High niche overlap between two successfully coexisting pairs of Lake Malawi cichlid fishes

Christopher H. Martin and Martin J. Genner

Abstract: Many species in high-diversity assemblages appear to coexist in similar ecological niches. It has been proposed that interspecific resource partitioning in these assemblages may only occur during periods of resource scarcity. We tested this hypothesis by measuring resource abundance, dietary overlap, foraging rate, and territoriality in a Lake Malawi rock cichlid assemblage over a period of 1 year. Our study examined two pairs of morphologically similar species, with each pair comprising one native species and one invader species that has successfully established after being translocated from another region of the lake. All four species changed their diet and foraging rate in response to seasonal variation in resource abundance. However, dietary overlap within both species pairs remained high in all seasons and was not influenced by resource availability. Similarly, territoriality did not decline during periods of low resource availability, suggesting no decrease in the strength of interspecific competition. These data suggest that these species pairs are successfully coexisting despite substantial niche overlap during resource scarcity. Thus, the coexistence of species within this radiation may not depend on the evolution of divergent resource use patterns.

Résumé : Plusieurs espèces appartenant à des peuplements de forte diversité semblent coexister dans des niches écologiques semblables. On a émis l'hypothèse selon laquelle la partition interspécifique des ressources dans ces peuplements ne se produit que dans les périodes de pénurie de ressources. Nous avons testé cette proposition en mesurant l'abondance des ressources, le chevauchement alimentaire, le taux de recherche de nourriture et la territorialité dans un peuplement de cichlidés des habitats rocheux du lac Malawi durant une période d'un an. Notre étude examine deux paires d'espèces à morphologie similaire, chaque paire comprenant une espèce indigène et une espèce envahissante qui s'est établie avec succès après avoir été déplacée d'une autre région du lac. Les quatre espèces changent toutes leur régime alimentaire et leur taux de recherche de nourriture en réaction à la variation saisonnière de l'abondance des ressources. Cependant, le chevauchement alimentaire dans chacune des deux paires d'espèces demeure élevé à toute saison et n'est pas influencé par la disponibilité des ressources. De même, la territorialité ne diminue pas durant les périodes de faible disponibilité des ressources, ce qui laisse croire qu'il n'y a pas de réduction de la force de la compétition interspécifique. Ces données indiquent que ces paires d'espèces coexistent avec succès malgré leur important chevauchement de niche durant la pénurie de ressources. Ainsi, la coexistence des espèces au sein de cette radiation peut ne pas dépendre de l'évolution de patrons divergents d'utilisation des ressources.

[Traduit par la Rédaction]

Introduction

There are many examples of closely related species that differ in secondary sexual traits but appear essentially indistinguishable in morphology and ecology (McPeek and Brown 2000; Mendelson and Shaw 2005; Ritchie 2007). These examples provide indirect support for the hypothesis that speciation can occur through sexual selection without the evolution of ecological divergence (Panhuis et al. 2001). Such species appear to violate classical coexistence theories suggesting that if resources are limited and species are ecologically indistinguishable, then one population should stochastically drift to extinction (Hubbell 2001) or suffer competitive exclusion if one species evolves dominance in the shared niche (Hardin 1960). This issue of long-term species coexistence is a major hurdle for models of sympatric speciation driven exclusively by disruptive sexual selection (Coyne and Orr 2004) and is also relevant to models of allopatric speciation driven primarily by divergent sexual selection and reinforcement on secondary contact.

Relatively recent translocations of Lake Malawi cichlids provide a "natural experiment" in which to examine mechanisms of species coexistence after secondary contact between ecologically equivalent species. The rocky-habitat cichlid fish assemblage of Lake Malawi is characterized by many species complexes with morphologically similar component species that are widely believed to have diversified via sexual selection (Allender et al. 2003; Streelman and Danley 2003; Genner and Turner 2005). Alpha diversity within these habitats is typically very high, often 40 or more species are present. In the 1960s, at least 13 species were translocated to Thumbi West Island from other regions

Received 10 July 2008. Accepted 10 February 2009. Published on the NRC Research Press Web site at cjfas.nrc.ca on 8 April 2009. J20664

C.H. Martin.[1] Section of Evolution and Ecology, University of California, One Shields Avenue, Davis, CA 95616, USA.
M.J. Genner. School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG, UK; Marine Biological Association, The Laboratory, Citadel Hill, Plymouth, PL1 2PB, UK.

[1] Corresponding author (e-mail: chmartin@ucdavis.edu).

Can. J. Fish. Aquat. Sci. 66: 579–588 (2009) doi:10.1139/F09-023 Published by NRC Research Press

of the lake, resulting in contact between many closely related and ecologically similar species that differ primarily in breeding colors (Ribbink et al. 1983; Genner and Turner 2005).

In contrast to expectations that species would be locally extirpated by random drift or competitive exclusion, repeated surveys have shown that the abundance of translocated species has gradually increased around the site of introduction, with no apparent influence on either the diversity or abundance of the indigenous species (Genner et al. 2006). This suggests an absence of prevalent interspecific competition for limited ecological resources in Malawi rock cichlids and supports observations on closely related species of rock cichlid species assemblages in general. For example, substantial overlap in diet and space use has been recorded between both coexisting morphologically similar species (Reinthal 1990; Genner et al. 1999*a*, 1999*b*) and those with greatly contrasting morphological features (Liem 1980; McKaye and Marsh 1983; Genner et al. 1999*b*).

One mechanism for the coexistence of resource competitors, in part motivated by observations of Malawi rock cichlid assemblages, is that resource partitioning may occur only during periods of resource scarcity (McKaye and Marsh 1983; Robinson and Wilson 1998). Niche differences between closely related translocated and native species might become apparent only during resource bottlenecks. Such a pattern would occur if both species share a preference for a seasonally abundant resource (e.g., zooplankton) but specialize on different resources when the preferred resource becomes scarce or unavailable, thus minimizing competition during lean times (Robinson and Wilson 1998). This pattern is well documented between several morphologically distinct species (Boag and Grant 1981; Luiselli 2006; Marshall and Wrangham 2007), including fishes (Ross 1986; Malmquist et al. 1992). However, temporal patterns of ecological overlap among high-diversity species assemblages rarely have been studied. To date, there have been no tests for the presence of ecological bottlenecks in African lake cichlids or their effect on trophic partitioning among species. No published diet studies have made temporal comparisons of trophic partitioning and resource abundance (e.g., Reinthal 1990; Bouton et al. 1997; Genner et al. 1999*b*). Stable isotope studies average diet across a time scale of many months, but they cannot uncover fine-scale temporal resource partitioning (Genner et al. 1999*a*). If niche differences do not exist, an alternative explanation for the successful coexistence of translocated and native species is that ecological selection on resource use patterns is weak. Such conditions may allow short-term coexistence of morphologically indistinguishable species.

Here we investigated diet and resource shifts between two closely related native and invader species pairs in a Lake Malawi rock cichlid assemblage over 1 year. We measured changes in plankton and epilithic algae abundance within a rocky littoral habitat and concurrently sampled diet, stomach content mass, foraging rate, territoriality, and microhabitat use. Our goals were (*i*) to measure seasonal variation in resource abundance, diet, stomach content mass, foraging rate, and territoriality, (*ii*) to quantify diet and microhabitat niche overlap, and (*iii*) to test for temporal fluctuations in diet overlap during periods of resource scarcity. We hypothesized that if resource partitioning during resource bottlenecks is contributing to the successful coexistence of these species pairs, then trophic overlap should decrease and species should specialize on different resources as preferred resources become less abundant. We also hypothesized that if species pairs are segregating resources during periods of scarcity, then aggression toward closely related species should decline.

Materials and methods

Study site and species

Mitande Rocks at Thumbi West Island, Lake Malawi (14°01′27″S, 34°49′24″E), contains a diverse rock cichlid mbuna assemblage of at least 44 species (Ribbink et al. 1983). We studied four sympatric species in two genera: *Metriaclima zebra*, *Metriaclima callainos*, *Tropheops* sp. “red cheek”, and *Tropheops tropheops* (following Konings (2007), previously *Tropheops* sp. “orange chest”). *Metriaclima* have wide terminal mouths with loosely packed bicuspid teeth and forage on either epilithic algae with scraping bites or plankton using suction feeding (McKaye and Marsh 1983). *Tropheops* have narrower subterminal mouths with dense rows of bicuspid teeth and forage mainly on epilithic algae with individual bites and rarely feed in the water column (C.H. Martin, personal observation). Year-round, males from all species defend breeding territories in which they feed. Although there is never substantial conspecific territorial overlap, there is often considerable overlap of heterospecific territories, resulting in a complex territorial mosaic. Females visit male territories to breed and then leave to brood fertilized eggs in their buccal cavity for approximately 1 month before releasing free-swimming fry (Genner and Turner 2005). We studied both native (*M. zebra* and *T. tropheops*) and translocated (*M. callainos* and *Tropheops* sp. “red cheek”) species that have coexisted at the site for over 40 years. Strong habitat philopatry of rock cichlid populations (Genner and Turner 2005) implies that our study populations are not maintained by migration from other habitats.

Sampling regime

All samples were collected within a 30 m × 30 m grid over homogeneous rocky habitat ranging from depths of 3 to 8 m. The four study species were among the top seven most abundant species in the study area (Supplemental Fig. S1).[2] Six sampling periods were spaced approximately 2 months apart over a full year (Supplemental Table S1)[2]. Underwater collections and observations were made using SCUBA and were conducted by a single observer (C.H.M.).

Plankton and algal sampling

A minimum of seven plankton trawls was taken during each sampling period, between 1200 and 1400 hours. For

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[2] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 3907. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/cms/unpub_e.html.

each trawl, a 53 μm mesh plankton net (7 cm diameter, 130 cm length) was towed horizontally for 100 m within the study site, starting at the surface and continuing to a depth of 8 m suspended approximately 1 m above the bottom. Samples were diluted in 100 mL of 15% formalin for storage. To quantify plankton diversity and abundance, the number and volume (1 volume unit = 1 *Anabaena* cell, the smallest plankton unit) of all components in eight equal subsamples were estimated using a haemocytometer under 400× light magnification. The volume of each plankton component in a sample was calculated from the mean of the eight subsamples. Phytoplankton were identified to genus using Bold and Wynne (1985). Zooplankton were identified to class.

The standing crop of epilithic algae was sampled from surfaces of horizontal, smooth, unshaded rocks at depths between 3 and 7 m. For each sample, a 25 cm^2 area was scraped clean and preserved in 15% formalin. Algal diversity (Supplemental Table S2)[2] was quantified as described below for dietary analyses. Total algal abundance was estimated by allowing samples to drain overnight and then measuring sample wet weight. Algae weight was positively associated with collection depth ($r^2 = 0.167$, $n = 113$, $P < 0.001$), and residual weights were used in analyses.

Diet sampling

During each sampling period, at least 10 male and 10 apparent female adult fishes from each study species were collected using a monofilament hand net. All fish were collected at depths ranging from 4 to 6 m at between 1000 and 1400 hours. On the surface, fishes were immediately euthanized by an overdose of MS-222 (tricaine methanesulfonate; Argent Laboratories, Redmond, Washington). Digestion was halted with an intraperitoneal injection of 15% formalin, and the fishes were preserved whole in 15% formalin. In the laboratory, fishes were sexed anatomically and standard length (SL) was measured with calipers (mean ± standard deviation (SD) cm: *M. zebra*, 8.43 ± 0.60; *M. callainos*, 8.62 ± 0.80; *T. tropheops*, 9.31 ± 0.69; *Tropheops* sp. "red cheek", 8.38 ± 0.55). There were no significant differences in SL across sampling periods in any species (one-way analysis of variance (ANOVA): *M. callainos*, $F_{[5,119]} = 0.844$, $P = 0.521$; *M. zebra*, $F_{[5,117]} = 0.299$, $P = 0.912$; *T. tropheops*, $F_{[5,115]} = 0.895$, $P = 0.487$; *Tropheops* sp. "red cheek", $F_{[5,115]} = 2.245$, $P = 0.055$). *Tropheops* stomach content wet weights were positively associated with SL (*T. tropheops*, $r^2 = 0.268$, $P < 0.001$; *Tropheops* sp. "red cheek", $r^2 = 0.278$, $P < 0.001$), and residual stomach content mass for each species was used in analyses. Individuals with empty stomachs ($n = 69$) were included and set equal to the lowest residual value for each species. *Metriaclima* stomachs were smaller and frequently empty, so only the proportion of empty stomachs was recorded. Stomachs undetectable during dissections were presumed empty and upper intestinal contents were sampled.

Dietary components of eight subsamples from each individual were quantified under 400× light magnification using a modified version of the points method (Hynes 1950), described in Genner et al. (1999*b*). Briefly, dietary components were assigned a predetermined number of points based on their relative volume within each subsample. The proportion of each dietary component was calculated from its point value relative to the total number of points assigned to the subsample. Proportions of dietary components in all eight subsamples were then averaged for each individual (Supplemental Table S2)[2].

Pairwise dietary differences among all individuals were calculated using the Bray–Curtis similarity index. We compared dietary overlap across sampling periods with analysis of similarities (ANOSIM) and multidimensional scaling plots using PRIMER-6 (PRIMER-E Ltd., Plymouth, UK). Inter- and intra-specific dietary overlap was computed using Schoener's index of resource use overlap (Schoener 1968). There were no dietary differences between the sexes in any species, so the sexes were pooled for dietary analyses.

Foraging rate

Foraging rate was estimated from the mean biting frequency of each study species during 2-min observation periods. In each sampling period, at least 10 males and 10 apparent females per species were observed. For each fish, the number of bites on benthic substrate and the number of bites in the water column were recorded.

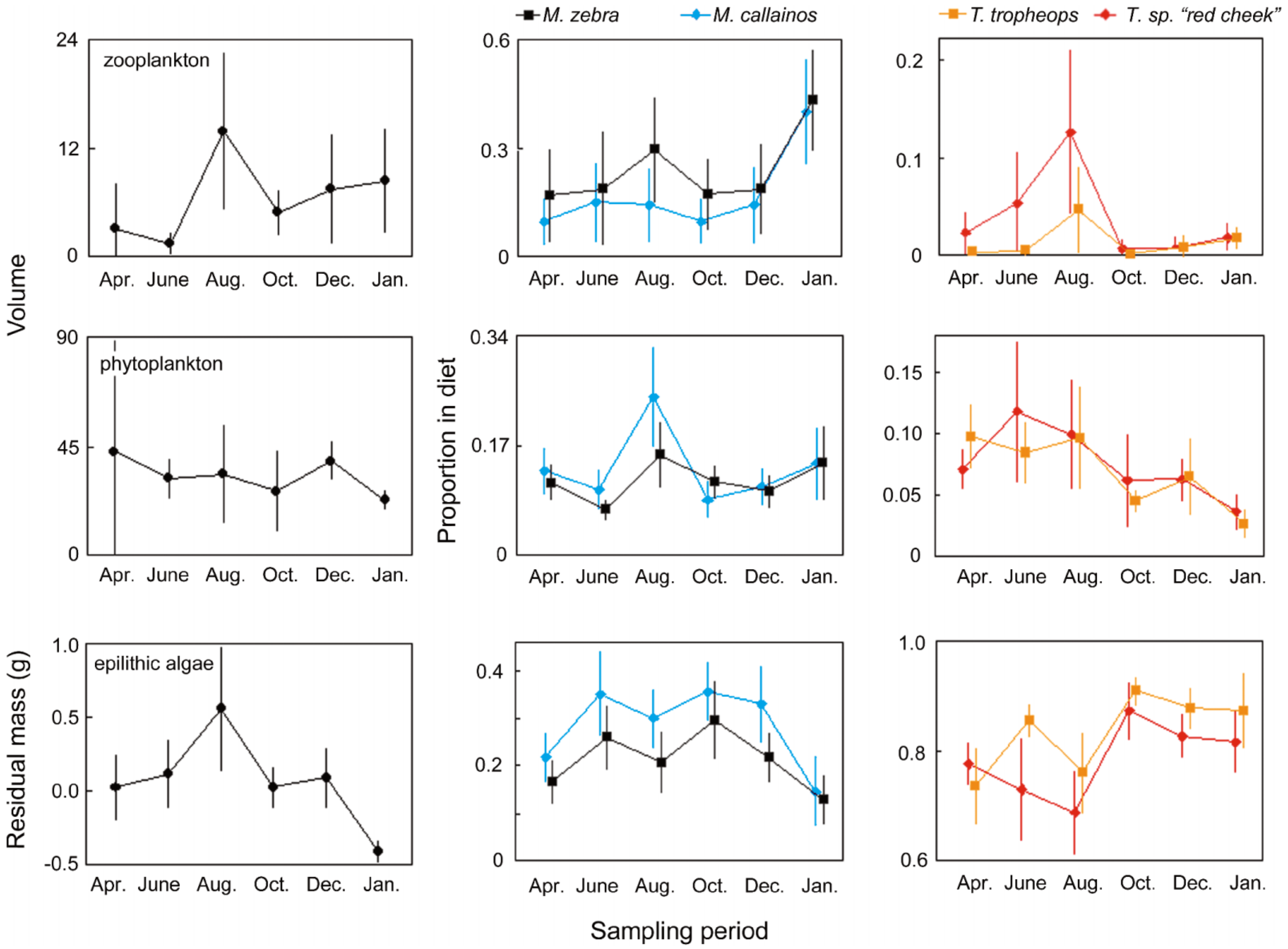
Male territoriality

During each sampling period, at least 10 territorial males from each species were observed within a 4 to 6 m deep transect. Prior to observations, a 70 cm × 70 cm reference quadrat was placed within the territory of the focal male for at least 5 min. All chases initiated by territorial males toward intruding fishes, and the number of intruding fishes surrounding each male in an area approximately equal to the reference quadrat, were recorded during 10-min observation periods. Intruding fishes from the four study species were identified to species and apparent sex, whereas other intruders were identified to genus following Konings (2007). Non-mbuna species were ignored. Total observed chases did not significantly vary across sampling periods in any focal species (one-way ANOVA, $\log_{10}$-transformed data: *M. zebra*, $F_{[5,76]} = 1.278$, $P = 0.282$; *M. callainos*, $F_{[5,72]} = 0.270$, $P = 0.928$; *T. tropheops*, $F_{[5,68]} = 1.059$, $P = 0.391$; *Tropheops* sp. "red cheek", $F_{[5,71]} = 1.030$, $P = 0.407$). The observed number of chases and intrusions were compared with expected values using the paired Wilcoxon's sign-rank test as detailed in Genner et al. (1999*c*). Briefly, the expected number of chases for each male was calculated from the proportional abundance of each species in his territory multiplied by the total number of chases initiated during the observation period. The expected number of intrusions was equal to the proportional abundance of each species in all observations multiplied by the total number of intruders recorded during the observation period.

Microhabitat

Following observations, rock number, sediment, and crevices within focal male territories were quantified. A 70 cm × 70 cm quadrat was marked with 25 even squares using intersecting grid lines. This quadrat was placed in the center of the territory and the number of squares containing mainly sediment, rock crevices, and algae-covered rocks was counted. Next, the number of small (<0.5 m), medium (<1 m), and large (>1 m) rocks completely or partially within the quadrat was recorded.

Fig. 1. Abundance (mean ± 95% confidence interval) of zooplankton, phytoplankton, and epilithic algae and their proportion in the diets of the study species (mean ± 95% confidence interval) during each sampling period. Plankton abundance was measured in arbitrary volume units = 1 *Anabaena* cell. Residual epilithic algal mass is the wet mass scraped from 25 cm^2 of rock surface, regressed on depth to remove depth effects.



Results

Resource abundance

We found significant variation in zooplankton, phytoplankton, and epilithic algae resources across sampling periods. Zooplankton abundance increased 10-fold in August from its lowest point during June–July (Welch's ANOVA, $F_{[5,62]} = 4.226$, $P = 0.007$; Fig. 1). Total phytoplankton abundance also significantly varied across sampling periods (Welch's ANOVA, $F_{[5,62]} = 2.938$, $P = 0.034$; Fig. 1). There were significant differences in the wet weights of epilithic algae samples throughout the year (ANOVA, $\log_{10}$-transformed residuals regressed on depth, $F_{[5,107]} = 10.453$, $P < 0.001$; Fig. 1). Epilithic algae mass peaked in August and was significantly lower in January than during other sampling periods (Tukey's honestly significant difference test (HSD), $P < 0.05$).

Dietary overlap

Resource abundance was not a significant predictor of dietary similarity within either of the species pairs (Table 1), and overall dietary overlap within species pairs did not significantly vary across sampling periods (Fig. 2), except between *Tropheops* species in June (Table 2). When using a pooled sample for the full year, significant interspecific dietary differences were detected within *Tropheops*, but not *Metriaclima* (Table 2).

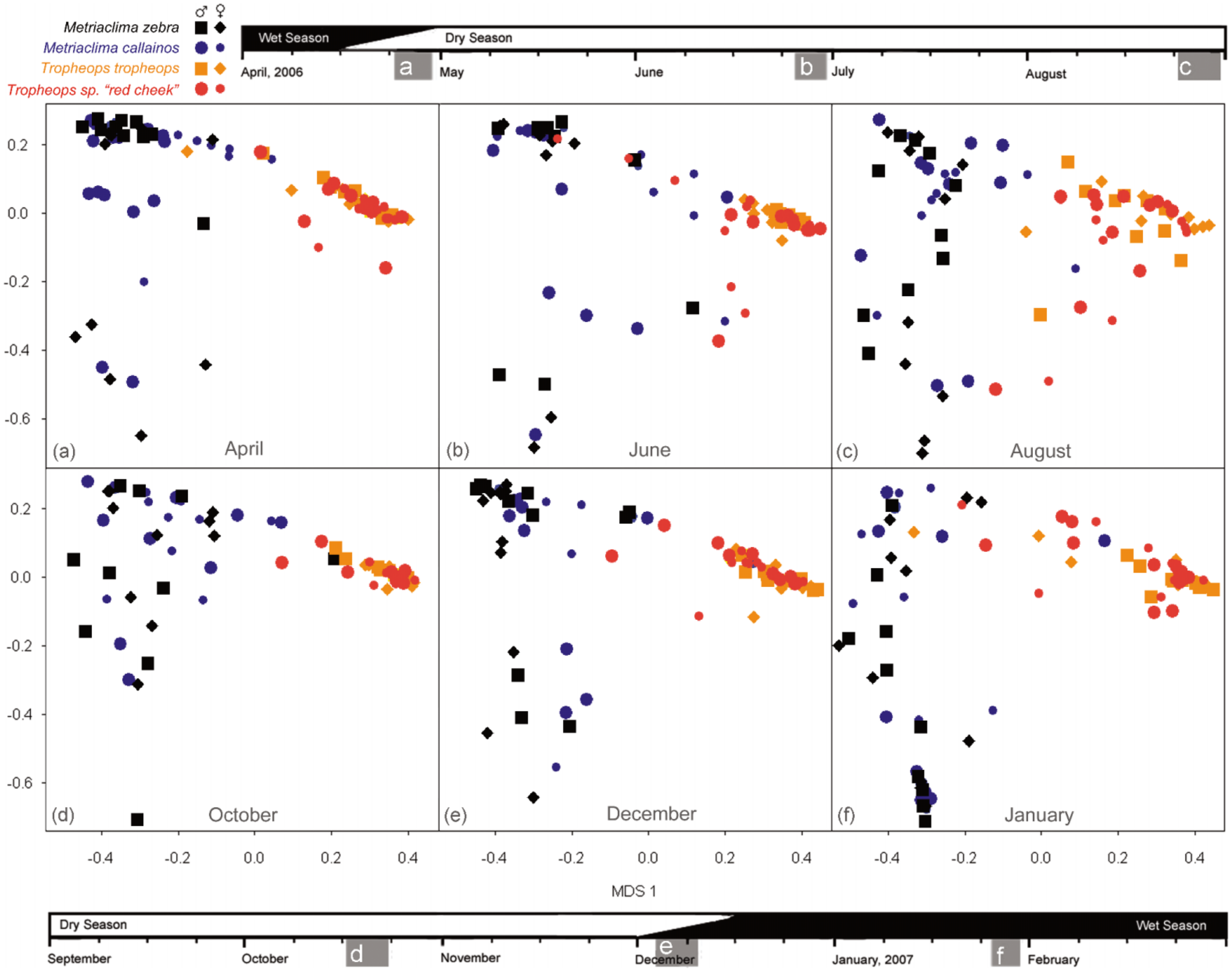
Table 1. Linear regression between resource abundance ($\log_{10}$-transformed) in each sampling period and dietary similarity ($\log_{10}$-transformed) within species pairs.

Species pair	Resource	r^2	*P*	Slope
Metriaclima	Zooplankton	0.161	0.431	0.098
	Phytoplankton	0.311	0.250	0.384
	Epilithic algae	0.571	0.082	0.210
Tropheops	Zooplankton	0.113	0.514	–0.139
	Phytoplankton	0.052	0.665	–0.136
	Epilithic algae	0.129	0.484	0.026

Note: Dietary similarity is the average Bray–Curtis similarity index of all interspecific pairwise comparisons within each species pair, calculated separately for zooplankton, phytoplankton, or epilithic algae dietary components.

Although there was a significant 5%–10% difference in zooplankton and *Calothrix* consumption between species

Fig. 2. Multidimensional scaling plots of the Bray–Curtis dietary similarity among all four study species plotted during each sampling period (stress = 0.11). Each individual is represented by one point, and closer points indicate greater dietary similarity. Shaded boxes on the time lines at the top and bottom indicate collection dates.



within each genus (two-way ANOVA, arcsin-transformed data: zooplankton: *Metriaclima*, $F_{[1,237]} = 4.124$, $P = 0.043$; *Tropheops*, $F_{[1,230]} = 6.788$, $P = 0.010$; *Calothrix*: *Metriaclima*, $F_{[1,237]} = 20.442$, $P < 0.001$; *Tropheops*, $F_{[1,230]} = 6.405$, $P = 0.012$; Fig. 1; Supplemental Fig. S2),[2] this difference was consistent across sampling periods, indicating no seasonal changes in trophic overlap occurred within species pairs (species by sampling period interaction: *Calothrix*: *Metriaclima*, $F_{[5,237]} = 0.851$, $P = 0.515$; *Tropheops*, $F_{[5,230]} = 1.359$, $P = 0.241$; zooplankton: *Metriaclima*, $F_{[5,237]} = 0.397$, $P = 0.851$; *Tropheops*, $F_{[5,230]} = 1.990$, $P = 0.081$). There were no significant differences between species within each genus in consumption of other abundant dietary components (Supplemental Fig. S2).[2]

Stomach content mass

Tropheops stomach content mass varied significantly across sampling periods (two-way ANOVA, $F_{[5,216]} = 10.477$, $P < 0.001$; Fig. 3). Stomachs contained significantly more mass in August, October, and December than in all other months (Tukey's HSD, $P < 0.05$). There was no significant interaction between sampling period and species on *Tropheops* stomach mass (two-way ANOVA, $F_{[5,216]} = 1.955$, $P = 0.087$). Sampling period had no significant effect on the frequency of empty stomachs in *Metriaclima* species (*M. callainos*, $n = 125$, $\chi^2 = 2.932$, $P = 0.710$; *M. zebra*, $n = 120$, $\chi^2 = 6.742$, $P = 0.241$; Fig. 3). Interspecific dietary overlap was not associated with stomach content mass in either *T. tropheops* ($r^2 = 0.0004$, $n = 76$, $P = 0.863$) or *Tropheops* sp. "red cheek" ($r^2 = 0.0004$, $n = 62$, $P = 0.877$).

Foraging effort

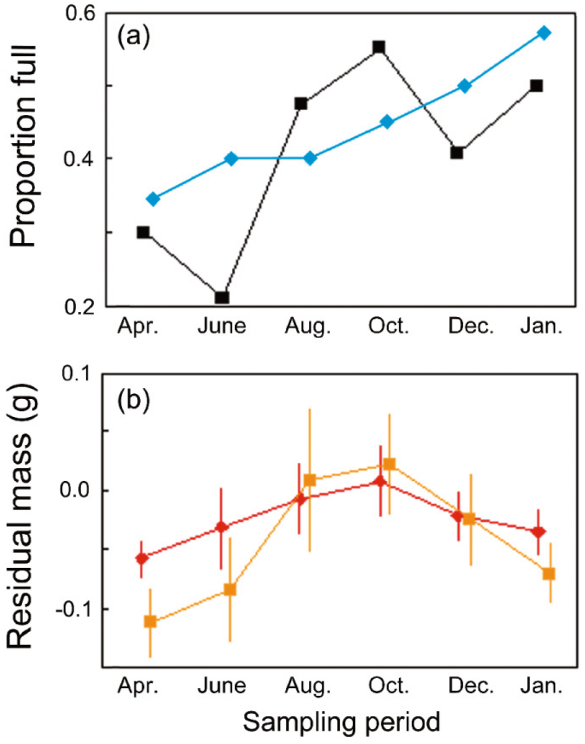
We found variable foraging rates in all four species across sampling periods, consistent with seasonal fluctuations in resource abundance. All four study species changed their benthic foraging rates across sampling periods (one-way ANOVA: *M. zebra*, $F_{[5,187]} = 4.959$, $P < 0.001$; *M. callainos*, $F_{[5,182]} = 4.392$, $P = 0.001$; *T. tropheops*, $F_{[5,174]} = 9.971$, $P < 0.001$; *Tropheops* sp. "red cheek", $F_{[5,175]} = 2.930$, $P = 0.015$; Fig. 4). Both *Metriaclima* species and *T. tropheops* also changed their planktonic foraging rates across sampling periods (Kruskal–Wallis rank-sum test, *M. zebra*, $\chi^2 =$

Table 2. Analysis of similarities (ANOSIM), intraspecific dietary overlap, and interspecific dietary overlap within species pairs.

Sampling period	Species	*n*	Intraspecific overlap	Species	*n*	Intraspecific overlap	Interspecific overlap	ANOSIM *R*	ANOSIM *P*
Global	MZE	123	0.58±0.02	MCA	126	0.58±0.02	0.59±0.03	0.007	0.284
April	MZE	21	0.59±0.04	MCA	26	0.63±0.04	0.61±0.04	–0.01	0.537
June	MZE	19	0.58±0.05	MCA	20	0.60±0.06	0.57±0.05	0.027	0.154
August	MZE	20	0.59±0.06	MCA	20	0.59±0.05	0.57±0.05	0.061	0.066
October	MZE	20	0.67±0.07	MCA	20	0.68±0.03	0.66±0.04	0.003	0.379
December	MZE	22	0.61±0.05	MCA	18	0.63±0.06	0.62±0.05	0.003	0.347
January	MZE	21	0.55±0.08	MCA	21	0.49±0.09	0.52±0.07	–0.032	0.905
Global	TRT	121	0.82±0.02	TRD	121	0.74±0.02	0.76±0.03	0.021	**0.039**
April	TRT	20	0.81±0.06	TRD	20	0.83±0.02	0.80±0.05	–0.019	0.780
June	TRT	21	0.85±0.02	TRD	19	0.72±0.06	0.81±0.02	0.177	**0.005**
August	TRT	20	0.77±0.05	TRD	21	0.72±0.06	0.76±0.04	–0.011	0.590
October	TRT	20	0.86±0.01	TRD	18	0.79±0.03	0.82±0.02	0.030	0.126
December	TRT	20	0.82±0.04	TRD	20	0.77±0.06	0.79±0.03	0.008	0.282
January	TRT	20	0.79±0.07	TRD	20	0.74±0.06	0.76±0.06	0.005	0.373

Note: Across sampling periods (global), the diets of each species pair were compared using two-way ANOSIM. Within each sampling period, species pairs were compared using one-way ANOSIM, critical $P = 0.008$ after Bonferroni adjustment. Significant *P* values are presented in bold. Dietary overlap is the mean (±95% confidence interval) of each individual's overlap with the intra- or inter-specific species mean, calculated by Schoener's resource overlap index (Schoener 1968). MZE, *Metriaclima zebra*; MCA, *M. callainos*; TRT, *Tropheops tropheops*; TRD, *Tropheops* sp. "red cheek".

Fig. 3. (*a*) Proportion of "full" stomachs in *Metriaclima zebra* (squares) and *M. callainos* (diamonds). (*b*) Residual stomach content mass (mean grams ± 95% confidence interval; regressed on standard length (SL) to remove size effects) in *Tropheops tropheops* (squares) and *Tropheops* sp. "red cheek" (diamonds) during all sampling periods. "Full" indicates that an individual's stomach was detectable during dissections. Only detectable *Tropheops* stomachs were weighed; undetectable stomachs were set equal to the lowest residual value for each species.



42.082, $P < 0.001$; *M. callainos*, $\chi^2 = 46.755$, $P < 0.001$; *T. tropheops*, $\chi^2 = 22.960$, $P = 0.0003$; *Tropheops* sp. "red cheek", $\chi^2 = 9.635$, $P = 0.086$; Fig. 4).

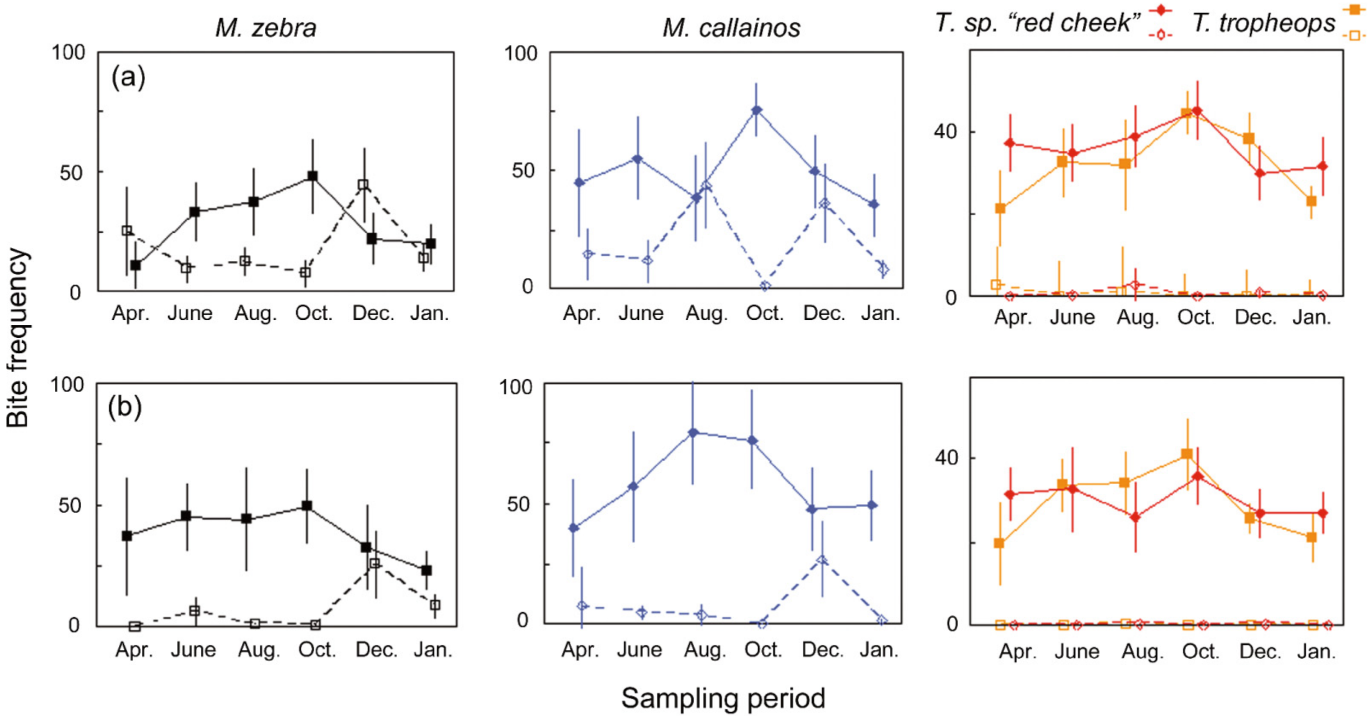
Male territoriality

During most sampling periods in all four species, conspecific females were chased more often than expected, and both conspecific males and females were present in male territories less often than expected by chance (Fig. 5), implying exclusion. Patterns of congeneric territoriality relative to resource abundance were equivocal. Both *Tropheops* species increased congeneric male and female exclusions from October through January (Figs. 5*c*, 5*d*), coinciding with higher trophic overlap and reduced trophic breadth (Fig. 2). However, this pattern must be interpreted with caution as *Tropheops* conspecific female chases also increased during these sampling periods, perhaps indicating increased courtship activity. *Metriaclima zebra* exclusions of other *Metriaclima* species increased during highest zooplankton abundance in August and January (Fig. 5*a*). However, *M. zebra* congeneric exclusions were also elevated during April, and *M. callainos* exhibited no congeneric aggression for comparison.

Overall, territoriality towards heterogeneric species was targeted against trophic competitors (Fig. 5). Within the generalist *Metriaclima* species overall, *M. zebra* excluded *Labeotropheus*, a genus of specialist epilithic algivores, whereas *M. callainos* excluded *Cynotilapia afra*, a translocated planktivore (Figs. 5*a*, 5*b*). Both algivore specialist *Tropheops* species were significantly more aggressive than expected towards the other algivore specialist genus *Labeotropheus*, while ignoring generalists *Metriaclima* and *Petrotilapia* and the plankton specialist *Cynotilapia afra*.

Territoriality against heterogeneric trophic competitors was unaffected by resource abundance, except in one case: during minimum zooplankton abundance in June, *M. zebra* showed more aggression than expected toward the plankton

Fig. 4. Benthic (solid lines) and planktonic (broken lines) foraging rates (mean ± 95% confidence interval) in all sampling periods for (*a*) females and (*b*) males, estimated from the frequency of biting on benthos or suction feeding in the water column during 2-min observation periods.



specialist *Cynotilapia afra* (Fig. 5*a*). During lowest epilithic algae biomass in January, there was no apparent change in aggression towards competing algivores in any species. During peak zooplankton and epilithic algae abundance in August, all focal species remained aggressive towards at least one group of algivores, either congeneric or heterogeneric (Fig. 5).

Microhabitat

Although there were significant male territory microhabitat differences between species in each genus, these differences did not reliably distinguish species. Only *Tropheops* species differed in the amount of rocks in male territories (one-way multivariate analysis of variance (MANOVA), $\log_{10}$-transformed data: *Metriaclima*, $F_{[1,131]} = 1.485$, $P = 0.225$; *Tropheops*, $F_{[1,120]} = 7.043$, $P = 0.009$; Table 3). Only *Metriaclima* species differed in the proportions of sediment and crevices in male territories (one-way MANOVA, arcsin-transformed data: *Metriaclima*, $F_{[1,144]} = 5.164$, $P = 0.025$; *Tropheops*, $F_{[1,130]} = 0.532$, $P = 0.467$; Table 3). Linear discriminant analyses using all five microhabitat variables could not distinguish between *Metriaclima* species in 40% of samples ($n = 133$; binomial test, $P = 0.024$) or between *Tropheops* species in 30% of samples ($n = 122$; binomial test, $P < 0.001$).

Discussion

We found extensive seasonal fluctuations in zooplankton, phytoplankton, and epilithic algae resources. All four study species shifted their diets, stomach content mass, and foraging rates in response to these changes. Dietary overlap between both morphologically similar species pairs was largely unaffected by changes in resource abundance throughout the year. Significant dietary differences between *Tropheops* species in June were linked not to partitioning distinct resources, but instead to the early adoption of a shared resource, zooplankton, by *Tropheops* sp. "red cheek". Similarly, significant dietary differentiation between *Tropheops* species throughout the year was linked not to strict partitioning of distinct resources, but instead to different relative consumption of shared resources. Thus, although small but significant dietary differences were observed in one species pair, there is no indication that these species occupied separate trophic niches.

If increased dietary partitioning during resource scarcity occurs, we predicted reduced territorial aggression due to reduced competition for resources. In contrast, male aggression toward both congeneric and heterogeneric fishes did not decrease during resource scarcity, and in one species, aggression toward a competing planktivore increased during zooplankton scarcity. These results support increased territorial aggression during resource scarcity, consistent with species competing for shared resources during lean times.

We cannot rule out that sampling may have occurred during an unusually resource-rich year and thus resources never became limiting for our study species. However, the 10-fold range and seasonality of zooplankton abundance observed was consistent with a previous 2-year study in the same lake region (Twombly (1983) reported a low of ~25 000 zooplankters·m^{-3} in January through April to a high of 250 000 zooplankters·m^{-3} in August). Similarly, a 2-year lake-wide survey found a sixfold difference in zooplankton

Fig. 5. Groups receiving significant aggression (via direct chases or territorial exclusions) from (*a*) *Metriaclima zebra*, (*b*) *M. callainos*, (*c*) *Tropheops tropheops*, and (*d*) *Tropheops* sp. "red cheek" territorial males during all sampling periods. Graphic presentation of data in Supplemental Tables S3–S6[2]. Global results may contain some pseudoreplication across sampling periods. MZE, *M. zebra*; MCA, *M. callainos*; MET, all other *Metriaclima* sp.; TRT, *T. tropheops*; TRD, *Tropheops* sp. "red cheek"; TRO, all other *Tropheops* sp.; LBD, *Labidochromis* sp.; MEL, *Melanochromis* sp.; CYA, *Cynotilapia afra*; PET, *Petrotilapia* sp.; LBO, *Labeotropheus* sp. LBO are absent from all chase analyses and TRO are absent from *Tropheops* chase analyses because of small sample sizes.

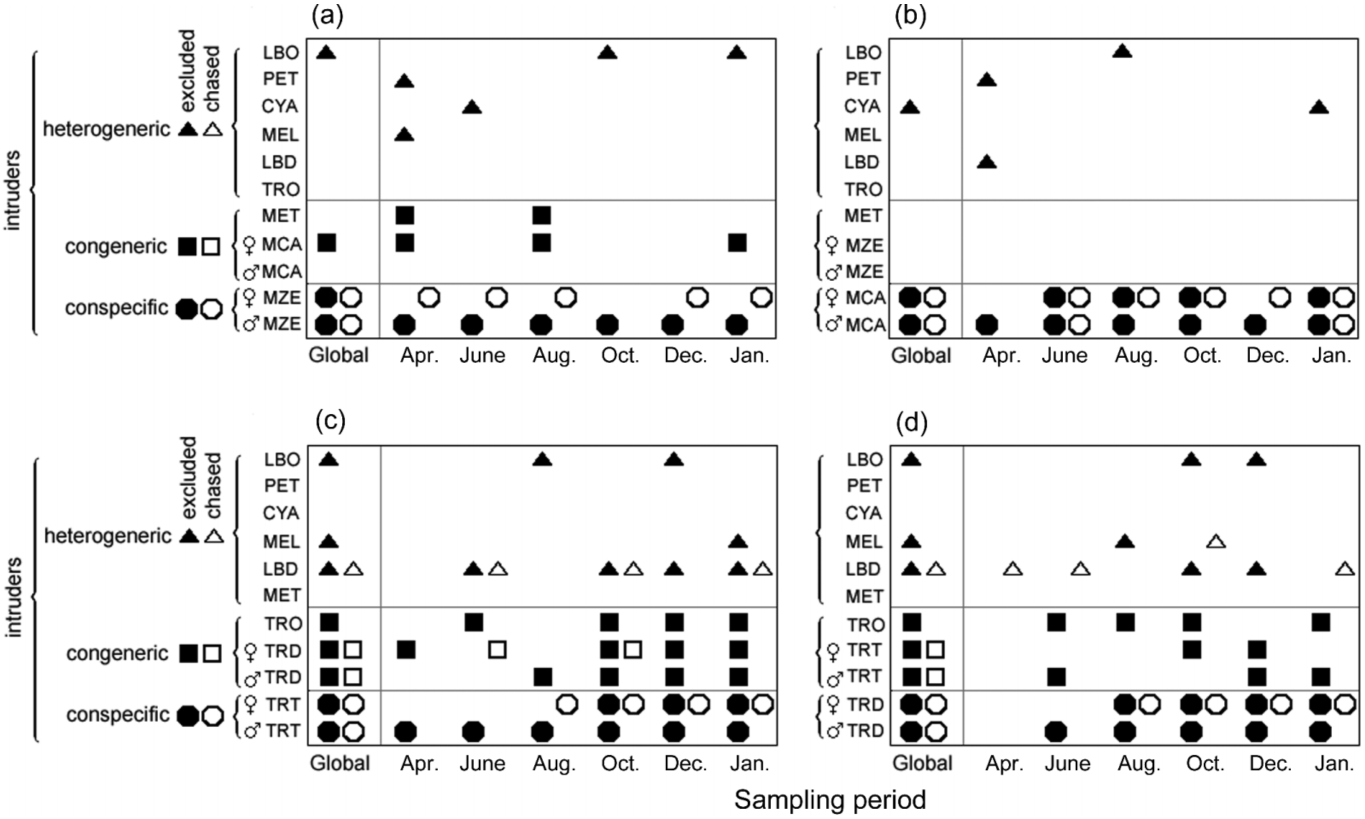


Table 3. Microhabitat differences among male territories quantified by 25 squares within a 0.7 m × 0.7 m quadrat.

	No. of rocks (mean ± 95% confidence interval)				
Species	Small	Medium	Large	% sediment	% crevices
Metriaclima zebra	7.6±1.1	1.8±0.4	1.9±0.4	10±2.9	18±1.9
Metriaclima callainos	6.4±1.0	2.0±0.4	1.6±0.3	7.3±3.0	16±1.9
Tropheops tropheops	4.7±1.0	0.9±0.3	2.4±0.3	13±3.7	12±1.6
Tropheops sp. "red cheek"	6.4±1.3	2.2±0.5	1.6±0.3	9.0±2.8	14±2.1

biomass, with lows in March–April and highs in July–August (Irvine and Waya 1999). This seasonal variability is due to seasonal mixing of the nutrient-rich hypolimnion during the dry season from May to November, when southeast winds accumulate surface waters in the northern end of the lake, creating a compensatory upwelling current in the south (Beadle 1981). If resources remained in excess during even the most resource-poor sampling periods in this study (June–July and January), we would expect to see constant foraging effort and stomach content mass across all sampling periods. However, we observed seasonal fluctuations in foraging rate and stomach content mass in all four study species, consistent with adjustments of foraging effort relative to changing resource abundance and quality.

In contrast to well-documented seasonal patterns in many taxa (Boag and Grant 1981; Luiselli 2006; Marshall and Wrangham 2007), overall, our results do not support predictions of greater trophic partitioning during resource scarcity (McKaye and Marsh 1983; Robinson and Wilson 1998). Although trophic partitioning in fish assemblages is undoubtedly widespread (Ross 1986; Robinson and Wilson 1994), recent dietary studies of several fish assemblages have also found high dietary overlap among sympatric species (Horstkotte and Strecker 2005; Barnett et al. 2006; Helland et al. 2008). In such cases, it is possible that species avoid competition through segregation on other niche axes; however, overlap in depth, microhabitat, and activity period was also high between species pairs in this study. Depth ranges of all four species in this study overlap considerably (Ribbink et al. 1983), and we sampled all fishes from approximately the same depth at which they co-occurred in high abundance. Congeneric species often show peak abundances at different depths, including the species in this study (Ribbink et al. 1983; Albertson 2008); however, this is not habitat partitioning in the strictest sense, as overlap in depth ranges among these species is substantial and the majority of species in rock-dwelling cichlid assemblages coexist within the depth range of 3–7 m studied here (Ribbink et al. 1983). Although we found some evidence of significant differences in male territory microhabitats, species could not be identified by these microhabitats alone. No evidence of temporal partitioning was observed; instead, all Lake Malawi rock cichlids are diurnal and tend to forage throughout the day.

These data suggest that two pairs of ecologically equivalent species co-occur at our study site with substantial overlap in resource use during periods of both resource scarcity and abundance. The recent invasions and rapid population expansions by one species in each pair without apparent detriment to the native species (Genner et al. 2006) further suggests that interspecific competition is not driving competitive exclusion. This pattern of invasive species increasing the diversity of native assemblages has been more widely found in global surveys of island birds and plants (Sax et al. 2002).

Successful coexistence of ecologically similar species pairs may be due to weak or absent divergent ecological selection within the species-packed Lake Malawi rock cichlid assemblages. Although evidence of divergent ecological selection is widespread in fishes (Ross 1986; Robinson and Wilson 1994), including cichlids (Hori 1993; Swanson et al. 2003), there is still no direct evidence of competition-driven divergent ecological selection operating among the haplochromine cichlids of the African lakes. We found no evidence of strong competition between *Tropheops* species, as individuals with more divergent diets did not have greater foraging success, as estimated from their stomach content mass. Nevertheless, close associations between trophic morphology of geographically separate conspecific populations and available diet is consistent with selection on tooth and jaw shape, although phenotypic plasticity is also likely (Bouton et al. 1999). In the absence of strong divergent ecological selection, nonecological processes may drive speciation, creating ecologically equivalent species. It is thus possible that weak divergent ecological selection among Lake Malawi cichlids has enabled the rate of speciation to outpace extinction (e.g., Gillespie 2004).

In conclusion, we found substantial trophic overlap persisting during seasonal resource fluctuations in two successfully coexisting pairs of Lake Malawi haplochromine cichlids, in contrast to predictions of ecological specialization during resource scarcity (McKaye and Marsh 1983; Robinson and Wilson 1998). High numbers of morphologically and ecologically similar sympatric species are not uncommon in rapidly speciating assemblages. This pattern is consistent with speciation by sexual selection generating ecologically equivalent incipient species. The apparent successful coexistence of such species in Lake Malawi adds plausibility to models of speciation that do not require ecological divergence.

Acknowledgements

We thank the Malawi Government for permission to conduct research in Lake Malawi National Park. U. Malindi, E. Morehouse, L.K. Banda, The Department of Aquaculture and Fisheries at Bunda College, the Billy Riordan Memorial Trust, and the Malawi Department of Fisheries provided valuable logistical support. Two anonymous reviewers provided helpful comments that improved the manuscript. R. Holzman, P. Wainwright, and K. McKaye provided helpful discussion. This work was funded by a US Student Fulbright Grant from the Institute of International Education and by an NSF Graduate Research Fellowship to C.H.M.

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