

Niche segregation among Lake Malawi cichlid fishes? Evidence from stable isotope signatures

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Abstract

The fish communities of the rocky littoral zone of Lake Malawi contain a large number of ecologically similar cichlid species. It has been suggested that dietary niche segregation may play a role in the coexistence of these species, but previous studies have yielded ambiguous results. Stable isotope analysis was used to determine whether five sympatric species are segregated by diet. Significant differences were found between the mean isotopic signatures of the study species, but there was considerable interspecific overlap between three species from the same subgenus that were anatomically almost indistinguishable. The implication that this was due to substantial dietary similarity was supported by stomach content analysis. We propose that ecological segregation may not always be necessary to allow coexistence of Lake Malawi cichlids.

Keywords

Cichlids, coexistence, ecology, Lake Malawi, niche differentiation, resource partitioning, stable isotopes.

Ecology Letters (1999) 2: 185–190

INTRODUCTION

Lake Malawi has a strikingly rich cichlid fish fauna. It is currently estimated that over 500 species are present (McKaye & Gray 1984; Ribbink 1994; van Oppen *et al.* 1998). The haplochromine flock, to which over 99% of the Lake Malawi cichlid taxa belong, is thought to have radiated within the last 700,000 years or less (Meyer *et al.* 1990). Many species within the lake have evolved unique anatomical and behavioural specializations (e.g. adaptations for fin biting and fry eating), but the majority of communities contain species that are found together with apparently little or no ecological differentiation. The means of coexistence of such species remains unclear.

The cichlids associated with the rocky littoral zone have been the most intensively studied. These communities may contain up to 35 species (Ribbink *et al.* 1983). It is currently believed that food niche partitioning is the principal factor allowing coexistence of sympatric species (e.g. Sharp 1981; Ribbink *et al.* 1983; Reinthal 1990). The same conclusion has been drawn from the ecologically comparable cichlid communities of Lake Tanganyika (Hori 1983; Sturmbauer *et al.* 1992) and Lake Victoria (Seehausen 1996; Bouton *et al.* 1997). However, the evidence is incomplete. While broad scale interspecific differences are easily found within communities, many sympatric species show large overlaps of diet (Reinthal

1990) and feeding sites (M. J. Genner, G.F. Turner & S.J. Hawkins, unpublished work).

A recent study by Bootsma *et al.* (1996) showed that stable isotope signatures could be used to identify interspecific differences of feeding strategies of Lake Malawi cichlids. Food sources often have specific isotopic signatures that are acquired by consumers (Fry 1988). If two individuals consume two separate food sources with differing isotopic compositions, they will have different isotopic signatures. However, interpretation of results is not always simple. Where food sources possess the same isotopic compositions, the signatures of consumers will be the same, even if diets are different. Also, if a number of food sources with different signatures are assimilated in differing proportions the overall isotopic composition of the consumers can potentially be the same. The primary benefit of stable isotopic signatures is that they represent the composition of assimilated food over a long time period, from weeks to years (Bootsma *et al.* 1996). In contrast, stomach content analysis is simply a “snap-shot” technique that enables analysis of the dietary intake of an individual over the previous few hours. Also, it is often difficult to identify masticated food items accurately (Hynes 1950).

We used a combination of stable isotope analysis and stomach content analysis to study feeding strategies of rocky habitat cichlid species. We tested the null hypoth-

esis that there was no dietary differentiation between the three sympatrically occurring sibling species of the *Pseudotropheus* (*Maylandia*) subgenus at Chirundu Point, Nkhata Bay, Malawi (see map in van Oppen *et al.* 1997). The three species are *P. (M.) zebra*, *P. (M.)* “gold zebra” and *P. (M.) callainos* (Ribbink *et al.* 1983; Meyer & Foerster 1984; Stauffer & Hert 1992). (Note: Stauffer *et al.* 1997 have proposed a new generic name *Metriaclima* to replace the *P. [Maylandia]* subgenus.) These species are anatomically almost indistinguishable but differ in body colouration. They have been shown to be reproductively isolated species in their natural habitats by microsatellite DNA allele frequency analysis (van Oppen *et al.* 1998) and also in laboratory assortative mating experiments (Knight *et al.* 1998). All three are known to feed upon both the epilithic algae and the plankton (Ribbink *et al.* 1983). We also studied *Cynotilapia afra* (Günther 1893), a known obligate planktivore, and *Pseudotropheus (Tropheops)* “deep” (Ribbink *et al.* 1983; Trewavas 1984), a benthic algal feeder (Ribbink *et al.* 1983). These two species were chosen because of their contrasting and comparatively strict diets. Their use enabled us to verify that isotope signatures are a product of dietary intake. All five study species coexist at depths between 7 and 25 m at Nkhata Bay (Ribbink *et al.* 1983).

METHODS

Using SCUBA, 10 males and 10 females of each of the five study species were collected from depths between 9 and 12 m. The collection site was approximately 100 m wide and consisted of sediment-rich rocky habitat. After capture, fish were brought to the surface and killed as quickly as possible by an overdose immersion in 2-phenoxyethanol. Samples of the primary food sources—plankton, epilithic algae and sediment—were taken. Plankton was collected in two hauls at 10 m depth from the inshore area of Chirundu point using a 50 µm mesh phytoplankton net. Samples of epilithic algae were taken from rocks brought to the surface from the fish collection site. Bootsma *et al.* (1996) found preliminary evidence that the algae growing on different slopes had different isotopic signatures, so algae were collected from rock surfaces of known slope. Sediment was collected by laying weighted bowls at the study site. Upon collection lids were sealed and the bowls were brought to the surface. All samples were collected between 30 June and 8 August 1997.

Stable isotope analysis

A fillet of the flank of each fish and the food samples were sun dried to constant weight. Subsequently, all samples were stored independently with desiccant (silica gel; 4–7

mesh) before being ground to a fine homogeneous powder using a pestle and mortar. Powdered samples weighing either 1 mg (± 0.05 mg) (for fish) or 3 mg (± 0.05 mg) (for foodstuffs) were placed into foil capsules that were then sealed. Isotopic analyses were carried out using a PDZ-Europa (Crewe, UK) ANCA 20/20 mass spectrometer with ANCA SL preparation unit at the Biomedical Mass Spectrometry Unit, University of Newcastle, UK. Samples were analysed in four runs of up to 50 samples. A cod (*Gadus morhua*) reference sample (for instrument drift correction) and a cod control sample (for run standard deviation) with known isotopic constituents were analysed at the start of each run and subsequently every fifth cichlid sample. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were determined following Bootsma *et al.* (1996). Standard deviations of the control samples in run 1 were $\delta^{15}\text{N}$ 0.13‰, and $\delta^{13}\text{C}$ 0.03‰, run 2 $\delta^{15}\text{N}$ 0.09‰ and $\delta^{13}\text{C}$ 0.02‰, run 3 $\delta^{15}\text{N}$ 0.27‰ and $\delta^{13}\text{C}$ 0.03‰ and run 4 $\delta^{15}\text{N}$ 0.06‰ and $\delta^{13}\text{C}$ 0.04‰. All runs were within the manufacturers precision of 0.3‰ and 0.1‰ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively.

Stomach content analysis

After fillets were removed, fish were tagged for identification and preserved in formalin (paraformaldehyde 1 g/L). Subsequently, stomachs contents were removed and again formalin preserved (paraformaldehyde 1 g/L). Dietary constituents were identified under $\times 100$ to $\times 400$ magnification and classified into broad categories (see Table 1). The volumetric proportions of food items within each stomach were calculated from an average of eight subsamples. Constituents within each subsample were quantified using a modified version of the points method of Hynes (1950). The dietary component with the highest volume was allocated 16 points.

Table 1 Categories used when quantifying stomach contents

Cyanophyta	Bacillariophyta
<i>Anabaena</i> sp.	<i>Aulacoseira</i> sp.
<i>Botryococcus</i> sp.	<i>Amphora</i> sp.
<i>Calothrix</i> sp.	<i>Cymbella</i> sp.
<i>Microcystis</i> sp.	<i>Cymatopleura</i> sp.
<i>Oscillatoria</i> sp.	<i>Gomphonema</i> sp.
	<i>Navicula</i> sp.
Chlorophyta	<i>Nitzschia</i> sp.
	<i>Pinnularia</i> sp.
<i>Cladophora</i> sp.	Centric Diatoms
<i>Mougoetia</i> sp.	
<i>Pediastrum</i> sp.	Miscellaneous
<i>Staurastrum</i> sp.	
	Chironomid larvae
	Crustacean zooplankton
	Silt
	Detritus

Subsequently every other dietary component was awarded 16, 8, 4, 2, 1 or 0 points depending upon its volume relative to the component with the highest volume. The percentage volumes within each subsample were calculated as follows:

$$\% \text{ volume of component } \alpha = \frac{\text{Number of points allocated to component } \alpha}{\text{Total points allocated to subsample}} \times 100$$

The computer package PRIMER (Clarke & Warwick 1994) was used to analyse stomach content data using the following techniques. A similarity matrix of all 100 individuals was constructed in CLUSTER using the Bray-Curtis coefficient (Bray & Curtis 1957). Multidimensional scaling (MDS) was then used to produce a two-dimensional ordination of the data from the similarity matrix. To statistically test the interspecific differences in stomach contents the nonparametric ANOSIM (analysis of similarities) was employed.

RESULTS

There were highly significant differences between the isotopic signatures of the five species (1-way ANOVA [$\delta^{15}\text{N}$, $F_{4,95} = 110.50$, $P < 0.001$] [$\delta^{13}\text{C}$, $F_{4,95} = 642.03$, $P < 0.001$]). Distinct species clusters were present. *Pseudotropheus (T.)* “deep” (low $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values) and *C. afra* (high $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values) were segregated from one another and the three *P. (Maylandia)* species (Fig. 1a). Isotopic signatures of individuals of the *P. (Maylandia)* subgenus overlapped considerably (Fig. 1b). The means of $\delta^{13}\text{C}$ differed significantly in pairwise comparisons between all species (Table 2). $\delta^{15}\text{N}$ means also differed significantly in pairwise comparisons of all study species with the exception of a nonsignificant difference between *P. (M.)* “gold zebra” and *P. (M.) zebra* (Table 2). Isotopic signatures were not significantly influenced by the sex of the individuals (1-way Nested ANOVA [$\delta^{15}\text{N}$, $F_{4,90} = 0.47$, $P = 0.758$] [$\delta^{13}\text{C}$, $F_{4,90} = 1.09$, $P = 0.365$]).

Stomach content analysis demonstrated *P. (T.)* “deep” to be an obligate epilithic algal feeding herbivore, whilst

Figure 1 (a) Mean isotopic composition (+ 95% confidence limits) of the five study species and potential food sources (the 95% confidence limits of all fish species are small and obscured by markers); each fish species $n = 20$, plankton $n = 2$, all epilithic algae and sediment $n = 4$. (b) Expanded section of Fig. 1(a), markers represent isotopic signatures of all *P. (Maylandia)* individuals ($n = 20$ each species), many markers overlap.

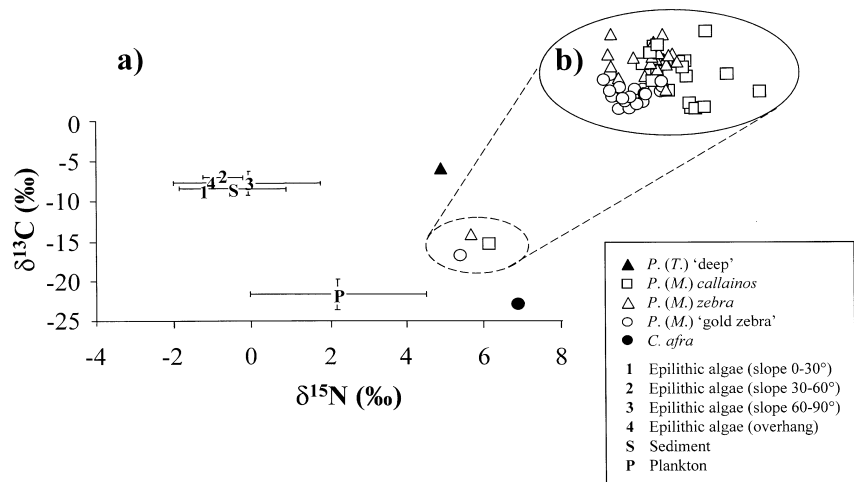


Table 2 Pairwise comparisons of mean isotopic composition (Tukey’s test) and stomach contents (Anosim)

	Species	<i>C. afra</i>	<i>P. (M.) callainos</i>	<i>P. (M.)</i> “gold zebra”	<i>P. (M.) zebra</i>
$\delta^{15}\text{N}$	<i>P. (M.) callainos</i>	*			
	<i>P. (M.)</i> “gold zebra”	*	*		
	<i>P. (M.) zebra</i>	*	*	–	
	<i>P. (T.)</i> “deep”	*	*	*	*
$\delta^{13}\text{C}$	<i>P. (M.) callainos</i>	*			
	<i>P. (M.)</i> “gold zebra”	*	*		
	<i>P. (M.) zebra</i>	*	*	*	
	<i>P. (T.)</i> “deep”	*	*	*	*
Stomach contents	<i>P. (M.) callainos</i>	*			
	<i>P. (M.)</i> “gold zebra”	*	*		
	<i>P. (M.) zebra</i>	*	–	–	
	<i>P. (T.)</i> “deep”	*	*	*	*

*Significant difference of means at $\alpha = 0.05$; –, nonsignificant difference $\alpha = 0.05$.

C. afra was principally a planktivore (Fig. 2). *Pseudotropheus* (*Maylandia*) species showed great similarity in their diets, consuming both epilithic and planktonic items. Stomachs of all three species contained large proportions of detritus and silt. Overall, there were significant differences in the diets of the five species (Anosim global test statistic $R = 0.593$, $n = 20$ for each species, $P < 0.001$). Pairwise comparisons revealed that only within the *P.* (*Maylandia*) subgenus were there nonsignificant differences in diet. *Pseudotropheus* (*M.*) *zebra* showed nonsignificant differences with both *P.* (*M.*) *callainos* and *P.* (*M.*) "gold zebra" (Table 2). Multidimensional scaling of the stomach content data confirmed this result by showing total differentiation between individuals of *P.* (*T.*) "deep" and *C. afra*, yet great similarity between the three *P.* (*Maylandia*) species (Fig. 3).

Food source analysis

All food sources had consistently lower $\delta^{15}\text{N}$ signatures than the fish species (Fig. 1). Plankton had lower $\delta^{13}\text{C}$ and higher $\delta^{15}\text{N}$ than both epilithic algae and sediment. The slopes upon which epilithic algae were growing did not significantly influence isotopic signatures, but sample sizes were small (1-way ANOVA [$\delta^{15}\text{N}$, $F_{3,12} = 0.878$, $P = 0.480$] [$\delta^{13}\text{C}$, $F_{3,12} = 1.304$, $P = 0.318$]).

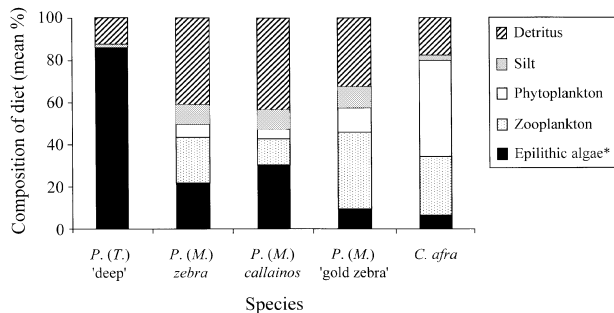


Figure 2 Summarized dietary composition of the five study species. Each species $n = 20$. *Includes chironomid larvae which were very infrequently present.

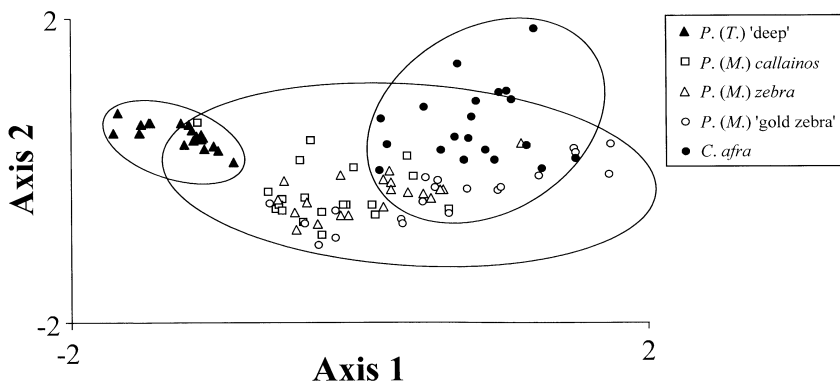


Figure 3 Multidimensional scaling (MDS) ordination plot of percentage dietary composition of the five study species; scaling uses raw data that includes all 22 categories of dietary component quantified; each species $n = 20$. Ellipses are fitted by eye.

DISCUSSION

Integration with previous work

The results indicate that $\delta^{13}\text{C}$ values represent a continuum of food sources between plankton (low values) and benthos (high values). Similar $\delta^{13}\text{C}$ results are reported by Bootsma *et al.* (1996), Hamilton & Lewis (1992) and Jennings *et al.* (1997). It is unknown whether the cichlid species in this study can selectively feed upon individual items within their bulk food sources, but both *C. afra* and *P. (T.)* "deep" had $\delta^{13}\text{C}$ values that matched the isotopic signatures of the bulk food reference samples, plankton and epilithic algae, respectively. This suggests that if selection of individual food items from bulk food sources does occur, it does not strongly influence the isotopic signatures of the study species. $\delta^{15}\text{N}$ values became enriched with the trophic level of the food sources and also proved to be good indicators of food sources, these findings support the results of Bootsma *et al.* (1996), Fry (1988), Hesslein *et al.* (1991) and Jennings *et al.* (1997). [Note: the samples used in the study by Bootsma *et al.* have recently been reanalysed and the new $\delta^{15}\text{N}$ values are an average of 1.15‰ higher than previously reported (SD = 0.03, $n = 33$). The $\delta^{13}\text{C}$ values remain unchanged. (H.A. Bootsma, personal communication.) These reanalysed results corroborate well with results reported in this study for ecologically comparable species.]

Ecological segregation among the *P. (Maylandia)* subgenus?

Isotopic signatures of all members of the *P. (Maylandia)* subgenus were clearly distinct from both *C. afra* and *P. (T.)* "deep". Considerable interspecific overlap was found between the species of *P. (Maylandia)* subgenus, although there were significant differences between means. These results imply that there are overall differences in the foraging strategies of these species, but the fact that the species to which an individual belongs cannot be determined from the isotopic signature means that these species have not fully ecologically diverged. The results of the stomach content analysis support this conclusion by

revealing substantial dietary similarity that cannot be taken as evidence of strong food resource partitioning. It is feasible that the species feed upon the same type of food resources, but utilize different feeding microsites. Data have not been collected from the depths where all three of the studied *P. (Maylandia)* coexist, but where *P. (M.) callainos* and *P. (M.) zebra* are found together in abundance in shallower waters, no statistically significant differences in the characteristics of feeding microsites have been found (M. J. Genner, G.F. Turner & S.J. Hawkins, unpublished work).

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BIOSKETCH

Martin Genner is currently a PhD student working with Dr George Turner, who is interested on the evolution of African cichlid fishes, and Prof. Stephen Hawkins, who is interested in aquatic community structure.

Editor, U. Sommer

Manuscript received 12 January 1999

First decision made 19 February 1999

Manuscript accepted 12 March 1999