Diet overlap among three sympatric African annual killifish species *Nothobranchius* spp. from Mozambique

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The diet patterns of three *Nothobranchius* species (*N. furzeri*, *N. orthonotus* and *N. rachovii*), small, short-lived annual killifish from temporary pools in African savannah were investigated. Four sites with contrasting fish density and water surface area were sampled in 2008 and 2009 in southern Mozambique. Stomach content analysis showed that all the species examined were generalists, with diets largely based on aquatic invertebrates. The same invertebrate prey categories were consumed by all three species, but their relative proportions varied across species. The largest species, *N. orthonotus*, showed the most distinct diet and consumed vertebrates (juvenile lungfish *Protopterus annectens* and larval Amphibia) and a relatively high proportion of Odonata, Coleoptera and Ephemeroptera larvae. The diet of the other two species (*N. furzeri* and *N. rachovii*) showed a stronger overlap, did not include vertebrates, but was rich in small crustaceans (Cladocera, Copepoda, Ostracoda and Conchostraca). Mosquito (Diptera) larvae formed only a negligible part of the diet of all the three species.
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Key words: coexistence; food niche; niche partitioning; similar morphology.

INTRODUCTION

The genus *Nothobranchius* (Cyprinodontiformes, Nothobranchiidae) consists of *c*. 70 small (typically standard length, L_S, 40–70 mm) species with striking sexual dichromatism; males are brightly coloured while females are dull (Neumann, 2008). The genus occurs in temporary pools throughout east Africa. Their life cycle is annual; the eggs that were laid on the substratum during the previous wet season hatch at the start of the rainy season. The fishes grow rapidly and reach sexual maturity in <1 month (Wildekamp, 2004; Neumann, 2008; Watters, 2009*a*). Their life span is restricted to a few months due to relatively rapid habitat desiccation and *Nothobranchius furzeri* Jubb is one of the vertebrates with the shortest life span, with some populations surviving only 9 weeks post-hatch (Valdesalici & Cellerino, 2003).

Nothobranchius spp. possess several characteristics making them extremely valuable in several branches of biological and biomedical research (Wildekamp, 2004).

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For example, the existence of three diapauses (a physiological state of dormancy with specific triggering and releasing conditions) during their embryogenesis makes them an ideal subject for developmental studies (Markofsky & Matias, 1977; Levels *et al.*, 1986). In toxicological research, *Nothobranchius* spp. were recognized as a useful tool for acute toxicity tests due to their sensitivity to toxic compounds and ability to be maintained at the diapause stage for prolonged periods without laboratory testing (Shedd *et al.*, 1999). Recently, *Nothobranchius* spp. (especially *N. furzeri*) have become a well-established model for ageing studies. They represent an excellent model for studying the effects of extrinsic mortality rate on the evolution of senescence (Genade *et al.*, 2005; Valenzano *et al.*, 2006; Terzibasi *et al.*, 2009). Finally, they are valuable models for ecological and evolutionary studies. The sympatric occurrence of several species and colour morphs (Reichard *et al.*, 2009) and the ease of their reproduction in captivity have stimulated the use of the *Nothobranchius* fishes as model systems for studies on sexual selection (Haas, 1976; Polačik & Reichard, 2009) and speciation (Reichard & Polačik, 2010).

Despite their extensive use and emerging new perspectives for research in *Nothobranchius* spp., there is a lack of basic information on the biology of wild populations in the scientific literature. Indeed, with the exception of Reichard *et al.* (2009), the only available information on distribution, habitat characteristics and colour morphs comes from a hobbyist literature (Nagy, 2009; Watters, 2009*a*, *b*). In sharp contrast with the intensive study of laboratory populations, most of the fundamental questions regarding the life history of *Nothobranchius* spp. in the wild (*e.g.* timing of hatching, growth rate, fecundity and natural lifespan) remain unresolved. Hitherto, there has been no quantitative study on the diet of any *Nothobranchius* species in the wild. Anecdotal evidence in hobbyist literature and an unpublished World Health Organization report on malaria control refer to *Nothobranchius* spp. as predators of smaller water organisms including mosquito and chironomid larvae (Vítek & Kadlec, 2001; R. H. Wildekamp, pers. comm.).

In this study, diet patterns of three *Nothobranchius* species that are widely used in experimental research on ageing and evolutionary ecology were investigated. Specifically the diets of *N. furzeri*, *Nothobranchius orthonotus* (Peters) and *Nothobranchius rachovii* Ahl from four sites and sympatric occurrence in southern Mozambique were compared. The species are morphologically similar, with marginal differences in body size (Skelton, 2001). The distribution of the three species overlaps in the range of *N. furzeri* (southern Mozambique) (Reichard *et al.*, 2009). The range of *N. orthonotus* and *N. rachovii* is larger and stretches from the area north of the Zambezi River in Mozambique to Kruger National Park in South Africa (Wildekamp, 2004; Watters, 2009*b*).

The aims of the present study were 1) to describe prey availability, diet patterns and prey selectivity in *N. furzeri*, *N. orthonotus* and *N. rachovii* in their natural habitats and 2) to test the hypothesis that despite their morphological similarities, their food niches are partitioned when they occur together.

MATERIALS AND METHODS

Samples were collected in Gaza Province in south-western Mozambique. In total, four sites with sympatric occurrence of *Nothobranchius* species were sampled (Table I). Site 1 (24° 3·8′ S; 32° 43·9′ E) and site 3 (23° 18·4′ S; 32° 32·1′ E) were situated in the Limpopo River basin
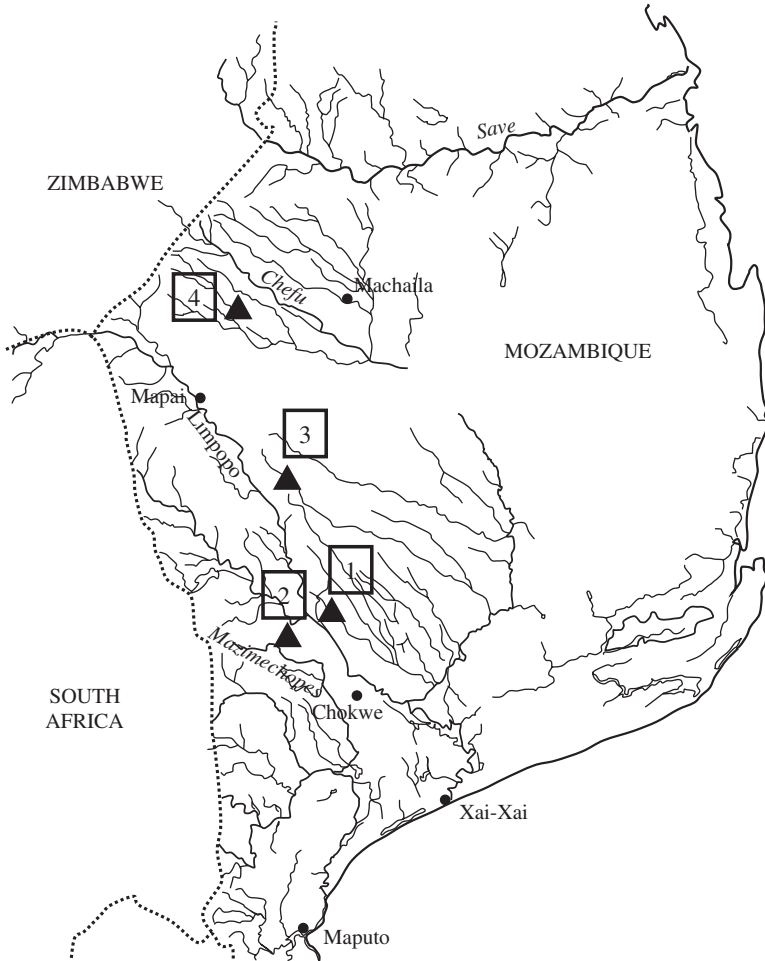
Fig. 1. Map of southern Mozambique showing the river network and important settlements. Sample sites (1 to 4) are indicated by triangles. Note that many rivers are temporary and may not form flowing sections every rainy season. International borders are indicated (·······). Named rivers are shown in italics.

and were sampled in February 2008 (second half of the rainy season). Site 2 (24° 11·1′ S; 32° 21·3′ E) situated in the Mazimechopes River basin and site 4 (22° 21·7′ S; 32° 19·9′ E) in the Chefu River basin (Fig. 1) were sampled in February 2009. All three *Nothobranchius* species co-occurred at sites 1, 2 and 3. At site 4 only *N. furzeri* and *N. rachovii* populations were found (Tables I and II). The sites were typical *Nothobranchius* temporary pools. They had muddy substrata and were partly overgrown with littoral and aquatic (mainly *Nymphaea* sp.) vegetation (Reichard *et al.*, 2009). The sites had contrasting water surface area (small at sites 1 and 2, large at sites 3 and 4; Table I) and *Nothobranchius* density (high at sites 1 and 4, low at sites 2 and 3; Table I). Only adult fishes were encountered in all of the sites examined. No species specific habitat use within a pool was identified among *Nothobranchius* spp. and all three species spatially overlapped. The only other fish species present was the lungfish *Protopterus annectens* Owen that occurred in low numbers at sites 1, 2 and 3.

Population samples of *N. furzeri*, *N. orthonotus* and *N. rachovii* were collected using a triangular dip-net (450 mm × 450 mm and mesh-size 5 mm) and seine (length 2·7 m,

TABLE I. Characteristics of the study sites and the *Nothobranchius* spp. community. *Nothobranchius* spp. density was estimated as number of fishes per dip with a standard dip net. Species ratio is expressed as the percentage of sampled fish of each species (*N. furzeri* : *N. orthonotus* : *N. rachovii*), with sample size (all fishes collected at site during quantitative sampling) shown (*N*)

Site	Habitat area (m^2)	Maximum depth (cm)	*Nothobranchius* spp. density (fish dip^{-1})	Species ratio	*N*
1	75	40	0·72	53:37:11	32
2	29	30	0·08	38:29:33	42
3	450	130	0·14	75:13:12	40
4	1500	30	0·65	68:0:32	99

depth 0·7 m and mesh-size 4 mm). The chosen mesh-size was sufficient to capture all *Nothobranchius* fishes present at sampling sites. The number of individuals analysed per site and species varied from seven to 13 (typically 12; Table II) and was chosen haphazardly from a total sample of fish of the particular species collected at a given site if more than the required number of fish was collected. The sample size varied slightly among species and sites (Table II) as it was not always possible to capture all three species in sufficient numbers. *Nothobranchius furzeri* was typically the dominant species, with fewer individuals of the other two species present (Reichard *et al*., 2009). The sex ratio was kept equal as far as possible, although female-biased sex ratios are a typical characteristic of *Nothobranchius* populations (Reichard *et al*., 2009). In general, *N. orthonotus* had the largest body size, followed by *N. furzeri* and *N. rachovii* (Table II). All species examined had the highest mean standard length (L_S) at site 3 (large pool, low fish density), but otherwise there was no consistent pattern in mean L_S across species and sites. Fish community characteristics are shown in Table I.

Immediately after capture, fishes were killed using an overdose of clove oil and fixed in 4% formaldehyde. In the laboratory, fishes were measured (to the nearest 0·1 mm) and weighed (0·1 g). The gut and its contents were removed from the preserved fishes and prey items recovered from the gut were grouped according to their taxonomic category (order or family

TABLE II. Summary of samples dissected for diet analysis. The number of fish analysed (*N*), their sex ratio (male:female), mean ± S.D. standard length (L_S) and total mass (M_T) are shown for each species

	Site	*N*	Sex ratio	Mean ± S.D. L_S (mm)	Mean ± S.D. M_T (g)
Nothobranchius furzeri	1	12	6:6	31·5 ± 2·5	0·8 ± 0·2
	2	12	6:6	33·7 ± 4·1	1·1 ± 0·4
	3	13	9:4	41·3 ± 4·6	2·0 ± 0·8
	4	10	5:5	31·5 ± 3·9	0·9 ± 0·4
Nothobranchius orthonotus	1	12	6:6	43·0 ± 6·7	2·0 ± 1·3
	2	12	6:6	40·4 ± 3·7	1·6 ± 0·4
	3	11	2:9	46·2 ± 3·4	2·7 ± 0·8
Nothobranchius rachovii	1	7	6:1	30·3 ± 1·8	0·7 ± 0·1
	2	12	4:8	24·8 ± 2·4	0·4 ± 0·1
	3	10	5:5	36·4 ± 2·5	1·3 ± 0·3
	4	10	5:5	26·0 ± 2·4	0·5 ± 0·1

level). Since the goals were to obtain a realistic estimate of prey importance (the mass of a given prey item in the gut of a given individual or population) and prey selectivity (numerical proportion of prey in the fish diet relative to its proportion in the environment), the analysis of gut contents was treated in two ways. First, to avoid overestimation of the importance of small but numerous prey items (Wallace, 1981), the percentage contribution of all taxonomic categories found in the gut to the entire gut content was estimated visually (Marrero & Lopez-Rojas, 1995; McMahon *et al.*, 2005). Second, to collect relevant data for prey selectivity index calculation, all prey items dissected from the gut were counted. The relativized index E^* of Vanderploeg & Scavia (1979), shown by Lechowicz (1982) to be superior to other methods, was used for assessing prey selectivity. The E^* was calculated as: $E_i^* = (W_i - n^{-1})(W_i + n^{-1})^{-1}$, where n is the number of prey types available and W_i is calculated by: $W_i = (r_i p_i^{-1}) \sum [(r_i p_i^{-1})]^{-1}$, where r_i and p_i are the percentage of prey type i in the diet and in the environment, respectively. The E^* ranges from -1 to 1. In theory, negative values indicate avoidance, positive values indicate selection and 0 represents no selection. It should be noted, however, that the maximum preference 1 can be attained only under unrealistic conditions where $r = 1$, $p = 0$ and the number of food types is infinite (Lechowicz, 1982). Hence, even a negative value may in fact represent a positive selection depending on the relative comparison with values for the remaining prey categories. Occasionally, a prey category occurred in the diet, but not in the corresponding invertebrate sample or *vice versa*. In such cases, when the prey comprised >5% of the diet, the selectivity value was set to 1 (Nunn *et al.*, 2007). Correspondingly, when the percentage of a (potential) prey in an invertebrate sample was >5% and this prey was not consumed, the selectivity value was set to -1. In cases when either the percentage of consumed prey not detected in the invertebrate sample comprised <5% of the diet or the percentage of unconsumed prey was <5% in the invertebrate sample, the selectivity value was set to 0. All such cases are well indicated. The same person analysed all samples.

A representative sample of prey availability (aquatic invertebrates present in different pools) was also taken at each site at the time fishes were collected. Given that the habitats sampled were rarely deeper than 40 cm, a rectangular hand-net (20 cm × 15 cm, 500 μm mesh-size) was used for qualitative sampling of benthic and pelagic invertebrates using kick sampling (Frost, 1971). After sampling, the entire contents of the hand-net were fixed in 4% formaldehyde. In the laboratory, invertebrates were separated from the substratum and organic debris, sorted into taxonomic categories (order or family level) and counted. The percentage of each taxonomic group was calculated, based on the sum of all invertebrates in the sample. Due to their ecologically similar lifestyles, Conchostraca and Ostracoda were pooled. Similarly, the ecologically similar larvae of Ceratopogonidae and Chironomidae were pooled in the analysis (Allan *et al.*, 1995; Meintjes, 1996).

Schoener's index (C) (Schoener, 1970) was used to evaluate food niche overlap between the *Nothobranchius* species. The index was calculated as: $C = 1 - 0{\cdot}5 \left(\sum |P_{xi} - P_{yi}| \right)$, where P_{xi} is the mean proportion of the biomass (Wallace, 1981) of food item i used by *Nothobranchius* species x and P_{yi} is the mean proportion of the biomass of food item i used by *Nothobranchius* species y. Zero value indicates no overlap, while 1 represents complete overlap. The index value is generally considered biologically significant when it exceeds 0·6 (Zaret & Rand, 1971; Wallace, 1981).

Non-metric multidimensional scaling (nMDS) was used to investigate interspecific differences in diet composition and diet overlap among the fish species. A matrix of Bray–Curtis indices of dissimilarity was constructed using faunal composition of the diet of each individual, subjected to two-dimensional solution (two dimensions were the best solution of data matrix based on scree plots) *via* principal component analysis (PCA) in nMDS and the two dimensions were plotted. The nMDS is an explanatory method and does not include any direct tool for statistical testing. Hence, scores for individual species on the first dimension of the nMDS-reduced space were tested using ANOVA (for normally distributed samples) or the Kruskall–Wallis test. Only the first dimension was tested because raw stress values of the first dimension were always at least twice as high as for the second dimension. The nMDS analysis was performed using Statistica 9.0 (www.statsoft.com).

RESULTS

In total, 11 invertebrate taxonomic categories were found in the samples of prey availability (Table III). The number of prey categories varied from 11 at site 3 to five at site 4. The sites differed considerably in terms of the relative proportions of prey categories. Crustaceans (including Cladocera, Copepoda, Ostracoda and Conchostraca) were abundant at all sites. Insect larvae were less abundant, with the exception of a high proportion of odonatan larvae at site 3 (Table III). Notably, culicid larvae occurred in particularly low abundance. Other prey categories occurred at most sites, though in varying proportions (Table III).

The gut content analysis showed that all three species were generalists. Their diet was mostly composed of aquatic invertebrates, both benthic and pelagic. Indeed, the entire spectrum of invertebrates detected in the pools was used at least as sporadic prey. In addition, terrestrial insects were occasionally found in the guts. *Nothobranchius orthonotus* was the only species whose diet included vertebrates, namely larval anurans and juvenile *P. annectens*. (Table IV).

The importance of individual prey categories (in terms of their proportion in the diet and frequency of occurrence) for each *Nothobranchius* species varied markedly between sites (Table IV). In some cases, the exploitation of a given prey category was variable despite its relatively stable proportion in the environment. For example, hemipterans were frequently consumed by *N. orthonotus* at site 2, but not at sites 1 and 3 (Tables III and IV). In other cases, the consumption of particular prey appeared to be linked to its abundance in the environment, *e.g.* odonatan larvae were consumed by *N. orthonotus* and *N. furzeri* when they were abundant in the pool (Tables III and IV).

Food selectivity indices indicated that *N. furzeri* and *N. rachovii* preferred to feed on small crustaceans (Cladocera, Copepoda, Ostracoda and Conchostraca). In contrast, *N. orthonotus* showed a preference for coleopteran and odonatan larvae.

TABLE III. Relative percentages of invertebrate taxonomic categories collected at respective sites sampled for fish stomach contents analysis. Taxonomic categories are grouped with respect to their ecological similarity; each ecological category is separated by a broken line

Invertebrate taxon	Site 1	Site 2	Site 3	Site 4
Cladocera	3·0	1·8	7·2	50·0
Copepoda	0·7	1·4	2·2	20·8
Ostracoda*	68·9	82·8	20·9	12·5
Culicidae l	0·7	—	0·7	—
Coleoptera l	2·2	—	3·6	—
Ephemeroptera l	—	2·7	1·4	—
Odonata l	5·9	0·5	39·6	—
Coleoptera ad	3·0	—	5·8	—
Hemiptera	3·7	3·6	7·9	—
Chironomidae l**	—	5·4	0·7	4·2
Oligochaeta	11·9	1·8	10·1	12·5

*Also includes Conchostraca.

**Also includes Ceratopogonidae.

TABLE IV. Mean percentage (*m*) and frequency of occurrence (*f*) of prey items in the guts of *Nothobranchius furzeri* (Nf), *Nothobranchius orthonotus* (No) and *Nothobranchius rachovii* (Nr)

| | Site 1 | | | | | | Site 2 | | | | | |
| | Nf | | No | | Nr | | Nf | | No | | Nr | |
Prey category	*m* (%)	*f* (%)	*m* (%)	*f* (%)	*m* (%)	*f* (%)	*m* (%)	*f* (%)	*m* (%)	*f* (%)	*m* (%)	*f* (%)
Cladocera (cla)	12·3	83·3	0·2	8·3	4·0	71·4	24·5	100·0	12·2	83·3	24·1	83·3
Copepoda (cop)	3·0	50·0	0·3	8·3	4·1	100·0	1·3	33·3	—	—	6·5	83·3
Ostracoda* (ost)	71·6	100·0	12·3	50·0	76·1	100·0	11·2	83·3	2·7	50·0	10·1	75·0
Chaoboridae l (cha)	—	—	—	—	—	—	—	—	—	—	—	—
Chironomidae p (chP)	0·2	8·3	0·8	8·3	—	—	5·4	33·3	7·1	8·3	—	—
Culicidae l (cuL)	—	—	—	—	—	—	1·7	8·3	—	—	—	—
Coleoptera l (coL)	—	—	0·8	16·7	10·0	42·9	19·5	75·0	5·4	50·0	28·7	75·0
Ephemeroptera l (eph)	0·4	8·3	1·3	16·7	—	—	12·5	58·3	14·6	66·7	23·6	58·3
Odonata l (odL)	12·3	16·7	48·7	58·3	0·0	0·0	0·0	0·0	1·7	16·7	3·3	8·3
Coleoptera ad (coA)	—	—	—	—	—	—	—	—	—	—	—	—
Hemiptera (hem)	—	—	—	—	4·3	14·3	9·0	58·3	53·5	100·0	—	—
Chironomidae l** (chL)	0·2	16·7	—	—	0·7	14·3	9·2	58·3	2·5	33·3	2·9	25·0
Oligochaeta (oli)	—	—	—	—	—	—	—	—	—	—	—	—
Gastropoda (gas)	—	—	5·0	8·3	—	—	—	—	—	—	—	—
terrestrial insects (teI)	—	—	7·5	8·3	0·7	14·3	5·8	8·3	0·4	8·3	0·8	8·3
Amphibia (amp)	—	—	6·6	8·3	—	—	—	—	—	—	—	—
Protopterus annectens (pro)	—	—	16·6	16·7	—	—	—	—	—	—	—	—

TABLE IV. Continued

Prey category	Site 3						Site 4			
	Nf		No		Nr		Nf		Nr	
	m (%)	f (%)	m (%)	f (%)	m (%)	f (%)	m (%)	f (%)	m (%)	f (%)
Cladocera (cla)	2·8	23·1	0·0	0·0	0·7	33·3	49·5	100·0	61·3	90·0
Copepoda (cop)	2·0	30·8	0·5	8·3	5·5	66·7	3·6	90·0	2·2	80·0
Ostracoda* (ost)	41·3	100·0	0·9	25·0	69·2	100·0	24·0	100·0	19·1	70·0
Chaoboridae l (cha)	—	—	—	—	9·2	40·0	—	—	—	—
Chironomidae p (chP)	—	—	—	—	—	—	4·0	10·0	—	—
Culicidae l (cuL)	0·4	7·7	—	—	—	—	—	—	—	—
Coleoptera l (coL)	1·3	15·4	8·6	8·3	2·0	6·7	7·9	10·0	—	—
Ephemeroptera l (epL)	4·6	7·7	0·1	8·3	—	—	—	—	—	—
Odonata l (odL)	38·9	69·2	49·5	66·7	10·7	46·7	0·0	0·0	0·0	0·0
Coleoptera ad (coA)	1·5	7·7	—	—	—	—	—	—	—	—
Hemiptera (hem)	0·4	7·7	—	—	—	—	—	—	—	—
Chironomidae l** (chL)	3·3	30·8	0·3	8·3	1·9	40·0	10·0	30·0	16·0	60·0
Oligochaeta (oli)	—	—	0·5	8·3	—	—	1·0	10·0	1·5	20·0
Gastropoda (gas)	—	—	—	—	—	—	—	—	—	—
Terrestrial insects (teI)	3·5	15·4	0·2	8·3	0·8	13·3	—	—	—	—
Amphibia (amp)	—	—	24·2	25·0	—	—	—	—	—	—
P. annectens (pro)	—	—	15·2	16·7	—	—	—	—	—	—

*Also includes Conchostraca.
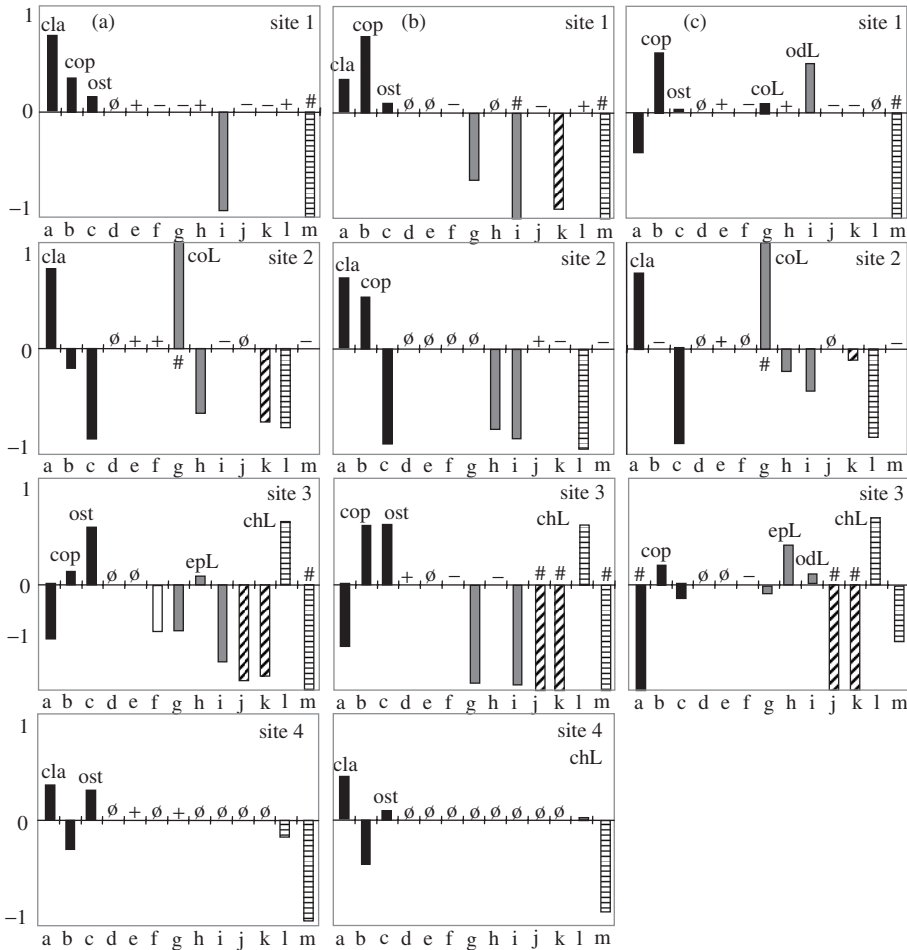**Also includes Ceratopogonidae. ad, adult; l, larvae; p pupae.

FIG. 2. Vanderploeg's prey selectivity indices for 13 prey categories found in the guts of (a) *Nothobranchius furzeri*, (b) *Nothobranchius orthonotus* and (c) *Nothobranchius rachovii*. Prey categories are grouped with respect to their ecological similarity (■, small planktonic and epibenthic crustaceans; □, pelagic insect larvae (stages); ▨ grey, epibenthic insect larvae; ▨, actively swimming coarse insects; ▤, substratum dwellers). Positively selected prey items are marked with prey: cla, Cladocera; cop, Copepoda; ost, Ostracoda; coL, Coleoptera larvae; odL, Odonata larvae; epL, Ephemeroptera larvae; chL, Chironomidae larvae. Some positions are marked for further specification; prey items neither detected in invertebrate sample nor consumed by fishes (Ø), prey item consumed by fish (and forming <5% of the diet) but not detected in the invertebrate sample of prey availability (+), prey items detected in the invertebrate sample (comprising <5%) but not consumed by fishes (−), prey items arbitrarily set +1 (>5% in the diet but lacking in prey availability samples) or −1 (>5% in the prey availability samples but lacking in the diet) (#). Prey items are indicated as: a, Cladocera; b, Copepoda; c, Ostracoda; d, Chaoboridae larvae; e, Chironomidae pupae; f, Culicidae larvae; g, Coleoptera larvae; h, Ephemeroptera larvae; i, Odonata larvae; j, Coleoptera adult; k, Hemiptera; l, Chironomidae larvae; m, Oligochaeta.

There was a general avoidance of adult coleopterans and oligochaetes by all three fish species and avoidance of hemipterans by *N. furzeri* and *N. rachovii* (Fig. 2).

There was a clear food niche separation between *N. orthonotus* and the other two species as revealed by nMDS and ANOVA (Fig. 3 and Table V). Food niche overlap

TABLE V. Differences among the diets of *Nothobranchius furzeri* (Nf) *Nothobranchius orthonotus* (No) and *Nothobranchius rachovii* (Nr) populations, with results of ANOVA tests for dimension 1 on nMDS-reduced space and Schoener's niche overlap indices. d.f., *F*-values and statistical significance (*P*) are shown for ANOVA tests. Significantly different pair-wise comparisons between species (*H*-test for unequal *N*) are indicated (*P < 0·05; ***P < 0·001; NS, not significant) and the values of Schoener's indices are given in parentheses

Site	d.f.	*F*	*P*	No-Nf		No-Nr		Nr-Nf	
1	2,28	1·2	***	***	(0·26)	***	(0·14)	NS.	(0·79)
2	2,33	4·6	***	*	(0·50)	***	(0·39)	NS	(0·71)
3	2,36	29.0	***	*	(0·42)	***	(0·14)	NS	(0·59)
4	1,18	3·8	NS						(0·82)

was consistently lower between *N. orthonotus* and *N. furzeri* and *N. orthonotus* and *N. rachovii* than between *N. furzeri* and *N. rachovii* as shown by Schoener's *C* values which were highest for the *N. furzeri* and *N. rachovii* comparisons at all investigated sites (Table V). The frequent utilization of large prey items (*e.g.* vertebrates) and hard-bodied insects and avoidance of very small prey (crustaceans) by *N. orthonotus* accounted for the difference (Table IV). The difference in the diet between *N. furzeri* and *N. rachovii* was less clear, but at sites 2 and 3 (with low fish density) niche separation was visually detectable (Fig. 3) and the difference was statistically significant at site 3 (ANOVA; Table V). In general, *N. furzeri* fed on larger and relatively hard-bodied prey (*e.g.* odonatan larvae; Table III) more often than *N. rachovii*. The larger and hard-bodied prey was typically avoided by *N. rachovii*, which instead consumed fine and small prey (*e.g.* Copepoda) at a higher frequency (Table IV).

DISCUSSION

All three *Nothobranchius* species were generalists. *Nothobranchius orthonotus*, the largest of the three species, showed the most distinct diet, with a consumption of larger prey items, juvenile *P. annectens*, anuran larvae, odonatan larvae and hemipterans. The diets of the other two species (*N. furzeri* and *N. rachovii*) were less separated and they fed on small crustaceans (Cladocera, Copepoda, Ostracoda and Conchostraca). The food niche separation was clearer at sites with low fish density than at sites with high fish density.

The generalist feeding strategy is an expected life history trait of *Nothobranchius* spp., given that they inhabit ephemeral habitats with variable conditions (Laufer *et al*., 2009; Watters, 2009*a*; R. H. Wildekamp, pers. comm.). *Nothobranchius* spp. habitats vary from non-vegetated, shallow, soft-substratum pools with very turbid water, to densely overgrown, relatively deep pools with clear water and sandy substrata (Reichard *et al*., 2009; Watters, 2009*a*, *b*). Temporary pools in southern Africa host a high abundance and diversity of aquatic invertebrates (Allan *et al*., 1995), but colonization of a pool is a partly stochastic process (Allanson *et al*., 1990). Hence, given the random component of prey available to each fish generation (*i.e.* each year) and variability among spatially close sites (with possible dispersal during floods in
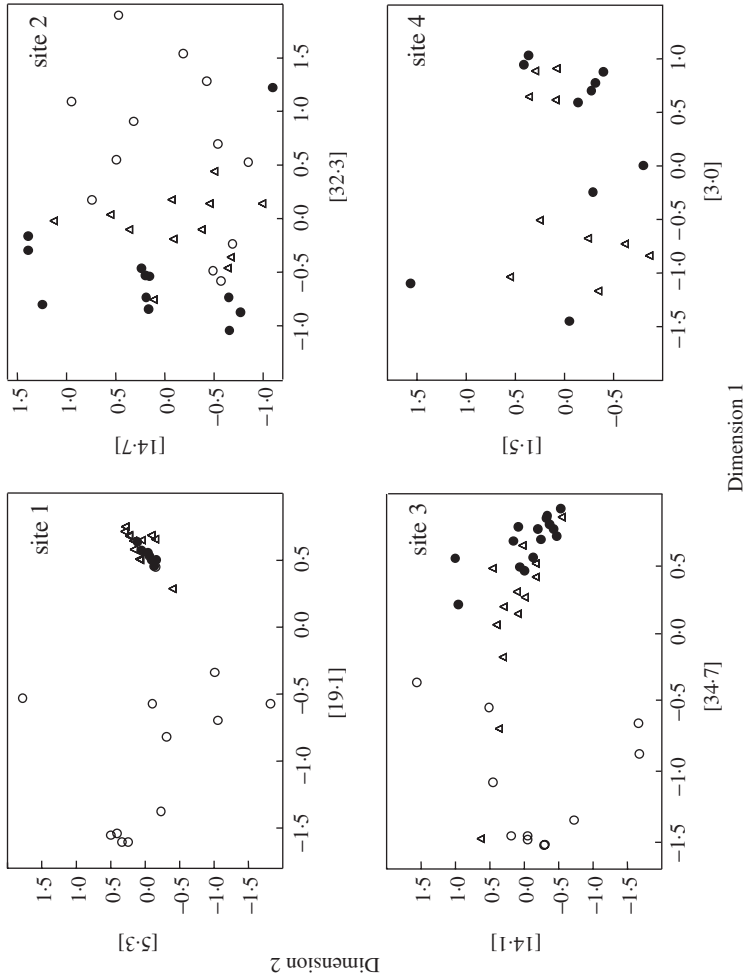
FIG. 3. Two-dimensional projection of diet similarity among *Nothobranchius* spp. individuals from nMDS analysis based on Bray–Curtis index, with *N. furzeri* (△), *N. orthonotus* (○) and *N. rachovii* (●) indicating individual fish. Raw stress values are shown for each dimension.

the rainy season), any diet specialization is likely to incur significant costs due to wide interannual and spatial variation in prey availability.

Prey selectivity is clearly a relative phenomenon since it is partly determined by availability of other prey items, as postulated by optimal foraging theory (Pyke *et al.*, 1977). This may explain the difference in the frequency of consumption of particular prey types. For example, hemipterans were more frequently consumed by *N. orthonotus* (100% frequency of occurrence, mean of 54% of gut contents) and *N. furzeri* (58% frequency of occurrence, mean of 9% of gut contents) at site 2 compared to sites 1 and 3 where hemipterans formed similar proportions of available prey as at site 2 (Tables III and IV). Presumably, hemipterans as hard-bodied prey were consumed only when alternative preferred prey was not available. This is confirmed by the observation that hemipterans are rarely eaten by captive *Nothobranchius* spp., including wild fishes captured at the field sites and during transport when they readily fed on other prey (chironomid larvae). Even if hemipterans were consumed, they were often regurgitated, which is otherwise very uncommon in *Nothobranchius* fishes (M. Polačik, pers. obs.).

Food selectivity indices showed consistency among sites for ecologically similar taxonomic groups. Aquatic insect larvae (mainly coleopterans, odonatans and ephemeropterans) were positively selected by *N. orthonotus*. Such a diet may be profitable in terms of trade-offs between item size, its body hardness and ease of catching. *Nothobranchius furzeri* and *N. rachovii* selected crustaceans, including both pelagic Copepoda and Cladocera and benthic and epiphytic Ostracoda and Conchostraca. This preference is less obvious in terms of favourable intake:output ratios since these organisms are small and provide little energy obtained per individual attack. A possible explanation may be their high abundance in African temporary pools (Meintjes, 1996), which significantly reduces foraging costs. The abundance of crustaceans in the diet matches their relative proportion in prey communities at sites 1, 2 and 4. At site 3 where the proportion of crustaceans was relatively low, *N. furzeri* and *N. rachovii* also consumed a high proportion of chironomid larvae. Alternatively, positive values of selectivity indices might also be a consequence of possible underestimation of crustacean abundance caused by the relatively large mesh-size of the net used for invertebrate samplings (Hwang *et al.*, 2007) and patchiness in zooplankton distributions at a small spatial scale (Cryer & Townsend, 1988; Kuczynska-Kippen, 2008). This could account for a possible bias in the estimates of relative abundance. Even if the actual magnitude of preference was lower, however, small crustaceans clearly dominated the diet of *N. furzeri* and *N. rachovii*.

Nothobranchius fishes are thought to prey on mosquito larvae and attempts have been made to use them for mosquito control (Skelton, 2001; R. H. Wildekamp, pers. comm.). In this study, the proportion of mosquito larvae in the diet of all three studied species was negligible (Table IV). They also comprised only a small portion of the invertebrate community in the pools (Table III), as reported also for the *Nothobranchius* habitats in Somalia (R. H. Wildekamp, pers. comm.). During the present survey, however, a high abundance of mosquito larvae, even in pools with no fishes, were never found. It can be hypothesized that mosquito larvae are not a typical component of *Nothobranchius* spp. habitats, at least in the study area comprising the savannah region of southern Mozambique with only sparse human settlement. Mosquito larvae may be more common in the habitats of *Nothobranchius* spp. elsewhere in east Africa, especially at sites near human settlement, or may fluctuate

in abundance during different succession stages of a temporary pool. Consequently, while the present study does not confirm that mosquito larvae are an important component of the diet of *Nothobranchius* spp., it cannot refute that they may be consumed by *Nothobranchius* spp. elsewhere.

The competitive exclusion principle (Gause, 1934) predicts niche separation among the three studied sympatric *Nothobranchius* species. Given the lack of any substantial meso and microhabitat segregation among the species studied (Reichard *et al.*, 2009), the difference in body size and partly also in morphology is the most likely mechanism for the observed food niche separation between *N. orthonotus* and the other two species. The diet of *N. orthonotus* differed significantly from the other two *Nothobranchius* species at each site, regardless of fish density or water surface area. *Nothobranchius orthonotus*, feeding on juvenile *P. annectens*, anuran larvae and coarse aquatic insects, is typically larger than *N. furzeri* and *N. rachovii* (Table II; Wildekamp, 2004). It is also characterized by a wider gape and more posterior position of the smaller anal and dorsal fins (Huber, 2000; Wildekamp, 2004), a morphological feature typical of predators. *Nothobranchius furzeri* and *N. rachovii* are of similar size and morphology. Their diets were similar, too, and differed significantly only at one of the four sites (site 3; Table V), a large pool with low fish density and the highest invertebrate diversity (Tables I and III). This is in contradiction to generally accepted theory of niche contraction with increasing interspecific competition (Zaret & Rand, 1971; Bouton *et al.*, 1997). It appears that *N. furzeri* and *N. rachovii* were able to choose their preferred diet under low competition conditions, but fed opportunistically at high fish densities, when the competition was higher and the fish tended to consume any available prey (Corrêa *et al.*, 2009).

The *Nothobranchius* community studied was not characterized by predator–prey relationships among particular species, in contrast to a comparable community of South American annual killifishes (Laufer *et al.*, 2009). No *Nothobranchius* spp. were found in the diet of any individual. Interspecific size differences in the study system were probably too small (Table II) to allow predation among sympatric *Nothobranchius* species.

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