undergoes during prey capture. We calculated trajectory length as the sum of Procrustes distances between successive motion shapes (Fig. 1B). For any cranial shape along a motion trajectory, its distance (D) from the next shape is given by the following equation

$$D(x, y) = \sqrt{\sum_{i=1}^{p} \left(\left(x_{i,j} - x_{i,j+1} \right)^2 + \left(y_{i,j} - y_{i,j+1} \right)^2 \right)}, \quad (1)$$

where x and y are the coordinates for the ith landmark (of p total landmarks) on the j^{th} and $j^{\text{th}}+1$ trajectory shapes. Kinesis values were averaged by individual and then by species. To visualize the distribution of kinesis across the cichlid phylogeny, we used the contMap function in the R package, "phytools" (Revell 2012).

Second, we described the kinematic efficiency of each feeding strike as the amount of trajectory nonlinearity relative to a linear baseline between start and end motion shapes (Fig. 1C). To assess divergence from linear for all shapes along a motion trajectory, we first generated corresponding shapes along a linear trajectory. Even though the shapes from feeding motions were sampled at approximately equal time intervals, the magnitude of kinesis produced within each interval varied (sometimes considerably) such that shapes along trajectories were irregularly spaced. Therefore, shapes on linear trajectories were created in a manner that replicated the relative spacing regime of those on their corresponding motion trajectory. The spacing regime consisted of a set of cumulative path lengths, extending from the start shape to each shape *j* along the motion trajectory, with values expressed as a proportion of total trajectory length. Next, we determined the total displacement that each landmark of the feeding apparatus experienced along a linear trajectory, simply by subtracting the coordinates of the end shape from those of the start shape. Shapes along a linear trajectory were estimated by sequential multiplication of these landmark displacements by each element of the spacing regime, followed by addition of start shape coordinates. Lastly, nonlinearity was assessed in two ways. One consisted of Procrustes distances that were calculated between corresponding sets of linear and nonlinear trajectory points (Fig. 1C), creating columns of a matrix of absolute linear deviations. For the other, each deviation was standardized by the linear path distance between start and end shapes, which resulted in a new matrix of relative trajectory nonlinearity that was comparable across strikes and provided a metric for kinematic efficiency. As with kinesis, values within these matrices were averaged by individual, then by species.

RELATIONSHIPS BETWEEN MORPHOLOGY AND MOTION

All comparative analyses in this study were done in geomorph. To adjust for phylogenic nonindependence we used a previously published ultrametric tree based on maximum likelihood phylogenetic reconstruction of the study species with ultraconserved elements (McGee et al. 2016). Statistical significance for all analyses was based on permutation tests with 10,000 iterations. First, we used phylogenetic partial least-squares (PGLS) regression to test whether absolute deviations of motion trajectory paths from linear were related to the lengths of linear baselines. We also used PGLS regression to evaluate relationships between interspecific cranial morphology and kinematic traits. For cranial data, we took the first shape from motion trajectories (i.e., the shape of the feeding apparatus with the mouth closed). A separate PCA was performed to examine major axes of static head shape diversity. Kinematic traits were then regressed on principle component axes with PGLS regressions. Note that we did not use multivariate head shape coordinates as the predictor variable, as regression of a univariate response variable (kinesis) on high-dimensional shape data resulted in an inflated variance explained with a high threshold to achieve significance. We also used phylogenetic two-block partial least-squares (2B-PLS) analysis to test for covariation between the multivariate kinematic efficiency data and cranial morphology (in full-dimensional shape space). Lastly, we used PGLS regression to evaluate relationships between lower jaw length and kinesis, kinematic efficiency, and the linear distance between start and end shapes. Jaw length was calculated from aligned and scaled head shapes, as the distance between the quadrate-articular joint and the distal end of the lower jaw.

COMPONENTS OF KINESIS

To provide additional context to observed patterns of kinematic diversity, we decomposed feeding motions relative to major morphological components of the feeding apparatus (Fig. S1). We used maximum angular displacements of aligned and scaled cranial landmarks to measure maxillary rotation, lower jaw rotation, and cranial elevation. Additionally, relative changes in landmark distances were used to calculate premaxillary protrusion and hyoid depression. Each component was then averaged by species. We performed a PCA of motion components and kinesis, all scaled to unit variance. Finally, we did PGLS regressions of kinesis on each of the components to gain a relative sense of contributions to overall feeding mobility.

TROPHIC ECOLOGY

We performed three separate phylogenetic ANOVAs to test whether species' primary diet item explained variation in head shape, kinesis, and kinematic efficiency. Based on an extensive literature search, we assigned cichlid species to one of five categorical diet groups (Table S1). The first group, "aufwuchs," included species that graze on benthic algal growth or otherwise feed from the micro-invertebrate communities that live among the algae. Other benthic feeders were separated into two groups; "zoobenthos 1" included species that eat snails, bivalves, and other small, slow moving invertebrates, while "zoobenthos 2" held species with diets that consisted of more evasive, generally larger invertebrates (e.g., shrimps, insects) and occasional small fishes. The "plankton" group referred to species feeding primarily or exclusively on small organisms from the water column, mostly zooplankton. Lastly, "fishes" were predatory species that fed mostly on other fishes, and more seldom on larger invertebrates. As the definitions of these trophic categories imply, boundaries of some groups are not fully discrete and some species could arguably be placed in more than one group. However, the overall objective was to categorize species by dietary classes that

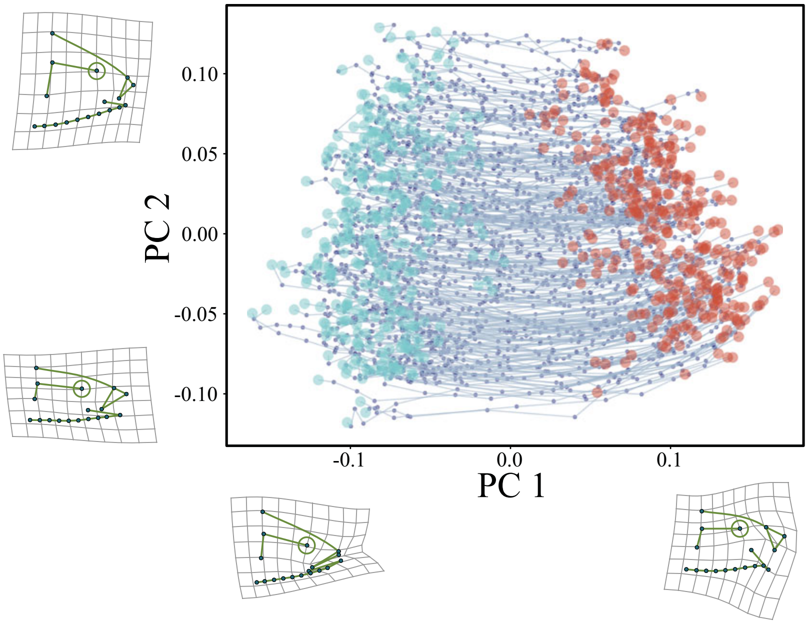

Figure 3. Principle components 1 and 2 for motion shapes from 326 suction feeding strikes in 56 species of African rift lake cichlid. PC 1 captures a common axis of kinesis, where PC 2 represents interspecific variation of cranial shape. Note that trajectory lengths generally increase with decreasing PC 2 values. Deviations of trajectories from linear may involve movements along PC axes not pictured, so this plot should not be used to infer differences in kinematic efficiency.

reflect a continuum of prey evasiveness, from immobile, like algae, to highly mobile, like fishes (see Document S1 for detailed justification).

Results

FEEDING MOTION TRAJECTORIES

After motion shapes were aligned, the resulting trajectories revealed major patterns of morphology and kinesis in rift lake cichlids (Fig. 3). The primary shape axis (PC 1) contained 36.9% of overall variation and was dominated by motion-related shape change (Fig. 3, warp grids). In contrast, the next largest shape axis (PC 2) explained 20.1% of total variance and was related to a static component of head and jaw morphology. A strongly stratified pattern of trajectories with respect to PC 2 suggests that much of this variation represents interspecific differences in head shape. This axis reflected variation from deep-headed and small-jawed species, oriented toward high PC 2 values, to shallow-headed species with elongate jaws, occupying lower values. Additionally, an overall pattern emerges in Figure 3, where trajectories generally increase in length with decreasing PC 2 scores. Several of the subsequent PC axes described different forms of interspecific cranial morphology and some additional signals of kinesis (Fig. S2).

INTERSPECIFIC CRANIAL DIVERSITY

A PCA of species' head shapes confirmed that the largest axis of static interspecific variation (PC 1; 35.5% of total shape variance) was, indeed, related to head depth and jaw length (Fig. S3). PC 2 contained 17.5% of shape variation, also displayed variation in mouth size, and was largely dominated by one morphologically extreme species, *Gnathochromis permaxillaris*, which has a particularly long maxilla that is used in distinctive ventrally directed feeding on prey suspended over sediment (Konings 2015). PC 3 represented 15.6% of shape diversity and characterized morphological variation from ventrally downturned to dorsally upturned mouths. In addition, the approximate location of the articulation between the supracleithrum and posttemporal (i.e., the cranial landmark posterior to the eye), was more anteriorly oriented at low PC 3 scores. This distinction has potential implications for kinesis, as the joint serves as the fulcrum for cranial elevation. A Phylogenetic ANOVA of head shapes grouped by diet was significant and explained 22% of overall morphological variation ($F_{4,51} = 3.69$; $P = 0.0004$).

MOBILITY OF THE FEEDING APPARATUS

The distribution of kinesis across study species was continuous, with no evidence of discrete breaks or modality. The magnitude of kinesis produced during suction feeding strikes varied by a factor of 2.24, from the species with the least dynamic strike, *Melanochromis auratus*, to the most, *Lamprologus fasciatus*. Intermediate levels of kinesis were most common and, based on ML reconstruction, constituted the ancestral condition on the tree. Several independent transitions to low and high kinesis occurred across the phylogeny, with the Tanganyikan tribe Lamprologini

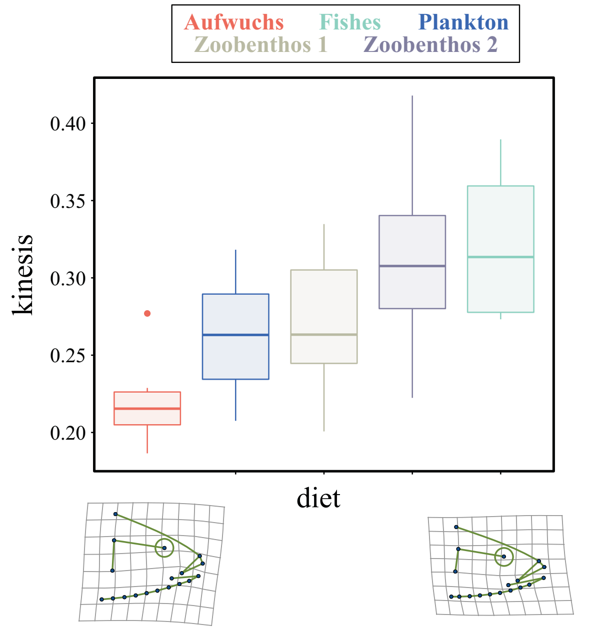

Figure 4. Boxplot of kinesis by diet category, displaying increasing levels of kinesis with more evasive prey types. Warp grids show mean shapes of cichlids that feed on aufwuchs (left) and fishes (right) relative to mean shape.

exhibiting the largest range (Fig. 2, clade "B"). Across all species, diet played an important role in these types of transitions (Fig. 4), explaining almost half of the variance in kinesis ($F_{4,51} = 11.62$; $P = 0.0002$; $R^2 = 0.48$). Here, the highest kinesis occurred in cichlids that eat large, evasive prey, and marked reductions were found in species that feed on sessile organisms and slow-moving prey.

Kinesis was significantly related to PCs 1 and 2 of interspecific cranial shape (Table S2), which were morphological axes along which variation in mouth size was a dominant feature (Fig. S3, warp grids). Phylogenetic regression of kinesis on lower jaw length confirmed that elongate jaws generated greater kinesis ($F_{1,54} = 16.15$; $P = 0.0018$; $R^2 = 0.23$). Additionally, increases in jaw size had particularly important implications for the linear distance between start and end shapes, or the minimum shape distance between closed mouth and full gape ($F_{1,54} = 56.94$; $P = 0.0001$; $R^2 = 0.51$).

TRAJECTORY NONLINEARITY AND KINEMATIC EFFICIENCY

The absolute deviations of trajectory shapes from their linear baselines were greater when the baseline itself was longer ($F_{1,54} = 9.34$; $P = 0.002$; $R^2 = 0.15$). This means that the increasing distance between start and end shapes for longer jaws, shown above, was also associated with trajectories that diverged further from linear. However, when trajectory deviations were corrected for the length of their linear baselines, providing a multivariate metric of kinematic efficiency, a different pattern emerged. Cichlids with short jaws were among the least kinematically efficient (i.e., most nonlinear), and those with longer jaws had greater efficiency ($F_{1,54} = 17.58$; $P = 0.0001$; $R^2 = 0.25$). This pattern was further supported by significant relationships between kinematic efficiency and overall cranial morphology (Fig. 5; $r_{PLS} = 0.67$; $P = 0.0001$), as well as the first three PCs of cranial shape variation (Table S3). Diet again displayed a significant relationship with feeding motions, explaining approximately one quarter of the variance in kinematic efficiency ($F_{4,51} = 3.93$; $P = 0.012$; $R^2 = 0.24$).

COMPONENTS OF KINESIS

A PCA of feeding components suggested that lower jaw rotation, hyoid depression, and cranial elevation are most closely aligned to the primary axis of kinematic diversity (PC 1) in rift lake cichlids (Fig. S4, Table S4). Relationships between kinesis and each of these three components varied in strength (R^2) from 0.47 to 0.51 (Table S5). In comparison, maxillary rotation and premaxillary protrusion were less aligned to overall kinesis (Table S4) and were largely driven by a jaw protrusion specialist, the planktivore *Cyprichromis pavo*. R^2 values of regressions of kinesis on maxillary rotation and premaxillary protrusion were 0.34 and 0.31, respectively. Moreover, there was evidence of apparent antagonistic relationships between motion components (Fig. S5). Species that had particularly large maxillary rotation had less rotation of the lower jaw and cranial elevation. In addition, cichlids with large hyoid depression tended to have minimal premaxillary protrusion.

Discussion

This study provides evidence that the evolution and diversity of suction feeding kinematics in rift lake cichlids has potentially resulted from morphological adaptations to prey evasiveness. Certainly, more detailed intraspecific work will be necessary to further support this adaptive framework, but the observed links between motion, morphology, and diet agree with our understanding of selective pressures in fish feeding systems. Strike motions varied from small amplitude cranial kinesis and kinematically inefficient suction in small-mouthed species, to highly dynamic and efficient strikes in species with larger mouths. Large mouths were indicative of species that eat large, evasive prey, allowing them to generate larger gapes that enable capture of fast-moving shrimps and fishes. Smaller mouths were found in species that feed from the aufwuchs or primarily on slow moving organisms, like snails. It is noteworthy that despite the presence of apparent trophic specialists, like algal grazers and planktivores, there were no discrete breaks in the distributions of kinematic traits. This matched the pattern observed in cranial morphology where, despite morphologically distinct species like *Gnathochromis permaxillaris*,

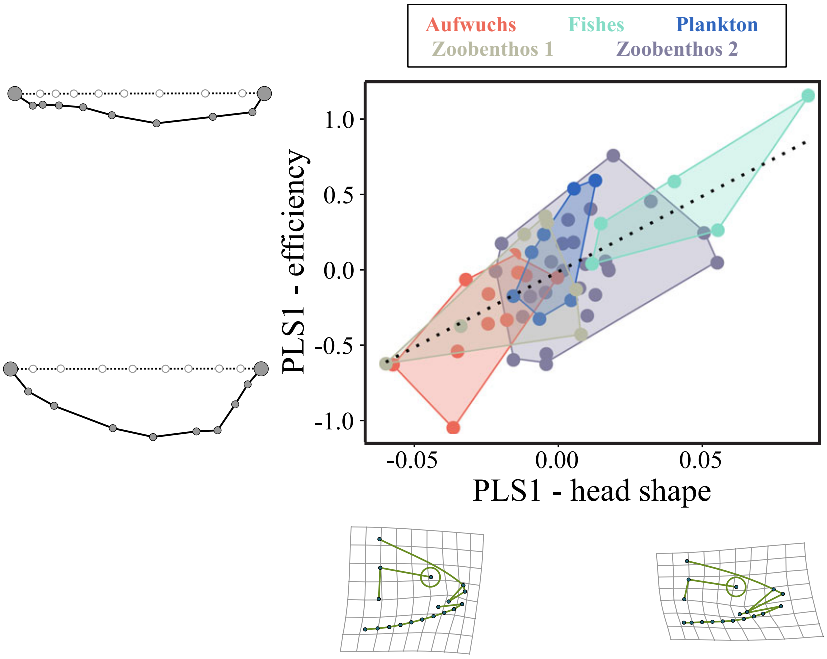

Figure 5. PLS plot of cranial shape versus kinematic efficiency, with observations colored by diet category. Axes represent maximum linear dimensions of covariation between the two multivariate datasets. Associated head morphologies and sample trajectories (scaled to the same linear baseline distance) are shown, for reference. More linear, kinematically efficient, motions are related to shallow heads, elongate jaws, and more evasive prey types.

variation was largely continuous (Fig. S3). Additionally, there appeared to be a level of integration across cranial morphology; species with long jaws tended to have more elongate and shallow heads while those with short jaws had deeper heads (Fig. 3, S3). Given this pattern, it is possible that the observed diversity of cranial morphologies represents both a constraint due to trait correlations as well as adaptations for prey evasiveness.

LINKING MORPHOLOGY, KINESIS, AND KINEMATIC EFFICIENCY

A primary challenge of our research was to interpret geometric shape trajectories as functionally relevant features of motion diversity. The link between jaw length and the magnitude of kinesis is clear; a larger mouth requires more kinesis simply to achieve full gape and is reflected by a large linear distance between start and end motion shapes. However, some components of feeding motions that are important contributors to overall kinesis, like hyoid depression and cranial elevation, are not directly related to oral jaw size. In addition to mouth size, overall kinesis is influenced by incorporation of temporal staging of feeding movements, causing nonlinearity in the motion trajectory. This is, again, related to the fact that by temporally isolating different parts of the motion, the feeding apparatus is undergoing different types of shape change and thereby changing direction in morphospace. And any morphology that can create a lot of kinesis simply by opening also has the capacity to diverge further from linear when its components are staged (see Fig. S6 for a graphical representation of relationships between jaw length, kinesis, and nonlinearity).

It is possible that kinematic efficiency serves as a compensatory adaptation that allows fishes with large mouths to maximize gape sizes for evasive prey capture, while limiting additional kinesis that comes from having a staged strike. Often, this staging was characterized by an early onset of lower jaw rotation and anterior jaw protrusion, followed by hyoid depression. Even though species with larger jaws did diverge more from linear in an absolute sense, when standardized by the linear distance between start and end shapes, their trajectories were shallower and more kinematically efficient than species with small jaws (Fig. 5). Interestingly, a major feature of suction feeding kinematics in fishes is that it occurs in a staged manner, normally described as an anterior-to-posterior wave of expansion, and it is key to their ability to synchronize peak suction flow speed with peak gape expansion during prey capture (Sanford and Wainwright 2002; Bishop et al. 2008; Wainwright et al. 2015). As such, we expected a level of nonlinearity to be characteristic of suction feeding kinematics. But why would a diet of slow or immobile prey result in less efficient suction kinesis? A likely reason is that the conservation of kinesis is less important to species that feed on less evasive prey, where an emphasis on other parts of the strike, such as mouth closing actions, may be emphasized. The evolution of algae scraping from open-water suction-feeding ancestors involves modifications that increase transmission of force through jaw levers, resulting in less transmission of motion (Wainwright et al. 2004; Westneat 2006; Cooper and Westneat 2009). Similarly, we find a trend away from efficient kinematics in species that do no specialize on large, elusive prey. Overall, we are just beginning to understand the implications of kinematic efficiency, as defined here, and more work will be necessary to gain insights on its relationship to factors like mechanical efficiency and energetics.

MODES OF FEEDING KINESIS

Our results show that a major axis of diversity in the African rift lake cichlid feeding mechanism–the degree of kinesis during prey capture–is linked to (and potentially driven by) adaptations to feeding on prey that fall along a gradient of size and elusiveness. At the upper limit, shrimps and fishes, both with quick and powerful escape responses, are among the most difficult-to-catch prey in rift lake systems (Muschick et al. 2012). Species that feed on large, mobile prey show the greatest cranial and jaw kinesis, but it is not always achieved in the same manner. Open-water, predatory species like *Bathybates minor* and *Rhamphochromis longiceps* have independently evolved a kinematic strategy with low jaw protrusion and high hyoid depression (Fig. S5). Here, minimal jaw protrusion is offset by elevated body ram that the fishes use to overcome prey.

Other predatory species, like *Lamprologus lemairii* and *Neolamprologus prochilus*, capture evasive prey by way of highly protrusible upper jaws and less hyoid depression (Fig. S5). In these and other suction-feeding species, maxillary rotation closes the lateral notch in the mouth opening, helping to form a circular and planar aperture that enhances water flow into the mouth during suction feeding (Skorczewski et al. 2010; Wainwright et al. 2015). Previous work has also shown that upper jaw protrusion is timed in such a way that the hydrodynamic forces exerted on prey by suction feeders are magnified by up to 40% (Holzman et al. 2008; Staab et al. 2012). For these reasons, high jaw protrusion and maxillary rotation in cichlids with elevated kinesis may be interpreted as adaptations that enhance suction feeding performance. Upper jaw protrusion has evolved at least five times in ray-finned fishes (Wainwright et al. 2015), but only the presence of the trait in spiny-rayed fishes (Acanthomorpha) appears to be associated with highly predatory activities that have increased through time (Bellwood et al. 2015). The strong association between diet and feeding motions that we observe in rift lake cichlids, raises the possibility that the evolution of high kinesis morphologies in spiny-rayed fishes, whether by protrusion or hyoid depression, may have been a response to the selective demands of an increasingly predatory lifestyle (Bellwood et al. 2015).

The divergent feeding strategies present in cichlids with predaceous lifestyles were not observed in species that eat nonelusive prey. Rather, there was evidence for kinematic and morphological convergence in the substrate-feeding specialists that comprise the aufwuchs diet group. These cichlids, often distantly related (Fig. 2), had deep heads and short jaws with reduced kinesis and low kinematic efficiency (Figs. 4 and 5). The same traits were also found in the molluscivore, *Chilotilapia rhoadesii*, suggesting that these species share a suite of adaptations for higher bite force, compared to the fast strikes of higher predators. These patterns are consistent with previous work on rift lake cichlids, where widespread morphological convergence has been documented (Kocher et al. 1993; Stiassny and Meyer 1999; Muschick et al. 2012). This might also suggest that the evolution of biting and scraping modes of feeding, common in reef and other benthic environments, could impose similar constraints on form and function.

RELATION TO PREVIOUS WORK

In many respects, this work demonstrates recurring themes of fish feeding mechanics that have been observed over decades of kinematic and functional research. These include, ecologically associated convergence and divergence in feeding kinematics (Norton and Brainerd 1993; Higham et al. 2017), strong links between modes of feeding kinesis and mouth size and morphology (Norton 1991), and the staging of distinct feeding movements (e.g., Lauder and Liem 1981; Cook 1996; Wilga and Motta 2000; Flammang et al. 2009). Given that the approach used in the current study integrates various morphological features that comprise the feeding apparatus, we expected (and found) important distinctions from previous research on the same system that focused on a single component, upper jaw kinesis (McGee et al. 2016). Our work showed that lower jaw rotation, cranial elevation, and hyoid depression are the largest drivers of kinematic diversity in rift lake cichlids, such that exclusion of these features does not fully capture the ecologically relevant variation in feeding motions. This distinction is important as it impacts the rank order of kinesis across species. *Cyprichromis pavo*, for example, had among the highest kinesis in McGee et al. (2016), but low kinesis in this study (Fig. 2), due to the fact that the species produces high jaw protrusion but nearly no change in other features (i.e., hyoid depression and cranial elevation). Furthermore, our treatment of feeding motions as trajectories explicitly placed the complex kinematics of cichlid prey capture in a temporal context that allowed for direct comparisons of the pattern and efficiency of kinesis that was not possible before.

CONCLUSIONS AND FUTURE APPLICATIONS

The magnitude of kinesis produced during prey acquisition and the efficiency at which it is done, define fundamental, and distinct qualities of feeding motions. The African rift lake cichlids exhibit a considerable range of trophic and feeding diversity, showcasing the capacity of geometric analyses of motion to address research questions concerning evolutionary relationships between kinematics, morphology, and ecology. Additionally, there is great potential to expand on our approach for future research on fish feeding kinematics, and also to explore motions in other systems and contexts. For example, in some fishes, body shape evolution has a significant impact on cranial morphology and jaw orientation (Martinez and Sparks 2017). Future work might seek to understand the indirect effects of body shape variation on feeding kinematics and the evolutionary trade-offs that result. Another area of interest is intraspecific variation in feeding motions, whether due to ontogeny (Cook 1996), diet (Liem 1979), or the physical environment (Rupp and Hulsey 2014). In these types of studies or others, we believe that further development of the methods used in this article may lead to breakthroughs in our understanding of evolution and diversity in functional systems.

AUTHOR CONTRIBUTIONS

CMM and PCW designed the study with input from MDM MDM and SRB took specimen videos. CMM collected data and did statistical analyses. CMM and PCW wrote the manuscript. All authors provided feedback during manuscript preparation.

ACKNOWLEDGMENTS

We thank Maxwell Rupp for help with video analysis. F. James Rohlf provided invaluable insight on morphometric motion trajectories, in addition to the initial motivation for CMM to pursue this line of research years ago in his geometric morphometrics course. We also thank James Soda for helpful conversations on analytical approaches for this work. Lastly, we thank Samantha Price, Sarah Longo, Sarah Friedman and the Wainwright lab at UC Davis for their advice and support during this study. This research was supported by a UC Davis Chancellor's Postdoctoral Fellowship to CMM and NSF grants IOS-0924489 and DEB-1061981 to PCW.

DATA ARCHIVING

The doi for our data is https://doi.org/10.5061/dryad.b8m8g7s.

LITERATURE CITED

Adams, D. C., and M. M. Cerney. 2007. Quantifying biomechanical motion using Procrsutes motion analysis. J. Biomech. 40:437–444.

Adams, D. C., and M. L. Collyer. 2009. A general framework for the analysis of phenotypic trajectories in evolutionary studies. Evolution 63:1143–1154.

Adams, D. C., and E. Otarola-Castillo. 2013. Geomorph: an R package for the collection and analysis of geometric morphometric shape data. Methods Ecol. Evol. 4:393–399.

Bellwood, D. R., C. H. R Goatley, O. Bellwood, D. J. Delbarre, and M. Friedman. 2015. The rise of jaw protrusion in spiny-rayed fishes closes the gap on elusive prey. Curr. Biol. 25:2696–2700.

Bishop, K. L., P. C. Wainwright, and R. Holzman. 2008. Anterior-to-posterior wave of buccal expansion in suction feeding fishes is critical for optimizing fluid flow velocity profile. J. R. Soc. Interface 5:1309–1316.

Brainerd, E. L., D. B. Baier, S. M. Gatesy, T. L. Hedrick, K. A. Metzger, S. L. Gilbert, and J. J. Crisco. 2010. X-ray reconstruction of moving morphology (XROMM): precision, accuracy and applications in comparative biomechanics research. J. Exp. Zool. 313A:262–279.

Burress, E. D., and M. Tan. 2017. Ecological opportunity alters the timing and shape of adaptive radiation. Evolution 71:2650–2660.

Collyer, M. L., and D. C. Adams. 2013. Phenotypic trajectory analysis: comparison of shape change patterns in evolution and ecology. Hystrix 24:75–83.

Cook, A. 1996. Ontogeny of feeding morphology and kinematics in juvenile fishes: a case study of the cottid fish *Clinocottus analis*. J. Exp. Biol. 199:1961–1971.

Cooper, W. J., and M. W. Westneat. 2009. Form and function of damselfish skulls: rapid and repeated evolution into a limited number of trophic niches. BMC Evol. Biol. 9:24.

Coughlin, D. J., and J. R. Strickler. 1990. Zooplankton capture by a coral reef fish: an adaptive response to evasive prey. Env. Biol. Fish. 29:35–42.

Drenner, R. W., G. L. Vinyard, M. Gophen, and S. R. McComas. 1982. Feeding behavior of the cichlid, *Sarotherodon galilaeum*: selective predation on Lake Kinneret zooplankton. Hydrobiologia 87:17–20.

Ferry-Graham, L. A., and G. V. Lauder. 2001. Aquatic prey capture in ray-finned fishes: a century of progress and new directions. J. Morph. 248:99–119.

Flammang, B. E., L. A. Ferry-Graham, C. Rinewalt, D. Ardizzone, C. Davis, and T. Trejo. 2009. Prey kinematics and four-bar linkages in the bay pipefish, *Syngnathus leptorhynchus*. Zoology 112:86–96.

Genner, M. J., and G. F. Turner. 2005. The mbuna cichlids of Lake Malawi: a model for rapid speciation and adaptive radiation. Fish Fish 6:1–34.

Higham, T. E., H. A. Jamniczky, K. Jagnandan, S. J. Smith, T. N. Barry, and S. M. Rogers. 2017. Comparative dynamics of suction feeding in marine and freshwater three-spined stickleback, *Gasterosteus aculeatus*: kinematics and geometric morphometrics. Biol. J. Linnean Soc. 122:400–410.

Holzman, R., S. W. Day, R. S. Mehta, and P. C. Wainwright. 2008. Jaw protrusion enhances forces exerted on prey by suction feeding fishes. J. R Soc. Interface. 5:1445–1457.

Kocher, T. D., J. A. Conroy, K. R. McKaye, and J. R. Stauffer. 1993. Similar morphologies of cichlid fish in Lakes Tanganyika and Malawi are due to convergence. Mol. Phylogenet. Evol. 2:158–165.

Konings, A. 2015. Tanganyika cichlids in their natural habitat, 3rd ed. Cichlid Press, El Paso, TX.

Lauder, G. V. 1980. The suction feeding mechanism in sunfishes (*Lepomis*): an experimental analysis. J. Exp. Biol. 88:49–72.

———. 1982. Patterns of evolution in the feeding mechanism of actinopterygian fishes. Am. Zool. 22:275–285.

Lauder, G. V., and K. F. Liem. 1981. Prey capture by *Luciocephalus pulcher*: implications for models of jaw protrusion in teleost fishes. Env. Biol. Fish. 6:257–268.

Liem, K. F. 1979. Modulatory multiplicity in the feeding mechanism in cichlid fishes, as exemplified by the invertebrate pickers of Lake Tanganyika. J. Zool. Lond. 189:93–125.

———. 1980. Adaptive significance of intra- and interspecific differences in the feeding repertoires of cichlid fishes. Am. Zool. 20:295–314.

Longo, S. J., M. D. McGee, C. E. Oufiero, T. B. Waltzek, and P. C. Wainwright. 2016. Body ram, not suction, is the primary axis of suction-feeding diversity in spiny-rayed fishes. J. Exp. Biol. 219:119–128.

Martinez, C. M., and J. S. Sparks. 2017. Malagasy cichlids differentially limit impacts of body shape evolution on oral jaw functional morphology. Evolution 71:2219–2229.

McComas, S. R., and R. W. Drenner. 1982. Species replacement in a reservoir fish community: silverside feeding mechanisms and competition. Can. J. Fish. Aquat. Sci. 39:815–821.

McGee, M. D., B. C. Faircloth, S. R. Borstein, J. Zheng, C. D. Hulsey, P. C. Wainwright, and M.E. Alfaro. 2016. Replicated divergence in cichlid radiations mirrors a major vertebrate evolution. Proc. R Soc. B. 283:20151413.

Meyer, A. 1987. First feeding success with two types of prey by the Central American cichlid fish, *Cichlasoma managuense* (Pisces, Cichlidae): morphology versus behavior. Environ. Biol. Fish. 18:127–134.

Muschick, M., A. Indermaur, and W. Salzburger. 2012. Convergent evolution within an adaptive radiation of cichlid fishes. Curr. Biol. 22:2362–2368.

Muschick, M., P. Nosil, M. Roesti, M. T. Dittmann, L. Harmon, and W. Salzburger. 2014. Testing the stages model in the adaptive radiation of cichlid fishes in East African Lake Tanganyika. Proc. R Soc. B. 281:20140605.

Nemeth, D. M. 1997 Modulation of attack behavior and its effect on feeding performance in a trophic generalist fish, *Hexagrammos decagrammus*. J. Exp. Biol. 200:2155–2164.

Norton, S. F. 1991. Capture success and diet of cottid fishes: the role of predator morphology and attack kinematics. Ecology 72:1807–1819.

Norton, S. F., and E. L. Brainerd. 1993. Convergence in the feeding mechanics of ecomorphologically similar species in the Centrarchidae and Cichlidae. J. Exp. Biol. 176:11–29.

Oufiero, C. E., R. A. Holzman, F. A. Young, and P.C. Wainwright. 2012. New insights from serranid fishes on the role of trade-offs in suction-feeding diversification. J. Exp. Biol. 215:3845–3855.

Powder, K. E., K. Milch, G. Asselin, and R. C. Albertson. 2015. Constraint and diversification of developmental trajectories in cichlid facial morphologies. Evodevo 6. https://doi.org/10.1186/s13227-015-0020-8.

R Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Revell, L. J. 2012. Phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol. Evol. 3:217–223.

Rohlf, F. J. 2015. The tps series of software. Histrix 26:9–12.

Rupp, M. F., and C. D. Hulsey. 2014. Influence of substrate orientation on feeding kinematics and performance of algae-grazing Lake Malawi cichlid fishes. J. Exp. Biol. 217:3057–3066.

Sanford, C. P. J., and P. C. Wainwright. 2002. Use of sonomicrometry demonstrates link between prey capture kinematics and suction pressure in largemouth bass. J. Exp. Biol. 205:3445–3457.

Seehausen, O. 2006. African cichlid fish: a model system in adaptive radiation research. Proc. R Soc. B 273:1987–1998.

Skorczewski, T., P. C. Wainwright, A. Cheer, and S. Cheung. 2010. Use of computational fluid dynamics to study forces exerted on prey by aquatic suction feeders. J. R Soc. Interface 7:475–484.

Staab, K. L., R. Holzman, L. P. Hernandez, and P. C. Wainwright. 2012. Independently evolved upper jaw protrusion shows convergent hydrodynamic function in teleost fishes. J. Exp. Biol. 215:1456–1463.

Stiassny, M. L. J. 1981. Phylogenetic versus convergent relationship between piscivorous cichlid fishes from Lakes Malawi and Tanganyika. Bull. Br. Mus. Nat. Hist. 40:67–101.

Stiassny, M. L. J., and A. Meyer. 1999. Cichlids of the rift lakes. Sci. Am. 280:64–69.

Vinyard, G. L. 1980. Differential prey vulnerability and predator selectivity: effects of evasive prey on bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) predation. Can. J. Fish. Aquat. Sci. 37:2294–2299.

———. 1982. Variable kinematics of Sacramento perch (*Archoplites interruptus*) capturing evasive and nonevasive prey. Can. J. Fish. Aquat. Sci. 39:208–211.

Wainwright, P. C., D. R. Bellwood, M. W. Westneat, J. R. Grubich, and A. S. Hoey. 2004. A functional morphospace for the skull of labrid fishes: patterns of diversity in a complex biomechanical system. Biol. J. Linnean Soc. 82:1–25.

Wainwright, P., A. M. Carroll, D. C. Collar, S. W. Day, T. E. Higham, and R. A. Holzman. 2007. Suction feeding mechanics, performance, and diversity of fishes. Integr. Comp. Biol. 47:96–106.

Wainwright, P. C., D. M. Kraklau, and A. F. Bennett. 1991. Kinematics of tongue projection in *Chamaeleo oustaleti*. J. Exp. Biol. 159:109–133.

Wainwright, P. C., M. D. McGee, S. J. Longo, and L. P. Hernandez. 2015. Origins, innovations, and diversification of suction feeding in vertebrates. Integr. Comp. Biol. 55:134–145.

Westneat, M. W. 2004. Evolution of levers and linkages in the feeding mechanisms of fishes. Integr. Comp. Biol. 44:378–389.

———. 2006. Skull biomechanics and suction feeding in fishes. *in* R. E. Shadwick, and G. V. Lauder, eds., Fish biomechanics: Fish physiology, vol. 23. Elsevier Academic Press, San Diego, CA.

Wilga, C. D., and P. J. Motta. 2000. Durophagy in sharks: feeding mechanics of the hammerhead *Sphyrna tiburo*. J. Exp. Biol. 203:2781–2796.

Winemiller, K. O., L. C. Kelso-Winemiller, and A. L. Brenkert. 1995. Ecomorphological diversification and convergence in fluvial cichlid fishes. Eviron. Biol. Fish. 44:235–261.

Zelditch, M. L., D. L. Swiderski, H. D. Sheets, and W. L. Fink. 2004. Geometric morphometrics for biologists: a primer. Elsevier Academic Press, San Diego, CA.

Associate Editor: S. Patek
Handling Editor: Mohamed A.F. Noor

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Major components of feeding motions captured by landmarks in this study.
Figure S2. Warp grids, displaying shape extremes on the first six PC axes for motion shapes representing all 326 cichlid feeding strikes.
Figure S3. Phylomorphospace of interspecific cranial shapes.
Figure S4. Biplot with principle components 1 and 2, from a PCA of major features of feeding motions and kinesis.
Figure S5. Biplot with principle components 2 and 3, from a PCA of major features of feeding motions and kinesis.
Figure S6. 3D plot of jaw length, kinesis, and maximum absolute trajectory deviation from linear.
Table S1. Diet, native lake, and sample sizes (in number of specimens examined and number of feeding strikes analyzed) for cichlid species in this study.
Table S2. Results of PGLS regressions of kinesis on PC axes of cranial shapes.
Table S3. Results of PGLS regressions of kinematic efficiency on PC axes of cranial shapes.
Table S4. Loadings from PCA of feeding motion components and kinesis in rift lake cichlids.
Table S5. Results of PGLS regressions of kinesis on components of feeding motions.
Document S1. Justification and citations for cichlid diet categories.