



Metazoan parasites of African annual killifish (Nothobranchiidae): abundance, diversity, and their environmental correlates

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ABSTRACT

Estimates of biodiversity and its global patterns are affected by parasite richness and specificity. Despite this, parasite communities are largely neglected in biodiversity estimates, especially in the tropics. We studied the parasites of annual killifish of the genus *Nothobranchius* that inhabit annually desiccating pools across the African savannah and survive the dry period as developmentally arrested embryos. Their discontinuous, non-overlapping generations make them a unique organism in which to study natural parasite fauna. We investigated the relationship between global (climate and altitude) and local (pool size, vegetation, host density and diversity, and diversity of potential intermediate hosts) environmental factors and the community structure of killifish parasites. We examined metazoan parasites from 21 populations of four host species (*Nothobranchius orthonotus*, *N. furzeri*, *N. kadleci*, and *N. pienaar*) across a gradient of aridity in Mozambique. Seventeen parasite taxa were recorded, with trematode larval stages (metacercariae) being the most abundant taxa. The parasites recorded were both allogenic (life cycle includes non-aquatic host; predominantly trematodes) and autogenic (cycling only in aquatic hosts; nematodes). The parasite abundance was highest in climatic regions with intermediate aridity, while parasite diversity was associated with local environmental characteristics and positively correlated with fish species diversity and the amount of aquatic vegetation. Our results suggest that parasite communities of sympatric *Nothobranchius* species are similar and dominated by the larval stages of generalist parasites. Therefore, *Nothobranchius* serve as important intermediate or paratenic hosts of parasites, with piscivorous birds and predatory fish being their most likely definitive hosts.

Key words: distribution patterns; fish intermediate host; habitat variation; Limpopo; Mozambique; *Nothobranchius furzeri*; paratenic host.

COEVOLUTIONARY RELATIONSHIPS HAVE A MAJOR EFFECT ON BIODIVERSITY ESTIMATES AND THEIR SPATIAL PATTERNS. Analysis of host-parasite relationships between vertebrates and their helminth parasites has shown that parasites display two times greater species richness than their hosts (Dobson *et al.* 2008). However, approximating parasite diversity from simple estimates is not straightforward, as parasite diversity fails to comply with the latitudinal gradient of biodiversity (Rohde 1999), perhaps because the coevolutionary framework adds complexity to general macroecological patterns (Poulin 2007). Parasites are also crucial components of biological networks and must be taken into consideration when inferring local ecological patterns and processes (Lafferty *et al.* 2008). Therefore, a better understanding of the role of parasites is crucial to fully understand the relationships in biological communities.

Fish parasites have been used as model groups to study macroecological patterns and diversity across spatial scales (Poulin 2007). At the global scale, helminth parasite communities in tropical freshwater fish are less species rich than those of temperate fish (Choudhury & Dick 2000). However, at the local scale, the role of environmental factors in the diversity, structure, and connectivity of parasite communities is largely unknown (Lima *et al.* 2012). Annual

killifish of the genus *Nothobranchius* (Cyprinodontiformes, Nothobranchiidae) are small fishes that inhabit annually desiccating savannah pools in east Africa (Wildekamp 2004). The ephemeral nature of their habitat has selected for an extremely short natural lifespan. At the beginning of the rainy season, the pools fill with rainwater and the fish hatch from desiccation-resistant eggs, grow rapidly, and become sexually mature in a few weeks (Blažek *et al.* 2013). Adult fish live only a few months, and all adults die when the pool desiccates. Only eggs survive the dry period buried in the dry sediment (Reichard 2015). Several species of *Nothobranchius* often co-inhabit the same savannah pool (Reichard *et al.* 2009). Hatching is synchronous among and within *Nothobranchius* species, with a single age cohort (Polačik *et al.* 2011). The diet of co-occurring species is largely overlapping (Polačik & Reichard 2010, Polačik *et al.* 2014a), and their spatial segregation within and among pools is negligible (Reichard *et al.* 2009). Other aspects of their ecology are also relatively well known (Reichard 2015, Cellerino *et al.* 2016), making this group of fish a useful model for ecological research. In this study, we built upon current knowledge of *Nothobranchius* ecology to investigate the natural parasite fauna using the metazoan parasite communities of 21 wild populations from four *Nothobranchius* species.

Similar parasites may be found in sympatric, closely related host species (Marques *et al.* 2011) such as in co-occurring cheilinin fishes (*Cheilinus chlorourus*, *C. trilobatus*) from the Great Barrier

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Reef (Muñoz & Grutter 2006). The life cycle of *Notobrancheius* does not allow for transmission of parasites between generations, while transmission of parasites from other fish hosts is very limited. Occasionally, other fish species can colonize the ephemeral pools during occasional floods that may connect the isolated pools with non-desiccating river and stream systems.

The parasite community structure depends on the local ecological setting (e.g., diversity, structure, and abundance of vegetation) and environmental conditions, the presence of potential intermediate and reservoir hosts, and host population structure (e.g., density). The four killifish species in this study are largely sympatric across a large part of southern and central Mozambique, a region that experiences a steep gradient in aridity. While the coastal region receives over 1000 mm of rainfall per year, the inland region may receive <400 mm. Rainfall in the dry region is also less predictable within and between years (Terzibas Tozzini *et al.* 2013, Polačik *et al.* 2014b). This altitudinal gradient of aridity (a shorter and less predictable rainy season and lower total annual precipitation with increasing altitude) is further enhanced by a latitudinal cline, with a further decrease in rainfall with increasing latitude (Fig. S1). Consequently, the environmental characteristics of *Notobrancheius* habitats vary along the climatic cline, which may factor into their parasite community structure (Moyer *et al.* 2002, Jex *et al.* 2007). Across their range, killifish inhabit pools that vary in size, structural heterogeneity (such as presence of vegetation), and community composition, including the presence of potential predators (Reichard *et al.* 2009, 2014, Reichard 2010). These local factors do not vary predictably across the gradient of aridity (Reichard *et al.* 2014). Thus, characteristics of the local habitat may structure parasite communities (Brouat *et al.* 2007) and override any clinal variation at a larger scale.

Disentangling the role of environmental and ecological factors on parasite load and parasite community structure is challenging due to the diffuse structure of most host populations. Migration and dispersal may mask the patterns arising from non-random parasite acquisition in different local habitats and populations (Poulin 2007). African annual killifish occur in isolated pools across a range of environmental conditions, which is an advantage for the study of parasite communities. Given that they contain only a single generation, examination of their parasites may provide direct insights into how variability in habitat conditions affects parasite communities. However, polyxenous parasites (with more than one host in their life cycle) may be dispersed by their intermediate, reservoir, or final hosts, and thus are not constrained by limited dispersal in *Notobrancheius* hosts. Especially, in allogenic species (life cycle includes a non-aquatic host), where definitive hosts such as birds and mammals are not limited by land barriers between water bodies, fish hosts may share parasites independently of connection between their localities, homogenizing parasite diversity (Karvonen *et al.* 2005). Similarities in parasite load and community structure should decay with geographic distance when only local dispersal is possible (Poulin & Morand 1999).

The aim of this study was to describe the metazoan parasite communities of four annual killifish species. Specifically, we compared parasite communities between and within host species and

tested the role of environmental factors on parasite community structure. We tested the following hypotheses:

1. Parasite communities do not differ between host killifish species living in sympatry. There are two main processes through which fish can be infected: either through direct contact or through dietary transmission. Because there is almost complete dietary overlap between the host species studied (Polačik *et al.* 2014a), all species prey on both benthic and limnetic prey (Polačik & Reichard 2010), and they are not separated into different microhabitats within the pool (Reichard *et al.* 2009), the degree of exposure to parasites should be similar.
2. Most killifish parasites are generalists. Specialist parasites often rely on the stable presence of their hosts in the environment. Annual desiccation of the killifish's environment results in the periodic disappearance of all hosts, limiting opportunities for specialist parasites to persist.
3. Parasite diversity increases from dry to humid regions. This trend will arise due to differences in pool duration, with the wet phase in the dry region (2–5 months) being 50 percent shorter than in the humid region (Terzibas Tozzini *et al.* 2013). As a result, intermediate and final hosts have a longer period of time to develop and transmit parasitic stages in the humid region. Furthermore, annual killifish co-occur with other fish species, more frequently in the humid region (Reichard *et al.* 2009), increasing the potential spectrum of parasite species.
4. Finally, we expect a strong association between the characteristics of parasite communities and environmental factors. Specifically, we predict higher parasite diversity in pools where other fish and invertebrate species are present due to an increase in potential definitive and intermediate hosts. We also predict a positive effect of vegetation and pool size on parasite diversity and abundance.

METHODS

HOST SPECIES.—Host species used were *Notobrancheius furzeri* Jubb, *Notobrancheius orthonotus* (Peters), *Notobrancheius kadleci* Reichard, and *Notobrancheius pienaar* Shidlovskiy, Watters & Wildekamp. *Notobrancheius furzeri* and *N. kadleci* are allopatric, closely related, and ecologically vicariant species (Reichard 2010, Dorn *et al.* 2011). *Notobrancheius orthonotus*, *N. pienaar*, and *N. furzeri* are sympatric and syntopic in southern Mozambique, while *N. orthonotus*, *N. pienaar*, and *N. kadleci* are widely sympatric and syntopic in central Mozambique (Bartáková *et al.* 2015).

STUDY SITES, COLLECTION OF FISH, AND ENVIRONMENTAL PARAMETERS.—This study was conducted in southern and central Mozambique across a steep gradient in aridity (Terzibas Tozzini *et al.* 2013) (Fig. S1). We collected fish at the end of the rainy season in March 2013 when their estimated age was 10–12 weeks, representing fully grown but not senescent adult fish. We sampled fish using a dip net, identified them to the species level, and took them to the laboratory for further examination.

We examined 80 *N. furzeri* from eight populations (mean standard length [SL] 42.2 ± 7.5 mm standard deviation [SD], range 31–65 mm), 77 *N. orthobotus* from eight populations (51.3 ± 9.4 mm, 35–82 mm), 38 *N. kadleci* from four populations (38.8 ± 9.7 mm, 27–67 mm), and 10 *N. pienaari* from a single population (28.6 ± 3.2 mm, 25–35 mm).

At each site, we recorded a range of local environmental and ecological predictors on parasite abundance and community structure: pool area (estimated to the nearest 10 m²), maximum depth (estimated to the nearest 10 cm), water turbidity (three categories: transparent, bottom visible; turbid, visibility 1–10 cm; and very turbid, visibility <1 cm), the presence of vegetation (separately for *Nymphaea* sp.; submerged aquatic vegetation; and grassy littoral vegetation within the pool, including its margins; all visually estimated as percentage cover), and the presence of other fish (*Barbus* spp.; *Tilapia* sensu lato [*Tilapia* sp., *Oreochromis mossambicus* (Peters)]; lungfish, *Protopterus annectens* (Owen); *Clarias gariepinus* (Burchell); recorded as present/absent) and invertebrate taxa (Hemiptera: Belostomatidae, Notonectidae; Odonata; Coleoptera: Dytiscidae, Gyrinidae). Data on fish species presence were expressed as “Fish host diversity” (combining *Nothobranchius* and other fish species), and data on non-vertebrate taxa (Invertebrate diversity) were used as an indicator of potential intermediate host diversity. Finally, we estimated the density of killifish (*Nothobranchius* density) based on semi-quantitative estimates of catch per unit effort data (low, medium, or high fish density, quantified as the number of fish captured per sampling effort).

The Aridity Index (AI) was established at each site and was calculated as the ratio between mean annual precipitation and mean annual potential evapotranspiration based on averaged data from 1950 to 2000 (Trabucco & Zomer 2009). Using the AI and our own data on seasonal patterns in habitat desiccation (Terzibasi Tozzini *et al.* 2013), we divided the study area into three regions based on the expected length of pool duration: dry (AI < 0.3), intermediate (AI = 0.3–0.5), and humid (AI > 0.5) (Fig. S1). Each locality represented a single, isolated pool and was named according to its position on the aridity gradient. This resulted in three localities in the dry region (Dry1–Dry3), five localities in the intermediate region (Med1–Med5), and six in the humid region (Wet1–Wet6).

FISH EXAMINATION AND PARASITE PROCESSING.—In the laboratory, the fish were held in species-specific containers with aerated water and examined within 3 days of capture. The spinal cord was severed behind the head, and the entire fish was examined under a binocular microscope for the presence of metazoan parasites using a standard protocol (Ergens & Lom 1970). All parasites were preserved for further examination and identification according to size and taxonomic classification in either 4% formaldehyde, 70% ethanol, a mixture of glycerin and ammonium picrate (GAP), or a mixture of glycerin and ethanol. A subsample of parasites was stored in 70% ethanol for identification via DNA sequencing. Parasites were subsequently identified by a combination of morphological and molecular methods. See Appendix S1 for information on parasite identification.

Given the large number of larval stages and the lack of known sequences at lower taxonomic resolution on the NCBI data base (BLAST search) for most parasite taxa recovered, reliable identification was often possible only to the family level. Nevertheless, despite our inability to assign exact species name, we were able to separate morphospecies within such families (Table 1).

PARASITE POPULATIONS AND COMMUNITIES.—We calculated ecological descriptors of parasite populations and communities for each host individual/species, for each host population, and across host populations. We estimated the prevalence (the percentage of hosts infected with a particular parasite species), mean abundance (mean number of individuals of a particular parasite species in a sample of a host species, including both infected and uninfected hosts), parasite species richness (the number of parasite taxa in a particular host individual), and the intensity of infection (the number of individuals of a particular parasite species in a host individual). Parasite infrapopulation includes all individuals of a parasite species in an individual host. Parasite infracommunity is a community of parasite infrapopulations in an individual host (Bush *et al.* 1997).

Parasite specificity refers to the restricted range of host species infected by a single species of parasite (Lymbery 1989). Parasite specificity was only determined for pools where multiple killifish species co-occurred (hence, *Emoleptalea* sp., Proteocephalidae, Hymenolepididae, and Glossiphoniidae found at localities with single killifish species were excluded). As we were unable to identify all parasites to the species level, we classified parasite species as apparent specialists (infecting a single killifish species) or generalists (infecting two or more killifish species). In addition, we classified parasites as autogenic (completes its life cycle in the aquatic system) or allogenic (uses at least one non-aquatic host, *e.g.*, a bird or mammal) (Esch *et al.* 1988).

DATA ANALYSIS.—We calculated parasite species richness, parasite abundance, and Brillouin index (a measure of diversity) (Magurran 2004) to measure different aspects of parasite community structure at the infracommunity level. These indices had a Global Variance Inflation Factor (GVIF) <3 and hence were statistically independent (Zuur *et al.* 2010). Finally, we calculated Bray–Curtis matrices of similarity (based on parasite abundance) and the Jaccard index of similarity (based on parasite presence/absence, *i.e.*, ignoring relative abundance) for each pool with more than one killifish species (individual host level) and between all population combinations (host population level).

To investigate variation among host species, we compared all within-pool conspecific and heterospecific pairwise contrasts from the Bray–Curtis dissimilarity matrix using two-way ANOVA (factors: pool identity, heterospecific vs. conspecific contrasts). The Jaccard index returned a qualitatively identical outcome (results not shown). For inter-population comparisons within each host species, we first tested whether there was any spatial autocorrelation in parasite community structure with a Mantel test using the matrices of geographic distance and similarity of parasite

TABLE 1. Prevalence (P), mean abundance (MA), and range of infection intensity for each parasite taxon.

Parasite	Location in/on host	<i>Nothobranchius furzeri</i> (n = 80)			<i>Nothobranchius orthonotus</i> (n = 77)			<i>Nothobranchius kadleci</i> (n = 38)			<i>Nothobranchius pienaari</i> (n = 10)		
		P (%)	MA ± SD	Range	P (%)	MA ± SD	Range	P (%)	MA ± SD	Range	P (%)	MA ± SD	Range
Trematoda													
Diplostomidae 1 (mtc)	Muscle	70	82.61 ± 128.19	1–595	55	65.9 ± 134.67	3–807	32	12.79 ± 23.56	6–107	100	7.5 ± 9.08	2–33
Diplostomidae 2 (mtc)	Muscle	48	6.93 ± 12.05	1–50	29	3.65 ± 9.76	1–66	8	0.13 ± 0.47	1–2	–	–	–
<i>Apatemon</i> sp. (mtc)	Cerebral cavity	9	0.53 ± 1.82	3–9	–	–	–	–	–	–	–	–	–
Echinostomatidae (mtc)	Gills	16	3.05 ± 9.32	4–57	8	4.26 ± 21.28	2–140	–	–	–	–	–	–
Strigeidae 1 (mtc)	Muscle	13	0.45 ± 1.79	1–12	9	0.27 ± 1.12	1–6	–	–	–	–	–	–
Strigeidae 2 (mtc)	Abdominal cavity	10	0.63 ± 2.11	3–13	4	0.1 ± 0.58	1–4	–	–	–	30	1.1 ± 2.13	1–6
Digenea 1 (mtc)	Muscle	–	–	–	3	0.03 ± 0.16	1–1	–	–	–	–	–	–
Digenea 2 (mtc)	Muscle	20	1.69 ± 5.39	1–39	3	0.16 ± 1.01	4–8	–	–	–	–	–	–
<i>Emmoleptalea</i> sp. (ad)	Intestine	13	4.49 ± 15.80	3–80	–	–	–	3	0.13 ± 0.81	5–5	–	–	–
Nematoda													
Camallanidae (larv)	Intestine	40	2.5 ± 5.74	1–30	32	4.3 ± 11.19	1–64	61	9.95 ± 14.52	1–58	40	0.7 ± 1.25	1–4
Gnathostomatidae 1 (larv)	Muscle	23	1.31 ± 5.33	1–45	13	0.53 ± 1.85	1–12	26	2.76 ± 6.59	1–30	10	0.1 ± 0.32	1–1
Gnathostomatidae 2 (larv)	Muscle	1	0.01 ± 0.11	1–1	21	1.99 ± 6.89	1–50	18	0.47 ± 1.18	1–5	–	–	–
Cestoda													
Proteocephalidae (larv)	Abdominal cavity	–	–	–	–	–	–	13	0.42 ± 1.18	2–5	–	–	–
Hymenolepididae (larv)	Abdominal cavity	1	0.03 ± 0.22	2–2	–	–	–	–	–	–	–	–	–
Cestoda 1 (larv)	Gallbladder	11	0.55 ± 2.57	1–21	1	0.01 ± 0.11	1–1	–	–	–	–	–	–
Cestoda 2 (larv)	Abdominal cavity	10	0.4 ± 1.61	1–12	16	1.17 ± 5.84	1–47	–	–	–	30	0.7 ± 1.34	1–4
Hirudinea													
Glossiphoniidae (ad)	Skin	–	–	–	3	0.03 ± 0.16	1–1	–	–	–	–	–	–

mtc, metacercariae; larv, larvae; ad, adult.

communities. Given that host size may confound parasite abundance and richness estimates, we tested the role of host body size on parasite load and parasite species richness using linear mixed models (LMMs), with host body size as the response variable and population identity as a random effect. This analysis was performed in the R environment (R Development Core Team, 2009) using the *lme4* package.

We tested the roles of global (aridity gradient and climatic region) and local (site-specific parameters) environmental and ecological correlates on parasite species richness, parasite abundance, and Brillouin index using LMMs. Given the limited number of host populations examined for each host species, we pooled all host populations (*i.e.*, across host species) into a single

analysis. To account for host species differences and repeated use of the same habitat, we assigned host species and pool identity as non-nested random factors. To avoid collinearity between predictor variables, we calculated the variance inflation factor (VIF) and removed collinear predictors that would bias model estimates (Zuur *et al.* 2010). Strong collinearity was detected between the amount of *Nymphaea* vegetation, submerged vegetation, and water turbidity (with water turbidity negatively associated with the two vegetation types). Therefore, we simplified the matrix by conducting principal components analysis (PCA) on these three variables. The first component (PC1) explained 63.4 percent of the variation (eigenvalue = 1.9) and was used as a composite measure for the three habitat variables. Vegetation was negatively (and water

turbidity positively) loaded onto the PC1. The AI was also strongly collinear with other variables. Given the direct relationship between the AI and climatic region (dry, intermediate, and wet), we deleted the AI from the set of predictor variables. Removing climatic region rather than AI did not eliminate collinearity from the dataset, probably due to an inherent association between the AI and other environmental correlates. The full set of environmental and ecological descriptors for each site, including those that were not used in the final model (due to collinear relationships), is presented in Supplementary Table 1.

We used the *lme4* package to build LMMs for each of the three response variables (parasite abundance, parasite species richness, and Brillouin index) with seven predictors tested against data from 21 host populations. Mean abundance was used as a measure of parasite abundance and was log-transformed prior to analysis to meet the assumptions of Gaussian distribution in residuals. Data on parasite species richness were corrected for the number of dissected hosts by rarefaction. Model assumptions were checked by plotting standardized residuals against fitted values. We also used a subset of host populations (pooled dataset for *N. furzeri* and *N. kadleci* host populations, $n = 12$) to validate general patterns between response variables and predictors.

RESULTS

DOMINANCE OF ENDOPARASITES AND IMPORTANCE OF NOTHOBRANCHIUS SPECIES AS INTERMEDIATE HOSTS.—We recorded 17 parasite taxa from a total sample of 205 host individuals taken from 21 killifish populations at 14 localities (Table 1). Twelve fish were free of parasites. Virtually all parasites were endoparasites, with trematode larval stages (metacercariae) being the most numerous at 75% prevalence. Larval cestodes and nematodes also occurred at high prevalence and abundance. The endoparasites mainly infected host muscle, often in extremely high numbers. Parasites were also found in the intestines, abdominal cavity, and occasionally the gills, gallbladder, and cerebral cavity. Most parasites were larval, encysted forms; hence, they were mostly identified to the family level only. The only endoparasite found in an adult stage was the trematode *Emoleptalea* sp. (Cephalogonimidae). Two glossiphoniid leeches (Hirudinea) were the only ectoparasites recorded.

Both allogenic and autogenic parasites were recorded. Trematodes were predominantly allogenic, except for autogenic *Emoleptalea* sp. (with fish as its definitive host). All nematodes and proteocephalid cestodes found were autogenic, while hymenolepid cestodes were allogenic. Both allogenic and autogenic life cycles were possible for the three trematode taxa (Echinostomidae, Digenea 1, and Digenea 2) and two cestode taxa (Cestoda 1 and Cestoda 2) that could not be identified at a lower taxonomic level.

No relationship was detected between host body size and parasite abundance (LMM using log-transformed data on parasite abundance: $F_{1,175} = 0.91$, $P = 0.342$) or parasite species richness (LMM: $F_{1,175} = 0.14$, $P = 0.707$). See Table 1 for

further details on the prevalence and abundance of each host-parasite combination and Table 2 for data on parasitism at individual localities.

INTER-SPECIFIC VARIATION.—Parasite communities were more similar among conspecific host individuals than among heterospecific host individuals (ANOVA on paired contrasts in Bray–Curtis dissimilarity: $F_{1,1131} = 13.05$, $P = 0.0003$). This relationship was driven by a significant difference in infracommunity structure between and within host species at sites Dry1 and Dry3 (site by species interaction: $F_{4,1131} = 3.51$, $P = 0.008$), where larval cestode 1 and strigeid metacercariae (Strigeidae 2) were more common in *N. furzeri* than other host species. At the other three sites, there was no difference between infracommunities of conspecific and heterospecific host fish individuals (Fig. 1).

In total, 13 different parasites (with the exclusion of *Emoleptalea* sp., Proteocephalidae, Hymenolepididae, and Glossiphoniidae) were found at localities with sympatric killifish communities, 11 being generalists (in most cases, they infected all killifish species at the locality) and two being apparent specialists for *N. furzeri* and *N. orthonotus* (*Apatemon* sp. and Digenea 1, respectively), both with very low prevalence and abundance (Table 1). Diplostomidae 1, Camallanidae, and Gnathostomatidae 1 infected all four killifish species (Table 1), often at high prevalence and abundance. In general, the frequency of parasite occurrence was low for less abundant and prevalent parasite species.

SPATIAL, ECOLOGICAL, AND ENVIRONMENTAL PREDICTORS OF PARASITE COMMUNITY STRUCTURE.—There was significant spatial autocorrelation in parasite abundance (Mantel test, $r = 0.189$, $P = 0.037$) but no spatial autocorrelation in parasite species richness ($r = -0.115$, $P = 0.907$) and Brillouin index ($r = 0.021$, $P = 0.345$).

Parasite abundance was not significantly associated with any ecological predictor, though it reached its highest levels in the intermediate region (Fig. S2). Parasite species richness and Brillouin index (comprising higher values for more diverse communities) were positively related to fish host diversity and pool size and tended to reach their highest levels in the intermediate region (Table 3; Fig. 2). In addition, the Brillouin index was negatively associated with the PC1 (it showed a positive association with vegetation and a negative association with turbidity), with the same (though non-significant) trend observed for parasite species richness (Table 3).

DISCUSSION

POSITION OF NOTHOBRANCHIUS SPP. IN PARASITE LIFE CYCLES.—African annual fish live in a periodically desiccating environment. In southern and central Mozambique, we found a total of 17 parasite taxa infecting *Nothobranchius* fishes. With the exception of two leeches, all were endoparasites, mainly metacercariae of trematodes located in the fish's muscle tissue. Dominance of larval endoparasitic species in the parasite community is typical for

TABLE 2. Data on parasitism in host populations.

Locality ^a	Fish species	N _{HOSTS} ^b	N _{PARASITE} SPECIES ^c	N _{PARASITE} INDIVIDUALS ^d	MA ^e ± SD	Brillouin index
Dry1	<i>Nothobranchius orthonotus</i>	7	5	508	4.03 ± 18.14	0.804
Dry1	<i>Nothobranchius furzeri</i>	10	5	413	2.29 ± 6.98	1.140
Dry2	<i>N. orthonotus</i>	10	1	16	0.09 ± 0.65	0
Dry2	<i>N. furzeri</i>	10	1	35	0.19 ± 1.21	0
Dry3	<i>Nothobranchius pienaari</i>	10	5	101	0.56 ± 2.74	0.811
Dry3	<i>N. orthonotus</i>	10	5	240	1.33 ± 6.22	0.474
Dry3	<i>N. furzeri</i>	10	6	148	0.82 ± 3.36	1.012
Med1	<i>N. furzeri</i>	10	8	518	2.88 ± 11.32	0.967
Med2	<i>N. furzeri</i>	10	7	1043	5.79 ± 30.97	1.178
Med3	<i>N. orthonotus</i>	10	6	2381	13.23 ± 59.80	0.434
Med3	<i>N. furzeri</i>	10	7	1460	8.11 ± 32.75	0.558
Med4	<i>N. orthonotus</i>	10	4	2482	13.79 ± 73.21	0.244
Med4	<i>N. furzeri</i>	10	5	2805	15.58 ± 74.70	0.314
Med5	<i>N. furzeri</i>	10	4	1990	11.06 ± 48.18	0.250
Wet1	<i>Nothobranchius kadleci</i>	10	3	78	0.43 ± 2.13	0.488
Wet2	<i>N. kadleci</i>	10	4	364	2.02 ± 8.32	0.576
Wet2	<i>N. orthonotus</i>	10	5	538	2.99 ± 13.94	0.977
Wet3	<i>N. kadleci</i>	10	3	543	3.02 ± 11.74	0.647
Wet4	<i>N. orthonotus</i>	10	3	146	0.81 ± 3.34	0.882
Wet5	<i>N. kadleci</i>	8	4	28	0.19 ± 0.73	0.923
Wet6	<i>N. orthonotus</i>	10	5	33	0.18 ± 1.33	0.902

^aSampling site code.

^bNumber of fish examined.

^cParasite species richness.

^dNumber of parasite individuals found.

^eParasite abundance (with Standard Deviation).

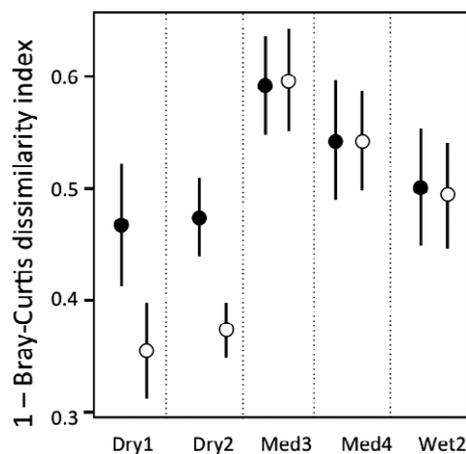


FIGURE 1. Mean values of similarity in community structure between conspecific (black circles) and heterospecific (empty circles) contrasts in five sites with at least two host killifish species. Whiskers represent 95% confidence intervals.

small-sized fish. Small fish often serve as prey for piscivorous predators and play a large role in transferring parasites to their definitive hosts (Dávidová *et al.* 2011). The remarkable scarcity of

ectoparasites was probably caused by the temporary nature of killifish habitat, since these parasites typically lack a desiccation-resistant stage that could survive during the dry season to become the source of infection for the new killifish generation. The leeches were only found in one locality (Wet6) and were probably transmitted by other fish species (*e.g.*, *Clarias* or *Tilapia*) during a temporary connection with a permanent water system during flooding.

Most parasites were found as immature, encysted forms, indicating that annual killifish are important intermediate or paratenic (substitute) hosts, as occurs with the non-annual North American spotfin killifish (*Fundulus luciae*) (Byrne 1978). Most of the parasites were allogenic trematode metacercariae, which is not surprising, as allogenic parasites use birds or other terrestrial animals as definitive hosts and therefore have a greater chance of being dispersed to new areas than autogenic parasites. By contrast, the adult trematode from the genus *Emoleptalea* (Proteocephalidae) found in the intestines of killifish at two localities utilizes fishes (probably *Clarias* fish species) as its definitive host (Vankara *et al.* 2014). Adult proteocephalid cestodes are known parasites of *Clarias gariepinus*, an omnivorous fish that is common in permanent waters throughout Mozambique and was found at two of our study sites (Wet5 and Wet6). All nematodes found

TABLE 3. Linear mixed models (with species and sites modeled as non-nested random intercepts) testing the effect of climatic and environmental predictors on parasite community structure. Full models (calculated for N = 21 host populations) are presented.

	Parasite abundance				Parasite richness				Brillouin index			
	nDF ^a	dNF ^b	F ^c	P ^d	nDF ^a	dNF ^b	F ^c	P ^d	nDF ^a	dNF ^b	F ^c	P ^d
Region	2	5.1	2.43	0.181	2	5.1	5.39	0.056	2	5.0	5.14	0.061
Pool size	1	5.2	0.63	0.462	1	5.7	6.76	0.043	1	7.6	7.29	0.028
PC1	1	5.2	0.44	0.537	1	5.0	4.42	0.089	1	4.9	12.75	0.017
Littoral vegetation	1	5.1	0.63	0.462	1	5.1	4.68	0.082	1	5.2	0.45	0.532
<i>Nothobranchius</i> density	1	5.1	0.63	0.462	1	4.9	0.62	0.468	1	4.6	1.32	0.308
Invertebrate diversity	1	5.1	0.54	0.495	1	5.1	0.77	0.420	1	5.1	3.43	0.122
Host fish diversity	1	5.1	0.20	0.673	1	5.0	8.30	0.034	1	5.0	14.92	0.012

^aNumerator degrees of freedom.

^bDenominator degrees of freedom (calculated using Satterthwaite approximation).

^cF-value.

^dStatistical significance, bold typeset indicates $P < 0.05$.

were autogenic. Camallanid nematodes predominantly infect fish intestines, with copepods serving as intermediate hosts, though several genera of this family also occur in amphibians and turtles. In our case, nematodes were always found at the larval stage, suggesting that killifish are not suitable definitive hosts for this parasite. Instead, killifish probably serve as paratenic hosts that transmit the parasite to larger predators in which maturity is attained (Anderson 1992). Adult gnathostomatid nematodes predominantly parasitize lower vertebrates (especially turtles), while third-stage larvae have been found encapsulated in various potential paratenic hosts, including fish (Hedrick 1935).

INTER-SPECIFIC VARIATION.—Host diet, behavior, and some life-history traits are often determinants of parasite fauna richness (Combes 2001, Poulin 2007). Sympatric species of *Nothobranchius* share similar habitats (Reichard *et al.* 2009) and food (Polačik & Reichard 2010, Polačik *et al.* 2014a) and are subject to the same risk of parasitism from the same spectrum of parasites. However, there were differences among host species in parasite infracommunity composition at two (Dry1 and Dry3) of five localities where two or more killifish species co-occurred. Closely related sympatric host species usually differ in their parasite fauna when infected with specialist parasites (Muñoz & Grutter 2006, Šimková *et al.* 2006, 2012). In our study, two generalist parasites (Cestoda 1 and Strigeidae 2) were found at high abundance and prevalence in *N. furzeri* over the other killifish species, indicating a degree of host preference in these parasite species. This significantly affected the similarity in parasite communities between sympatric host species. Nevertheless, generalist parasites tend to dominate in temporary and unpredictable habitats (Holmes & Price 1980, Holm 1988) with host species having similar parasite communities. Indeed, most of the parasite taxa found in this study infected all killifish species available; hence, we considered them to be generalists despite being unable to identify them beyond the family level. A comparison of communities consisting

mainly of generalist parasites indicated that parasite species richness and abundance did not differ between sympatric host species. This may correspond to the intensive infection by larval trematodes and nematodes, which tend to be generalists (Poulin 1992, Combes 2001).

SPATIAL, ECOLOGICAL, AND ENVIRONMENTAL PREDICTORS OF PARASITE COMMUNITY STRUCTURE.—At a global scale, positive spatial autocorrelation in community structure is often related to the distributions of individual species (Austin 2002), leading to a decay in the similarity of community structure with geographic distance (Poulin & Morand 1999, Poulin 2003). In our study, positive spatial autocorrelation was apparently driven by the extremely high abundance of diplostomid metacercariae in the intermediate aridity region (Tables 1 and 2), affecting parasite abundance rather than species composition. The predominance of allogenic species in *Nothobranchius* spp. parasite communities may explain the absence of spatial autocorrelation in parasite species richness. At a local scale, the parasite component community (at the host population level) in fish often shares allogenic parasites, independent of the distance separating the study sites, as definitive hosts are not limited by land barriers between water bodies (Karvonen *et al.* 2005).

It appears that high local densities of avian fish predators support high infection rates between intermediate and final hosts of trematodes and consequently a high abundance of larval stages. Potential definitive hosts of trematodes (fish-eating birds such as kingfishers, hammerheads, and herons) are thought to be a major source of mortality of *Nothobranchius* killifish (Haas 1976, Reichard *et al.* 2014). Therefore, the higher parasite abundance observed in the intermediate region does not appear to be a consequence of climatic conditions; rather it apparently arises from high abundance of definitive hosts for a subset of killifish parasite taxa. Host fish from three adjacent localities in the intermediate region (Med3, Med4, and Med5) were all heavily infected

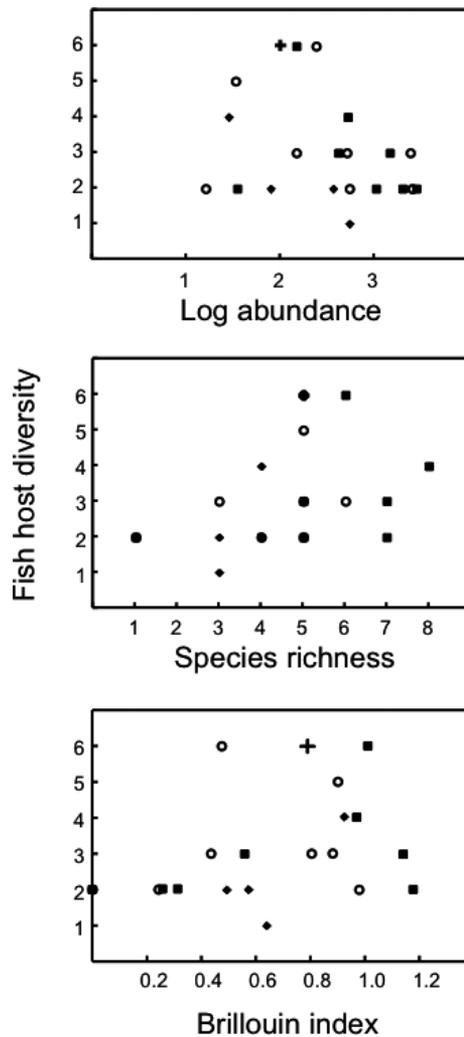


FIGURE 2. Relationship between fish host diversity and three descriptors of parasite communities visualized as simple bivariate plots (Table 3) (○ *Nothobranchius orthonotus*, ■ *Nothobranchius furzeri*, ◆ *Nothobranchius kadleci*, + *Nothobranchius pienaar*).

with trematode metacercariae (Tables 1 and 2), which drove the overall pattern of highest parasite abundance in the intermediate region.

Our results suggest that the diversity of host fishes was the primary predictor of parasite diversity. There is a relationship between the number of host species of a given taxon in a habitat and the number of parasite species exploiting that taxon, especially in parasites with low host specificity (Watters 1992, Poulin 2007). The presence of numerous fish species may result in a larger set of available parasite species, thereby facilitating an increase in the species richness of given communities (Poulin 2007); we also observed this trend from species-poor host communities. High species richness and population density of large benthic invertebrates (not necessarily hosts) has been reported as being positively correlated with diversity of trematode parasites in snail hosts, the benthic invertebrates attracting birds that are the

source of the trematode larvae infecting snails and, subsequently, fish (Hechinger *et al.* 2006). In this study, we observed no effect of invertebrate taxonomic diversity on parasite community structure; however, we did not estimate invertebrate population density.

We also predicted a positive effect of pool size and the presence of vegetation on parasite abundance and diversity. A positive relationship usually exists between habitat area and parasite community composition and richness (*e.g.* Marcogliese & Cone 1991, Dávidová *et al.* 2011). The presence of macrophyte vegetation may also contribute to an increased abundance of trematode metacercariae, which use aquatic snails as their first intermediate host (Marcogliese *et al.* 2001, Ondračková *et al.* 2004). We confirmed the positive roles of pool size and aquatic vegetation on parasite diversity, but not parasite abundance (Table 3).

CONCLUSION

Our study indicates that African killifish play an important role as an intermediate and paratenic host for many parasite species. Despite some differences between sympatric host populations, their parasite specificity was relatively low, and most parasite taxa are likely shared with a wider fish host spectrum. Hence, while *Nothobranchius* species provide an important link between aquatic and terrestrial habitats via parasite transmission between these habitats, the contribution of their parasite fauna to estimates of overall parasite diversity is negligible. Observed parasite diversity did not vary across a gradient of aridity but was associated with local habitat characteristics.

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DATA AVAILABILITY

Data deposited in the Dryad Repository: <http://dx.doi.org/10.5061/dryad.mk815> (Nezhybová *et al.* 2016)

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

APPENDIX S1. Details of parasite identification.

TABLE S1. *Environmental and ecological characteristics of the sample sites.*

FIGURE S1. Sampling sites for host populations in southern and central Mozambique with respect to an aridity index.

FIGURE S2. Mean number of parasite individuals for each climatic region, with 1 standard deviation and data range.

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