RESEARCH ARTICLE

Influence of substrate orientation on feeding kinematics and performance of algae-grazing Lake Malawi cichlid fishes

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ABSTRACT

Lake Malawi cichlids have been studied extensively in an effort to elucidate the mechanisms underlying their adaptive radiation. Both habitat partitioning and trophic specialization have been suggested to be critical ecological axes underlying the exceptional diversification of these fishes, but the mechanisms facilitating this divergence are often unclear. For instance, in the rock-dwelling mbuna of Lake Malawi, coexistence is likely tightly linked to how and where species feed on the algae coating all the surfaces of the rocky reefs they exclusively inhabit. Yet, although mbuna species often preferentially graze from particular substrate orientations, we understand very little about how substrate orientation influences feeding kinematics or feeding rates in any group of organisms. Therefore, for three species of mbuna, we quantified feeding kinematics and inferred the rates that algae could be ingested on substrates that mimicked the top, side and bottom of the algae-covered boulders these species utilize in Lake Malawi. A number of differences in feeding kinematics were found among species, and several of the kinematic variables were found to differ even within species when the fish grazed from different surface orientations. However, despite their preferences for particular microhabitats, we found no evidence for clear trade-offs in the rates that the three species were inferred to be able to obtain algae from different substrate orientations. Nevertheless, our results indicate microhabitat divergence linked to differences in feeding kinematics could have played a role in the origin and maintenance of the vast diversity of co-occurring Lake Malawi mbuna species.

KEY WORDS: Adaptive radiation, Cichlidae, Many-to-one mapping, Niche subdivision

INTRODUCTION

Adaptive radiations provide unparalleled systems for understanding the functional basis of organismal diversification. However, these radiations often challenge our ability to elucidate the mechanisms facilitating niche subdivision within communities composed of such remarkable numbers of closely related species. For instance, within Lake Malawi in Africa, up to 1000 species of cichlid fishes have diversified extensively over the past 2 million years to feed on virtually every available prey type in the lake (Fryer and Iles, 1972; Pauers, 2010). Yet, although habitat partitioning and trophic specialization have obviously played a role in this divergence (Bouton et al., 1998; Danley and Kocher, 2001), it is not clear in many instances whether the huge number of co-occurring species are ecologically distinct. For instance, in the most species-rich Malawi cichlid group, the mbuna, several hundred species all obtain their nutrition primarily from the algal mats that cover the rocky shores they exclusively inhabit. This considerable overlap in trophic habits and feeding locations among the mbuna calls into question what factors, if any, are facilitating the coexistence of so many unique species (Bouton et al., 1999; Genner et al., 1999; Genner and Turner, 2005; Siepielski and McPeek, 2010). However, microhabitat divergence, even within these algivorous mbuna species, does occur. For example, many species feed mainly from the tops of rocks while others preferentially obtain food from the side and bottom of boulders (Holzberg, 1978; Ribbink et al., 1983a; Ribbink et al., 1983b; Stauffer and Posner, 2006). This variation in the exploitation of substrate orientations could also interact with the extensive variation among mbuna trophic morphologies to facilitate previously underappreciated functional feeding specialization (Purcell and Bellwood, 1993; Collar et al., 2008; Wainwright et al., 2008; Afeworki et al., 2013). Although the mbuna might all be effectively utilizing a single ecological food type, elucidating the many ways that they have diverged within and among species to efficiently obtain this same resource could help clarify what processes facilitate their coexistence (Sanders and Gordon, 2003; Behmer and Joern, 2008).

The ability to exploit environments in multiple dimensions is likely a key component of animal diversification. For instance, divergence in locomotory and feeding performance often mediates the ability to exploit novel habitat dimensions and favors species-specific microhabitat usage (Higham, 2007). Likewise, many highly successful groups like birds and bats are able to exploit prey not only from the substrate but also from the air, a third dimension inaccessible to many other groups (Dudley and Yanoviak, 2011). Similarly, the evolution of subdigital toe pads in geckos and other lizards facilitated the ability to feed from the tops as well as the side of trees and rocky outcrops and the underside of branches and boulders that characterize their complex habitats (Irschick et al., 1996; Higham and Jayne, 2004; Foster and Higham, 2012). In terrestrial environments, the overriding influence of gravity has clearly created strong functional gradients that require specialized abilities in order to efficiently utilize different microhabitats (Duch and Pflüger, 1995; Astley and Jayne, 2009; Fujiwara et al., 2011; Schmidt and Fischer, 2011). However, because many aquatic organisms are neutrally buoyant, there might be few trade-offs associated with utilizing all three dimensions of aquatic environments. Yet, there are a number of aquatic organisms, including brine and fairy shrimps (Artemiidae), back swimmers (Corixidae), jellyfish (Cassiopeidae), the upside-down catfish (Mochokidae) and even back-stroking humans, that exhibit morphological and kinematic specializations associated with swimming in unusual orientations (Chapman et al., 1994; Blake and Chan, 2007; Hamlet et al., 2011). In Malawi cichlids, the ability, or inability, of mbuna species to efficiently feed from multiple surface orientations on rocky reefs could have a number of functional consequences.

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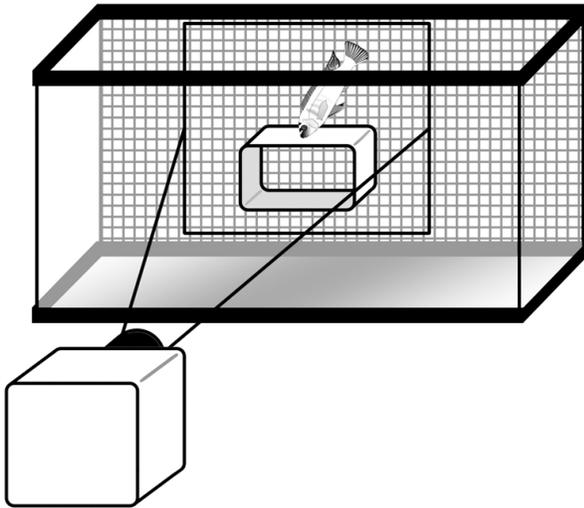*Author for correspondence (DarrinHulseyMinck1@outlook.com)

Fig. 1. Experimental filming setup. Fish were filmed scraping algae from the top, side and bottom of a PVC rectangle until five feeding events were recorded for each of the three surface orientations.

Herbivorous cichlids have the potential to consistently alter, or modulate, their oral jaw kinematics in response to the functional demands of obtaining food from different locations (Liem, 1979). This modulation could be a general mechanism promoting the ability of cichlid fishes to efficiently exploit multiple types of trophic resources (Herrel et al., 1999; Stauffer and Posner, 2006; Iriarte-Díaz et al., 2011). This modification of kinematics might be especially advantageous if feeding abilities were generally uncorrelated because a single performance trait could readily be varied without substantially influencing other kinematic events (Hulsey et al., 2006; Hulsey et al., 2007). However, the need for

individuals to modify their kinematics could also point to strong trade-offs associated with certain challenges faced during routine feeding (e.g. grazing particular substrate orientations) (Matott et al., 2005). Furthermore, if individual fish change their bites per unit time or body orientation in response to environmental challenges such as the orientation of the substrate, those abilities might result in ecological differentiation among mbuna species. For instance, these variables could be associated with documented microhabitat specialization of mbuna species like *Labeotropheus trewavasae* that preferentially feeds on the side and underside of rocks (Ribbink et al., 1983a), and the preference of many *Petrotilapia* species to feed on the tops of rocks (Stauffer and Posner, 2006). Therefore, identifying the kinematic traits that substrate orientation influences within and between mbuna species could illuminate the mechanisms responsible for the microhabitat specialization found among many herbivorous Lake Malawi cichlids.

However, whether the diverse jaw morphologies that characterize different Lake Malawi cichlids commonly result in substantial kinematic or functional diversity during feeding is also unclear. All of the algae-feeding cichlids in Lake Malawi brood their young in their mouth for several weeks following hatching and many of these cichlids lock jaws during aggressive interactions (Fryer and Iles, 1972). Therefore, variation in traits such as gape size or jaw angle could primarily reflect largely non-ecological processes such as sexual selection and these morphological differences could have little association with feeding abilities. However, mbuna species with different trophic morphologies likely vary in a number of kinematic variables that could differentiate how they procure algae from various substrate orientations (Figs 1, 2). For instance, we might expect fish to have clear associations between how frequently they bite the substrate and how frequently they beat their fins. When individuals scrape algae from a surface, the force generated during mouth closing tends to push the fish away from the substrate.
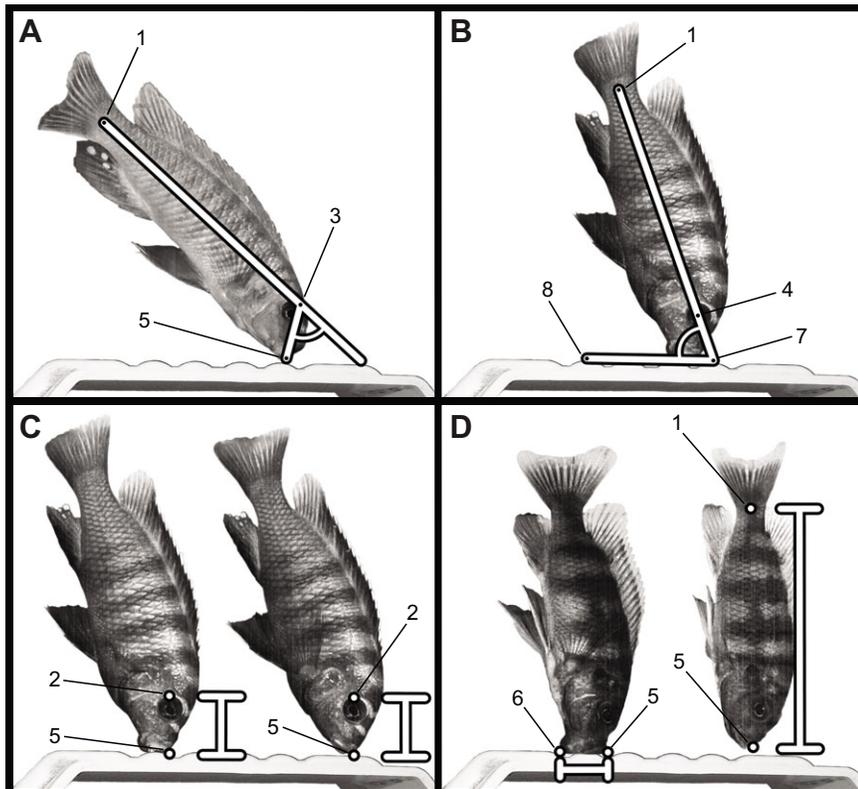

Fig. 2. Kinematic measurements. The panels demonstrate the measurement of (A) protrusion angle, (B) body angle, (C) protrusion distance and (D) gape length and standard length.
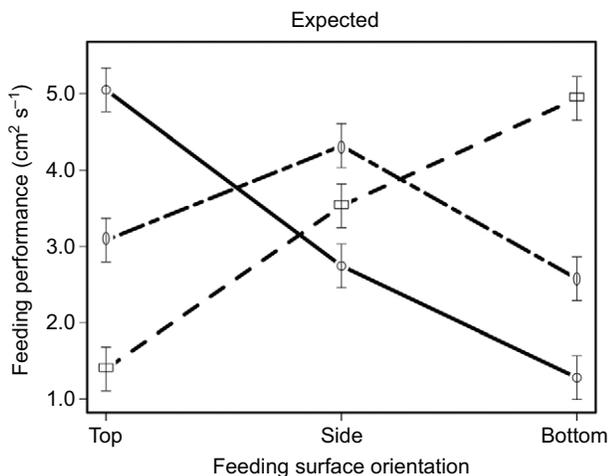
Fig. 3. Expected trade-offs in feeding performance. *Petrotilapia chitimba* (solid line with circular points) was expected to be able to graze the greatest area of algae per unit time on the top surface and graze the least amount of area on the bottom surface. *Labeotropheus trewavasae* (dashed line with rectangular points) was expected to have the exact opposite pattern of *P. chitimba* and feed at the greatest rate on the bottom surface. *Metriaclima patricki* (short–long dashed line with elliptical points) was expected to have intermediate feeding performance on the top and bottom surfaces but, of the three species, graze the greatest area of algae on the side of the feeding block.

Because grazing cichlids often use their pectoral fins to provide the necessary propulsion to bring their mouth back into contact with the feeding surface, cichlids that bite more frequently might be predicted to exhibit more rapid fin beats. The angle that mbuna protrude their jaw also clearly differs among species (Albertson et al., 2003; Stauffer and Posner, 2006) and might be related to the angle of the fish's body to the feeding surface. The orientation of both the mouth and the body of fish species might also be critical to ecological differentiation. Smaller body angles relative to the feeding surface might be essential to allowing fish to feed in more spatially constrained areas such as the underside of algae-covered rocks (Kassam et al., 2003a; Stauffer and Posner, 2006).

Herbivore communities, ranging from North American grasshoppers to the large mammalian herbivores on the plains of East Africa, could be mechanistically structured in part because of differences in feeding rates among species (Rejmanek, 1992; Chase, 1996). Likewise, the rate that mbuna are able to ingest algae could influence species coexistence, and an estimate of the amount of algae these fish are capable of ingesting could be straightforwardly inferred from knowledge of their gape size and bite frequencies. Gape size and bite frequency are commonly modulated during fish feeding (Higham, 2007; Kane and Higham, 2011; Holzman et al., 2012). Additionally, the existence of mbuna species with high bite frequencies and small gapes, as opposed to species with low bite frequencies and large gapes, could represent both alternative ways

to obtain the same amount of algae per unit time and could also generate trade-offs in the rate at which different species acquire algal resources (Kassam et al., 2003a; Kassam et al., 2003b). For example, when accounting for the influence of substrate orientation, one species with a particular mouth size and bite frequency might be functionally specialized for scraping more algae from the bottom of rocks whereas another species might alternatively be able to more rapidly procure algae from the tops of rocks. This divergence could provide a functional basis for feeding rate trade-offs among combinations of herbivorous mbuna species (Fig. 3). Therefore, if divergence in mbuna feeding kinematics resulted in trade-offs in the rate that different species graze algae from different microhabitats, the apparent narrow trophic divergence in the mbuna might not preclude trophically mediated coexistence.

To better understand the mechanistic factors underlying mbuna microhabitat partitioning and diversity in kinematics when obtaining algae, we examined the feeding kinematics of three phenotypically disparate mbuna species. By examining eight kinematic variables, we first determined whether there were intraspecific differences associated with the orientation of the feeding substrate. To establish how these traits were modulated intraspecifically, we also examined the intraspecific correlations among these variables. Finally, after controlling for the observed intraspecific differences due to substrate orientation, we determined whether the mbuna species commonly showed substantial interspecific differences in their feeding kinematics and trade-offs in their inferred grazing rates when scraping algae from different substrate orientations.

RESULTS
Intraspecific kinematic variation
The two displacement variables protrusion distance and gape length never exhibited intraspecific differences due to feeding surface orientation (Table 1). However, six of the variables showed intraspecific differences (significance threshold of $P \leq 0.05$) with respect to surface orientation within at least one species (prior to adjustment for multiple comparisons; Table 2). All three species exhibited significant orientation-induced differences in body angle, and all showed an increase in body angle with respect to the substrate as they moved from top to side to bottom feeding surfaces. *Petrotilapia chitimba* Konings 1990 exhibited a mean body angle of 78.8±2.4 deg on the top feeding surface and 85.9±1.9 deg on the bottom surface with an average difference of 7.04 deg. However, the body angle on the side (81.7±1.6 deg) was not significantly different from that of either the top or bottom surface. *Metriaclima patricki* Konings 1990 displayed a mean body angle of 67.8±4.7 deg on the top feeding surface, 75.2±2.6 deg on the side surface and 82.3±2.4 deg on the bottom surface, and significant differences were found for all pairwise comparisons of feeding surface orientation. The third species, *Labeotropheus trewavasae* Fryer 1956, showed a mean body angle of 48.9±3.4 deg on the top feeding surface, 57.6±2.1 deg on the side surface and 62.8±2.5 deg on the bottom feeding surface. All pairwise comparisons between orientation treatments were significant.

Table 1. Variables that showed no significant intraspecific differences but were significantly different among the three species

Kinematic variable	*P. chitimba*	*M. patricki*	*L. trewavasae*	*P*-value
Protrusion distance (% SL)	−0.006±0.004[a]	0.013±0.003[b]	0.017±0.002[c]	<0.001 (<0.001)*
Gape length (% SL)	0.187±0.005[d]	0.111±0.007[e]	0.052±0.003[f]	<0.001 (<0.001)*

For each species, we give the mean ± s.e.m. of the variable. Because of the number of comparisons being made, we have included the Holm's test corrected *P*-values in parentheses in addition to the raw *P*-values. Significant values are denoted with an asterisk, and superscript letters denote the results of the *post hoc* test.
SL, standard length.

Table 2. Six variables showed intraspecific kinematic differences

	Top	Side	Bottom	P-value
Body angle (deg)				
P. chitimba	78.8±2.4[a,x]	81.7±1.6[a,b,x]	85.9±1.9[b,x]	0.009 (0.146)
M. patricki	67.8±4.7[d,y]	75.2±2.6[e,y]	82.3±2.4[f,x]	0.001 (0.015)*
L. trewavasae	48.9±3.4[g,z]	57.6±2.1[h,z]	62.8±2.5[i,y]	0.001 (0.014)*
P-value	<0.001	<0.001	<0.001	
Protrusion angle (deg)				
P. chitimba	26.8±3.3[a,x]	22.3±3.2[a,x]	22.4±2.5[a,x]	0.093 (0.651)
M. patricki	40.5±3.4[d,y]	36.2±3.2[e,y]	35.2±2.1[e,y]	0.019 (0.258)
L. trewavasae	55.4±2.6[g,z]	51.6±2.5[g,z]	51.4±3.0[g,z]	0.286 (1.000)
P-value	<0.001	<0.001	<0.001	
Fin beat rate (beats s⁻¹)				
P. chitimba	3.89±0.21[a,x]	4.05±0.32[a,x]	3.89±0.25[a,x]	0.542 (1.000)
M. patricki	4.35±0.46[d,x]	4.04±0.46[d,e,x]	3.89±0.49[e,x]	0.055 (0.476)
L. trewavasae	5.86±0.35[g,y]	5.30±0.24[h,y]	5.52±0.34[h,y]	0.011 (0.167)
P-value	0.001	0.005	0.003	
Bite rate (bites s⁻¹)				
P. chitimba	2.94±0.18[a,x]	2.92±0.19[a,x]	2.67±0.17[b,x]	0.021 (0.258)
M. patricki	3.91±0.43[d,y]	3.72±0.45[d,y]	3.63±0.45[d,y]	0.161 (0.965)
L. trewavasae	5.54±0.25[g,z]	5.16±0.24[h,z]	5.34±0.24[g,h,z]	0.018 (0.258)
P-value	<0.001	<0.001	<0.001	
Bites per beat				
P. chitimba	0.76±0.05[a,x]	0.74±0.07[a,b,x]	0.69±0.05[b,x]	0.053 (0.476)
M. patricki	0.90±0.04[d,y]	0.92±0.04[d,y]	0.94±0.04[d,y]	0.257 (1.000)
L. trewavasae	0.95±0.03[g,y]	0.97±0.03[g,y]	0.98±0.04[g,y]	0.463 (1.000)
P-value	0.001	<0.001	<0.001	
Feeding performance (cm² s⁻¹)				
P. chitimba	5.0±0.8[a,x]	4.9±0.8[a,b,x]	4.3±0.8[b,x]	0.027 (0.300)
M. patricki	2.7±0.8[d,y]	2.7±0.9[d,y]	2.8±1.1[d,y]	0.574 (1.000)
L. trewavasae	1.9±0.4[g,z]	1.7±0.3[h,z]	1.8±0.2[g,h,z]	0.040 (0.400)
P-value	<0.001	<0.001	<0.001	

For each species, we give the mean ± s.e.m. of the variable. Because of the number of comparisons being made, we have included the Holm's test corrected P-values in parentheses in addition to the raw P-values. Intraspecific comparisons are shown horizontally and are marked with an asterisk if significant. Interspecific comparisons are shown vertically and all remained significant following adjustments for multiple comparisons. Superscript letters a–i denote the results of the *post hoc* test for intraspecific kinematic differences on each of the different feeding surface orientations for each individual species: *P. chitimba* (a–c), *M. patricki* (d–f) and *L. trewavasae* (g–i). Superscript letters x–z denote the interspecific results of the *post hoc* test for kinematic differences among species on each of the different feeding surface orientations.

Additionally, *M. patricki* differed in protrusion angle as a function of feeding surface orientation. On the top surface, *M. patricki* had a protrusion angle of 40.5±3.4 deg, while on the side and bottom surfaces its protrusion angle was 36.2±3.2 and 35.2±2.1 deg, respectively.

Effects of feeding surface orientation on fin beat rate were recovered for *M. patricki* and *L. trewavasae*. On the top feeding surface, *M. patricki* exhibited a fin beat rate of 4.35±0.46 beats s⁻¹, which was significantly different from that of the side (4.04±0.46 beats s⁻¹) and the bottom (3.89±0.49 beats s⁻¹). Similarly, *L. trewavasae*'s fin beat rate on the top (5.86±0.35 beats s⁻¹) was found to be higher than that on both the side (5.30±0.24 beats s⁻¹) and the bottom (5.52±0.34 beats s⁻¹) surfaces. Differences in bite rate with surface orientation were found in both *P. chitimba* and *L. trewavasae*. In *P. chitimba*, the bite rate on the top (2.94±0.18 bites s⁻¹) and the side (2.92±0.19 bites s⁻¹) differed from that on the bottom (2.67±0.17 bites s⁻¹). Lastly, *P. chitimba* showed orientation-induced differences in the number of bites per fin beat. The bite per beat ratio on the top surface (0.76±0.05 bites s⁻¹) was not different from the ratio on the side (0.74±0.07 bites s⁻¹), but was different from the bite per beat ratio on the bottom surface (0.69±0.05 bites s⁻¹).

Intraspecific kinematic correlations

To test for correlations between kinematic variables within each species, we generated a correlation matrix (Table 3). This matrix shows the correlation coefficients (*r*) for all pairs of kinematic variables. Body angle was significantly correlated with protrusion

angle for *M. patricki* and *L. trewavasae* (*r*=–0.82, *P*≤0.0001 and *r*=–0.57, *P*=0.0194, respectively). In contrast, *P. chitimba* showed little association (*r*=–0.31) between these variables (*P*=0.218). In addition, fin beat rate was correlated with bite rate for *M. patricki* and *L. trewavasae* (*r*=0.97 and *r*=0.79 respectively, *P*≤0.0001 for both species). Again, however, no correlation was found in *P. chitimba* (*r*=–0.10, *P*=0.704). Additional interactions were found between kinematic variables such as protrusion distance and gape length as well as bite rate and gape length. On the top feeding surface, *P. chitimba* exhibited the largest gape length and the slowest bite rate (*r*=0.04, *P*=0.872), *M. patricki* showed a 40% smaller gape length and a 33% faster bite rate (*r*=–0.55, *P*=0.018) than *P. chitimba*, and *L. trewavasae* had a 73% smaller gape length than *P. chitimba* but with an 89% greater bite rate (*r*=–0.43, *P*=0.114).

Interspecific kinematic variation

Significant differences among the three species were detected for all eight variables (Tables 1, 2). All means and s.e.m. given in this section are for the top feeding surface, but the comparisons made for the other two surfaces showed very similar differences among the three species. *Petrotilapia chitimba* consistently exhibited the largest body angle (78.8±2.4 deg) of the three species, followed by *M. patricki* (67.8±4.7 deg) and then *L. trewavasae* (48.9±3.4 deg). All changes in body angle were in the same direction for all three species as the orientation of the feeding surface changed. Protrusion angle differed among all three species for all feeding surface

Table 3. Pairwise correlations (*r*) between the kinematic variables

	Body angle	Protrusion angle	Protrusion distance	Gape length	Fin beat rate	Bite rate	Bites per beat
Body angle		−0.31	−0.06	−0.19	−0.45	−0.09	0.24
		−0.82	−0.02	0.68	−0.24	−0.29	−0.21
		−0.57	0.35	−0.31	0.22	0.14	−0.12
Protrusion angle	0.218		−0.11	−0.23	0.26	−0.16	−0.27
	<0.001		−0.03	−0.65	0.30	0.37	0.33
	0.019		−0.07	−0.14	0.16	0.42	0.20
Protrusion distance	0.818	0.652		−0.36	0.26	−0.17	−0.32
	0.935	0.914		0.02	0.13	0.08	−0.14
	0.162	0.701		−0.67	0.37	0.45	0.00
Gape length	0.459	0.368	0.140		0.01	0.04	0.06
	0.002	**0.003**	0.933		−0.46	−0.55	−0.51
	0.261	0.630	**0.007**		−0.40	−0.43	0.16
Fin beat rate	0.061	0.302	0.297	0.975		−0.10	−0.73
	0.336	0.222	0.602	0.058		0.97	0.11
	0.694	0.316	0.408	0.139		0.79	−0.67
Bite rate	0.720	0.536	0.495	0.872	0.704		0.74
	0.236	0.133	0.742	**0.018**	**<0.001**		0.34
	0.902	0.064	0.302	0.114	**<0.001**		−0.09
Bites per beat	0.328	0.286	0.202	0.824	**0.001**	**<0.001**	
	0.397	0.184	0.574	0.574	0.668	0.163	
	0.733	0.602	0.926	0.569	**0.004**	0.406	

All three species values are given in each cell with *P. chitimba* being the top value, *M. patricki* being the middle value and *L. trewavasae* being the bottom value. Corresponding *P*-values can be found below the diagonal and are ordered in the same format as the correlation coefficients. Significant *P*-values are in bold. Feeding performance (area s^{-1}) is not included as it is a composite variable.

orientations in a similar manner to body angle. *Petrotilapia chitimba* displayed the most terminal protrusion angle (26.8±3.3 deg), *M. patricki* exhibited a slightly more subterminal protrusion angle (40.5±3.4 deg), and *L. trewavasae* had the most subterminal protrusion angle (55.4±2.6 deg) of the three species. *Labeotropheus trewavasae* had the greatest size-standardized protrusion distance (0.017±0.002% standard length, SL). *Metriaclima patricki* showed slightly less protrusion (0.013±0.003% SL) and, interestingly, *P. chitimba* showed negative protrusion distance (−0.006±0.004% SL). This negative value of protrusion distance accurately reflects the fact that the tip of *P. chitimba*'s upper jaw actually moves slightly posteriorly at maximum gape length. Gape length differed significantly among the three species, with *P. chitimba* having the largest size-standardized gape length (0.187±0.005% SL). *Metriaclima patricki* had the next largest gape (0.111±0.007% SL) and *L. trewavasae* had the smallest gape length (0.052±0.003% SL). Fin beat rate also differed significantly among the species, with *P. chitimba* exhibiting the lowest fin beat frequency (3.89±0.21 beats s^{-1}) and *L. trewavasae* beating its fins at the highest rate (5.86±0.35 beats s^{-1}). *Metriaclima patricki* fell in between with an average fin beat rate of 4.35±0.46 beats s^{-1}. *Metriaclima patricki* and *L. trewavasae* both exhibited tightly coupled ratios of bites per fin beat (0.90±0.04 and 0.95±0.03 bites beat^{-1}, respectively), while *P. chitimba* exhibited a relatively uncoupled ratio of 0.76±0.05 bites beat^{-1}. Finally, the bite rate of *P. chitimba* (2.94±0.18 bites s^{-1}) was the lowest of the three species. *Labeotropheus trewavasae* had the highest bite rate (5.54±0.25 bites s^{-1}), and again *M. patricki* fell in between the other two species (3.91±0.43 bites s^{-1}).

Orientation-induced trade-offs in algae removal rate
Bite rate alone effectively determined our ability to make inferences of feeding performance (the area scraped per unit time) at different substrate orientations because the three mbuna species did not modulate gape size. Significant intraspecific differences in feeding performance were recovered for two of the three species, *P. chitimba*

and *L. trewavasae*. On the top surface, *P. chitimba* covered an average of 5.0±0.8 cm^2 s^{-1}, which was different from the rate on the bottom (4.3±0.8 cm^2 s^{-1}). In *L. trewavasae*, the grazing rate on the side of the feeding surface (1.7±0.3 cm^2 s^{-1}) was significantly different from that on the top feeding surface (1.9±0.4 cm^2 s^{-1}). Though the inferred area scraped differed intraspecifically for *P. chitimba* and *L. trewavasae* at different substrate orientations (*P*=0.027 and 0.040, respectively), this performance metric did not exhibit a clear interspecific trade-off among the species with respect to substrate orientation. Based on kinematics, *P. chitimba* would always have the greatest average rate of algae removal, *M. patricki* always had the second greatest rate, and *L. trewavasae* consistently exhibited the lowest rate of algae removal at all three orientations.

DISCUSSION
The Lake Malawi mbuna radiation has likely diversified substantially in how they obtain algae (Fryer and Iles, 1972; Genner et al., 1999). The interspecific and intraspecific kinematic divergence related to substrate orientation indicates there is likely variation in mbuna feeding abilities associated with particular algae-covered microhabitats. Notably, all eight variables we examined differed between at least two of the three cichlid species studied. Although most of the mbuna species exploit the same algal mats that coat the boulder fields they inhabit, they are clearly using divergent feeding kinematics and likely different abilities to obtain this food resource. This type of performance-mediated coexistence, whereby multiple species can exploit the same resource but in functionally different ways, could be characteristic of many adaptively radiating lineages (Wainwright et al., 2005; Parnell et al., 2008).

The modulation of mbuna feeding kinematics is potentially critical for the maintenance of elevated feeding efficiency in different environmental contexts. We found that several kinematic variables differed within the three species with respect to substrate orientation. Body angle changed substantially for each species among the three surface orientation treatments (Fig. 4). In general, as each species moved from the top to the bottom of the algae-
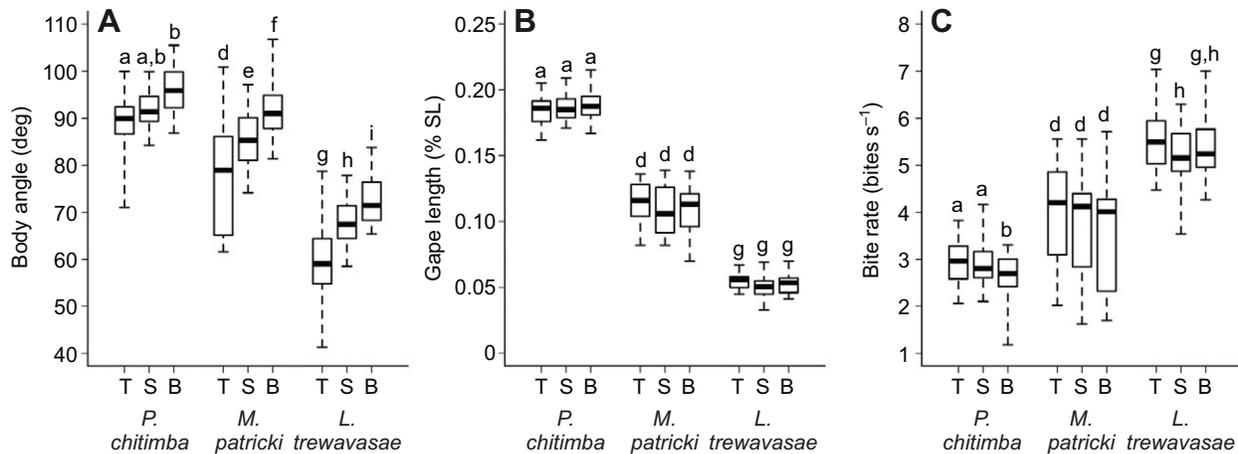
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Fig. 4. Comparison of kinematic variables. (A) Body angle, (B) gape length and (C) bite rate. The labels on the *x*-axis designate the orientation of the feeding surface (T, top, S, side; B, bottom) and the species. The *post hoc* analyses of intraspecific differences are designated with letters above the error bars.

covered surfaces, their body angle increased. In part, this was likely due to gravity acting on the fish's center of mass to influence its body orientation (Standen and Lauder, 2007). Body angles on the top surface were likely the smallest because gravity generally pulls the fish's body towards the feeding surface. However, on the bottom surface, gravity likely acts to pull the fish's body away from the feeding surface and thereby increase the observed body angle. This increased body angle could have clear performance consequences as it likely determines how spatially constrained algae-grazing species can be and remain effective in feeding on the underside of rocks. For instance, species like *L. trewavasae* that have a shallow body depth and a highly subterminal mouth are likely morphologically modified to exhibit a small body angle to the substrate and thus can efficiently feed in these spatially constrained areas. In addition to exhibiting changes in body angle, *M. patricki* and *L. trewavasae* also modulated protrusion angle in relation to the different substrate orientations. Interestingly, as body angle increased, protrusion angle tended to decrease. As observed in high-speed videos, the tips of the maxilla and dentary typically contact the feeding surface simultaneously. This means that the change in protrusion angle is not simply an effect of the jaws hitting the surface and being forced to an adjusted angle. These kinematic adjustments likely help to maintain optimal contact between the trophic apparatus and feeding surface, regardless of the substrate orientation from which the fish is feeding.

There are also clear relationships among several of the other kinematic variables when the mbuna were grazing algae. A good example is the interplay between bites per beat and gape length (Table 3). The closing of the mouth during grazing pushes the fish away from the feeding surface and, therefore, some forward force must be applied to bring the trophic apparatus back to the surface for each subsequent bite. During feeding, the mbuna routinely exhibited extensive use of the pectoral fins in producing this forward propulsion. Our original prediction was that the ratio of bites to pectoral fin beats should be very close to one-to-one as each pectoral fin beat might be predicted to produce enough force to bring the fish back to the feeding surface. For *M. patricki* and *L. trewavasae*, this does appear to be the case as both species displayed bite per beat ratios of roughly one-to-one, and the correlation between fin beat rate and bite rate was highly significant (Tables 2, 3). However, *P. chitimba* exhibited a relatively decoupled ratio of around three bites to every four fin beats. In some instances, it would take the

relatively large-mouthed *P. chitimba* individuals more than one beat of their pectoral fins to get back to the feeding surface. Individuals of *M. patricki* and *L. trewavasae* moved noticeably shorter distances from the feeding surface after each bite and this might be why they were able to maintain the one-to-one ratio of bites to fin beats. In general, the three-dimensional kinematics of fish fins and swimming are receiving increasing attention (Chapman et al., 1994; Blake and Chan, 2007) and our results indicate that understanding the dimensionality of the feeding habitat as well as the links between locomotion and feeding might both be key to elucidating the mechanistic basis of how jaws and fins have diversified in many fish groups (Collar et al., 2008).

Much of the interspecific variation in feeding kinematics we observed is likely associated with the different trophic morphologies of the three species. Gape length almost certainly plays a key role in determining bite and fin beat rates. Studies have shown that gape size influences traits such as prey capture and jaw movement speeds (Wainwright and Richard, 1995; Venesky et al., 2013), and its link to feeding kinematics is likely ubiquitous. Among the three mbuna species we examined, there is an inverse trend in gape length and bite rate. It appears that as gape length increases so does the length of the gape cycle, and this trend holds for not only the top but also the side and bottom feeding surfaces. Previous studies have also shown correlations between gape length and other kinematic variables during feeding (Wainwright et al., 2001; Higham et al., 2007). The large gape length of *P. chitimba* likely allows more algal biomass to be obtained with every bite when compared with *M. patricki* and *L. trewavasae*. Similar functional trade-offs have been proposed in the kissing gourami *Helostoma temminckii* and armored suckermouth catfishes of the family Loricariidae (Adriaens et al., 2009; Ferry et al., 2012).

However, further investigation into the amount of algae that each species was inferred to be able to remove per unit time revealed that kinematically mediated trade-offs associated with feeding surface orientation cannot alone provide a mechanistic basis for coexistence among these three mbuna. We originally thought that of the three species examined, *P. chitimba* would exhibit the highest feeding performance (algal area ingested per second) on the top feeding surface and *L. trewavasae* would, in contrast, have the highest observed feeding performance on the bottom feeding surface (Fig. 3). Additionally, we thought *M. patricki* would have been able to graze a greater amount of algae per unit time than the other two
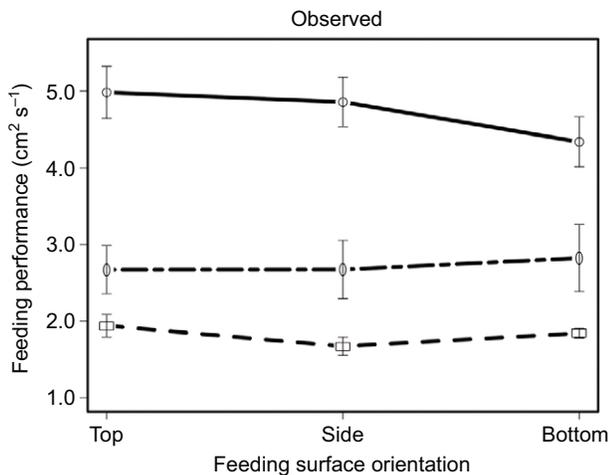
Fig. 5. Observed trade-offs in feeding performance. *Petrotilapia chitimba* (solid line with circular points) grazed a much greater area of the substrate per unit time than either of the other two species at all feeding surface orientations. *Metriaclima patricki* (short–long dashed line with elliptical points) showed no intraspecific differences in performance based on feeding surface orientation, and grazed a greater area of the substrate than *L. trewavasae* (dashed line with rectangular points) at all orientations. *Labeotropheus trewavasae* grazed significantly less of the substrate per unit time on the side as compared with the top orientation, with no difference in performance between the top and bottom or side and bottom feeding surfaces.

species on the side surface. However, we did not find support for either of these hypotheses. Instead, we found that *P. chitimba* had greater feeding performance than either *M. patricki* or *L. trewavasae* at all feeding surface orientations, and *L. trewavasae* likewise exhibited a lower feeding performance than *M. patricki* at all surface orientations (Fig. 5). Contrary to our hypothesis, the amount of algae ingested per unit time and its association with substrate orientation cannot alone explain the coexistence among these types of morphologically distinct mbuna species.

Importantly, our inferred feeding rates could provide a poor measure of the actual amount of algae that mbuna species remove from the substrate, process prior to digestion and ultimately assimilate. Tooth and jaw morphology likely both play a role in determining how mbuna feed on algal mats and how much and what kinds of algae are acquired with each bite (Bouton et al., 1998; Hulsey et al., 2007). For instance, the tricuspid teeth and short, robust jaw morphology of *L. trewavasae* could make it exceptionally efficient at scraping off greater amounts of attached algae with each bite. In contrast, *M. patricki* has a more terminal mouth and bicuspid teeth, and these traits could makes this species less efficient at scraping than *L. trewavasae* but more efficient at transporting the algae using suction (Bouton et al., 1999; Albertson et al., 2003). Additionally, the particular types of algae being grazed could result in differences across the species in the quantity of energy obtained with each bite. It has been noted that *P. chitimba* typically 'brushes' algal mats with its numerous rows of small teeth in order to gather loose fibers of filamentous algae, while *L. trewavasae* typically uses a rapid 'mowing' or 'nibbling' approach, which could be more efficient at gathering more nutritious attached algae (Ribbink et al., 1983b). Without measurements of the type and exact amount of algae removed from the feeding surface after individual bites, a completely integrative measure of feeding efficiency is extremely difficult to quantify. However, future experiments on algae feeding performance could make use of invasive stomach lavage techniques

that pump the fish's stomach and quantify the algae ingested following individual feeding bouts (Light et al., 1983).

Assimilation efficiency of algal resources that have passed through the entire digestive tract would also need to be taken into account in order to accurately measure feeding efficiency among herbivorous species. If there are differences in the amount of protein, nutritional composition and processing of the different types of algae the fish feed upon, it could have a direct impact on the amount of nutrients assimilated and also provide evidence of species-specific nutritional niches (Maldonado-García et al., 2012; Behmer and Joern, 2008). Additional morphological and physiological factors such as gut length and retention time could also affect the degree of absorption of algal nutrients (Cleveland and Montgomery, 2003), thus complicating the use of any single measure of feeding performance. Trade-offs in retention time and prey processing are also known to interact with assimilation efficiency (Clauss et al., 2009) because greater amounts of prey processing and high retention time can both result in increased assimilation of nutrients. It is feasible that while *L. trewavasae* obtains algae from a smaller surface area per unit time, this species could have greater assimilation efficiency than the other two species examined.

Another factor affecting feeding performance that is difficult to quantify in the laboratory is the ecological interaction among algae-grazing species (Ribbink et al., 1983b; Purcell and Bellwood, 1993). Our measure of area grazed per unit time assumes that feeding occurs as an uninterrupted process that takes place under isolated conditions. However, in the wild, factors such as predation and competition certainly have an effect on the ability of mbuna species to feed. For example, there are a large number of predatory species in Lake Malawi including piscivores, fin-nippers and scale-eaters that all cause disruptions in the feeding rhythms of the mbuna as they graze (Fryer and Iles, 1972). These disruptions generally lower trophic performance as mbuna would need to spend time avoiding these other species in lieu of feeding. Territorial aggression among heterospecifics is also common among the mbuna (Danley and Kocher, 2001). While there is some overlap in feeding space, most species have relatively defined feeding territories (Maruyama et al., 2010). Interspecific territoriality among mbuna might be especially relevant to the continued existence of species such as *L. trewavasae* that specialize in feeding on the bottom and side of rocks where there is far less competition. Feeding on the underside of rocks likely also limits encounters with predators. The ability of *L. trewavasae* to navigate and feed in such spatially constrained areas therefore likely allows it to graze relatively unhindered by pressures that other mbuna species more commonly face in Lake Malawi's species-rich communities and complex feeding microhabitats.

The ability to exploit multiple habitat dimensions is vital to the success of many groups (Irschick and Losos, 1999; Higham et al., 2001). Innovations such as wings and the toe pads of geckos have likely allowed the groups possessing these functional abilities to diversify in three dimensions rather than the two dimensions previously available to their ancestors. Similarly, the ability of the mbuna to exploit algae growing on the top, side and bottom of rocks has likely been one factor that has facilitated their exceptional diversification (Stauffer and Posner, 2006). For instance, variation in body angle and pectoral fin locomotion could be associated with *L. trewavasae*'s documented specialization of feeding on the side and underside of rocks (Ribbink et al., 1983a), and *P. chitimba*'s preference for feeding on the top and side of rocks (Stauffer and Posner, 2006). The substantial variation in how organisms utilize particular substrate orientations (Cartmill, 1985; Higham and Jayne, 2004; Foster and Higham, 2012) is clearly not constrained to

terrestrial systems as substrate orientation does influence how the mbuna feed. Nevertheless, all three mbuna species were capable of grazing algae from all three orientations and not all of their kinematics appeared to be extensively modulated with respect to surface orientation. It seems likely that there are fewer trade-offs associated with feeding from multiple substrate orientations in aquatic systems as compared with gravity-dominated terrestrial systems. Additional understanding of whether substrate orientation greatly impacts resource acquisition in aquatic environments could provide substantial insight into both major differences and similarities in the factors structuring ecological divergence within aquatic and terrestrial systems. The varying functional demands of different feeding surface orientations should be investigated further as this could be one of the driving forces behind the origin and persistence of the incredible diversity of algae grazing Lake Malawi cichlids.

MATERIALS AND METHODS

Fish species

We examined kinematics in three Malawi species: *P. chitimba*, *M. patricki* and *L. trewavasae*. These species were chosen because they likely represent some of the most morphologically disparate lineages of the algae-grazing mbuna clade in Lake Malawi. For instance, *P. chitimba* has a circular terminal mouth, *M. patricki* has an elliptically shaped and slightly subterminal mouth, and *L. trewavasae* has a very subterminal mouth and is one of the few fish species to have a rectangular mouth. If any kinematic differences in feeding and pectoral fin use exist among the many species of mbuna, we would expect them to be readily detectable among these three species. All fish were of adult size and obtained commercially with SLs ranging from 6.8 to 10.1 cm. *Petrotilapia chitimba* had a mean SL of 7.8 cm, *M. patricki* had a mean SL of 7.8 cm and *L. trewavasae* had a mean SL of 8.8 cm. Six individuals of each cichlid species were filmed in aquaria maintained at a temperature of 28±2°C. Prior to filming, fish were fed tropical fish flake food *ad libitum*. All experimental procedures followed protocols approved through the University of Tennessee's Institutional Animal Care and Use Committee (IACUC).

Experimental setup

To measure algae-grazing kinematics, we obtained high-speed video (500 frames s^{-1}) with a Phototron Fastcam 1024PCI video camera using Phototron Fastcam Viewer version 3171. For filming, each fish was isolated to the front 10 cm of a 20 gallon tank using an opaque Plexiglas barrier marked with 1 cm grid lines. The barrier was inserted into the tank orthogonal to the camera and used to calibrate all kinematic measurements (Fig. 1). Individuals were filmed while feeding on algae-covered rectangular PVC blocks. The PVC blocks had a depth of 6.4 cm and were 10.3 cm on the top and bottom, and 6.6 cm on each side. The feeding blocks were fixed to the center of the front surface of the tank using suction cups, and were carefully placed to ensure that the surface of the water and the other walls of the tank did not spatially constrain the ability of the fish to feed. To quantify kinematic variables, we filmed seven feeding events for each individual on each of the three surface orientations (top, side and bottom). For the purposes of our analyses, we only recorded feeding bouts that consisted of at least five uninterrupted bites from the substrate. Additionally, to limit any potential confounding effects of motivation and feeding fatigue, video recordings of individual cichlids were limited to five feeding bouts per day for a given individual. To increase the accuracy of our kinematic measurements, we also restricted our analyses to videos in which the fish was lateral and the feeding bout was confined to a single side of the rectangular feeding block.

Kinematic measurements

All videos were saved as a stack of TIFF images that were used to digitize kinematic landmarks using ImageJ (Schneider et al., 2012). Maxillary protrusion angle (Fig. 2A) was measured from the center of the caudal fin origin to the top edge of the eye, then from the top of the eye to the tip of

the fully protruded maxilla (line 1–3–5). Body angle (Fig. 2B) was quantified as the angle between the feeding surface and the line running through the horizontal axis of the fish's body. This horizontal axis was measured from the center of the caudal fin origin through the center of the eye and to the substrate (line 1–4–7–8), and was only quantified after the third bite from the substrate to ensure the fish had settled into a 'natural' feeding posture. Maxillary protrusion distance (Fig. 2C) was determined as the difference in distance from the back of the eye to the tip of the upper jaw when the jaw was fully protruded minus the distance from the back of the eye to the tip of the upper jaw when it was fully retracted (line 2–5). Gape length (Fig. 2D) was measured as the distance between the tip of the upper jaw and the tip of the lower jaw when the jaw was fully protruded (line 5–6). Standard length (Fig. 2D) was measured as the length (cm) of the body from the center of the caudal fin origin to the tip of the upper jaw while the jaw was fully closed (line 1–5). For analyses, protrusion distance and gape length were standardized as a proportion of SL to remove the effects of body size.

Three timing variables were also measured from each video. The fin beat rate was calculated as the number of fin beats during a feeding bout divided by the time (in seconds) from when the upper jaw first made contact with the feeding surface (time 0) until the jaw came into contact with the feeding surface for the last time. A fin beat was defined as a change from an abducting motion of the fin to an adducting motion. The fin beat rate was calculated using the total number of video frames from the jaw's first contact to its last contact with the feeding surface. The bite rate was similarly calculated as beginning when the upper jaw of the fish first made contact with the feeding surface to the moment of the jaw's final contact with the surface during the feeding bout.

We inferred feeding rates using a combination of bites per unit time and gape area. To determine gape areas of the three species, we modeled their gapes as three different geometric shapes. Using gape lengths (L) as the diameter of each fish's mouth taken from video sequences, we assumed *P. chitimba*'s mouth was symmetrical and modeled it as a circle: $\pi \times (L/2)^2$. The gape length of both *M. patricki* and *L. trewavasae* was taken from video. However, because the mouths of these species are asymmetrical, we estimated gape width (W) using dial callipers for a number of *M. patricki* (N=17) and *L. trewavasae* (N=10) that spanned the range of individuals examined with high-speed video. The standard length-adjusted estimate of gape width for the videoed fish was then determined. Then, we used the video-obtained gape length and inferred gape width for each fish to model the mouth of *M. patricki* as an ellipse: $\pi \times (L/2) \times (W/2)$, and the mouth of *L. trewavasae* as a rectangle: ($L \times W$). Gape areas were then multiplied by the bite rate during each individual feeding bout at each feeding surface orientation in order to estimate the area of algae each species could theoretically scrape per unit time (cm^2 s^{-1}). There were minimal differences in mean SL between *P. chitimba* and *M. patricki* (7.8 cm), but *L. trewavasae* had a slightly larger mean SL (8.8 cm; see above). To account for this, all gape areas for *L. trewavasae* were isometrically adjusted to an average SL of 7.8 cm.

Statistical analyses

Statistical analyses were performed using R v2.14.2 (R Development Core Team, 2011). To test for associations in pectoral fin and jaw kinematics, the data were partitioned in three different ways: (1) intraspecific by surface orientation, (2) species and (3) orientation and species. First, we checked for within-species variation in feeding kinematic variables grouped by the different orientations of the feeding surface (top, side and bottom). To account for repeated measurements within individuals, we implemented a linear mixed-effects (LME) model, which considers the correlation between the non-independent measurements within and among individuals. Additionally, LME models describe the variation of individuals with respect to the population mean (Pinheiro and Bates, 2000). Maximum likelihood models were constructed using the lme function in the nlme library (Pinheiro et al., 2013), and for each model, the slope and intercept were allowed to vary for all individuals. To test for significant differences between the three pairwise comparisons of surface orientation, we used the glht function in the multcomp library (Hothorn et al., 2008), specifying 'Tukey' as the method for linear *post hoc* hypothesis testing.

Correlations among the eight kinematic variables were calculated using the cor function in R. *P*-values were obtained for their corresponding correlation coefficients using the cor.test function. Marginal means for each kinematic variable were found for each individual at the three different feeding surface orientations. The marginal means were then used to determine the correlations among the different variables. Means within each species were not partitioned by feeding surface when calculating correlation coefficients.

If there were no detectable intraspecific kinematic differences among the orientation treatments for the species, all feeding events within each individual were combined and statistically compared among the three species using an LME model that did not nest feeding surface within individual. If there were intraspecific kinematic differences among the orientation treatments for any of the species, only the feeding events for that kinematic variable at a particular orientation were statistically compared among the three species. All *P*-values were subsequently adjusted for multiple comparisons by applying a Holm's correction to estimates of significance. This was performed in R using the function 'p.adjust'.

Author contributions
M.F.R. and C.D.H. both contributed to the conception, design, interpretation of the findings being published, drafting, as well as the revising the article. M.F.R. executed the experiments detailed in this article.

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