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Distribution, colour polymorphism and habitat use of the African killifish *Nothobranchius furzeri*, the vertebrate with the shortest life span

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Intensive collection in southern Mozambique across and outside the potential range of *Nothobranchius furzeri*, the species with the shortest recorded life span among vertebrates used as a model in ageing research, revealed that, contrary to previous data, it is a widespread species. It occurs in small freshwater pools south of the Save River and north of the Incomati River, including basins of the Limpopo, Changane, Chefu, Mazimechopes and Vaneteze Rivers. During collection in February 2008 (the second part of the rainy season), populations were strongly female biased (mean, 28% of males across 19 populations), and there was a spatial pattern in female bias among metapopulations. Populations varied in the proportion of male colour morphs. Fourteen populations were composed exclusively of the red male phenotype, three populations of the yellow male phenotype and 12 populations were mixed. Overall, the red phenotype was more common, but there was strong geographical variation in morph proportion, with yellow males more abundant at the periphery and red male dominance in the centre of the range of *N. furzeri* in the Limpopo basin. *Nothobranchius furzeri* was sympatric with *Nothobranchius orthonotus* (35% of investigated pools) and *Nothobranchius rachovii* (27% of sites). Analysis of habitat use of *N. furzeri* is presented; *N. furzeri* was associated with pools containing a soft muddy substratum and turbid water.

Key words: adult sex ratio; Cyprinodontiformes; fish-habitat association; geographic range; male colour polymorphism; species coexistence.

INTRODUCTION

The genus *Nothobranchius* (Cyprinodontiformes, Nothobranchiidae) is a group of small (range 30–150 mm, median 50 mm standard length, L_S), short-lived (3–12 months) fishes that inhabit isolated pools throughout the savannah region of eastern and central Africa. Their distribution ranges from southern Sudan to KwaZulu Natal in South Africa (Wildekamp, 2004). The genus includes

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c. 50 currently described species, separated into five well-defined clades (Huber, 2000; Wildekamp, 2004). The life history of *Nothobranchius* is adapted to annual desiccation of their habitat. Fishes hatch after the start of rainy season, grow rapidly and become sexually mature within a few weeks. After reaching sexual maturity, they reproduce daily and females lay five to 50 eggs each day (Haas, 1976*a*). Eggs are spawned into a substratum and remain there after pool desiccation. While the habitat is dry, embryos survive in a developmental diapause until the next rainy season (Wourms, 1972; Wildekamp, 2004). All *Nothobranchius* fishes are extremely sexually dimorphic and dichromatic; males are robust and colourful, and females are pale yellow or brown. The bright male colouration is sexually selected (Haas, 1976*b*) and species specific (Huber, 2000). Many species occur in several colour forms that are either sympatric or allopatric (Huber, 2000; Wildekamp, 2004). Several *Nothobranchius* species may co-occur in sympatry in the same pool (Huber, 2000).

Nothobranchius furzeri Jubb has the shortest recorded life span amongst all vertebrates (Valdesalici & Cellerino, 2003). In nature, its life span is limited by habitat desiccation, but its survival is similarly short in captivity, with a sharp increase in mortality at the age of 6 weeks and a maximum post-hatch life span of <12 weeks (Valdesalici & Cellerino, 2003). Given that mortality in *N. furzeri* is intrinsic and ageing fish show severe tissue degradation (*e.g.* accumulation of lipofuscin in the liver), and a sharp decrease in cognitive functions and locomotor activity (Valenzano *et al.*, 2006*a*), *N. furzeri* has been established as a model species in ageing research (Genade *et al.*, 2005; Valenzano *et al.*, 2006*b*). It has been used for pharmacological studies of life-span extension (Valenzano & Cellerino, 2006) and offers potential for investigating the genetic mechanisms controlling ageing. While the laboratory line shows a life span of <3 months (Valdesalici & Cellerino, 2003), there is supposedly large variation in life span and life-history traits among different *N. furzeri* populations (Terzibasi *et al.*, in press).

Current knowledge of the distribution and natural history of *N. furzeri* is deficient. The species was described from the Gona Re Zhou Game Reserve in Zimbabwe, where fish were collected in the Sazale Pan in March 1968, December 1968 and January 1969 (Jubb, 1971). Sazale Pan is located 25 km from the border with Mozambique and it drains into the upper Guluene River, a tributary of the Chefu River flowing to Mozambique, an important tributary of the Chingovo River basin. The Chingovo River basin never reaches the ocean as it disappears in a series of shallow swamps and inland lakes on a large flat plain at an altitude of *c.* 80 m above sea level (masl). The topography of the area, however, suggests that this river system may have been connected to the Limpopo River basin in humid periods of the Pleistocene in its downstream part *via* the Changane River (Jubb, 1971; Skelton, 2001).

Since its description, *N. furzeri* has never been collected in the Gona Re Zhou Reserve again (the reserves conservation status has currently been elevated to National Park) and, until recently, only the captive strain collected in the late 1960s was maintained by hobbyists. Gona Re Zhou, originally designed as an extension of Kruger National Park, South Africa, in the 1960s to protect migrating elephants, became a centre of guerrilla activists and subjected to poaching soon after the discovery of *N. furzeri* and has not been accessible for sampling thereafter. In 1999, three new populations of *N. furzeri* were discovered along the north bank of the Limpopo River in southern Mozambique (Wood, 2000). Interestingly, the new populations differed markedly in colouration and were considered new, as yet undescribed species (Wood, 2000). While the type population of *N. furzeri* consists exclusively of males with a yellow stripe at the margin of the caudal fin (Jubb, 1971), all three populations (adjacent to each other) collected in the Limpopo River basin were composed exclusively of males with red caudal fins. Only when the F1 captive generation produced a mixture of red and yellow phenotypes, the fish were recognized as a new phenotypic morph of *N. furzeri*. Between 2004 and 2007, when *N. furzeri* was established as a model species for ageing research (Valdesalici & Cellerino, 2003) and potential field sites in Mozambique become accessible to sampling, a total of six collection trips was completed. During those collections, several other populations were discovered, including populations with a mixture of red and yellow phenotypes. The sampling has largely been conducted with a sole aim of exporting wild fish and establishing captive populations either as a hobby or in research laboratories (Nothobranchius Maintenance Group, 2008; B. Watters, pers. comm.). Unfortunately, no collection was systematic and no precise data on distribution, demographic variables or habitat preference are available.

Data on the distribution, ecology and demography of *N. furzeri* are essential for their full appreciation in studies investigating the evolutionary origin and consequences of ageing (Genade *et al.*, 2005). In the present study, the results of a systematic survey of 28 *N. furzeri* populations are presented. Specifically, (1) the range extent of *N. furzeri* was examined along 10 transects within the potential range of *N. furzeri* in Mozambique, (2) sex ratio and male colour polymorphism were investigated in most sampled populations and (3) habitat preference and habitat segregation between *N. furzeri* and other *Nothobranchius* spp. (sympatric with *N. furzeri*) were studied.

MATERIALS AND METHODS

The study area in southern Mozambique (from 25°50′ to 19°17′ S, *i.e.* between the rivers Incomati and Pungue) was divided into 10 transects with respect to particular river basins (Appendix). Transects followed roads accessible to a 4WD vehicle (Fig. 1). The area included basins of the Incomati (comprising basins of the Mazimechopes and the Vaneteze Rivers), Limpopo (including the Changane basin), Save, Gorongosa, Buzi and Pungue Rivers that flow into the Indian Ocean and of the endorheic Chefu River basin that desiccates in a large flat pan east of the Banhine National Park (Fig. 1). Several small coastal basins were also investigated. Along transect 1 [T1, road between Chibuto and Chicualacuala (a town at the border with Zimbabwe), along the Limpopo River typically 5–20 km from the left bank of the river] 23 habitats were sampled. Along transect 2 (T2, between the main road along the Limpopo River and the Nuaneteze River channel), no suitable pool was found. All 22 sites sampled along transect 3 (T3, from Mapai towards Massagena) were within the Chefu River basin (not connected to the Limpopo River basin). Along transect 4 (T4, the Changane River basin), 13 sites were investigated along the road between Chirrunduo and Chigubo. Seven sites were sampled along transect 5 (T5, the Incomati River basin including the Vaneteze and the Mazimechopes River basins, south-west of the Limpopo River). At the lower Limpopo River (transect T6, downstream from the start of T4 where the Changane River meets the Limpopo River, *i.e.* <100 km from the ocean, measured

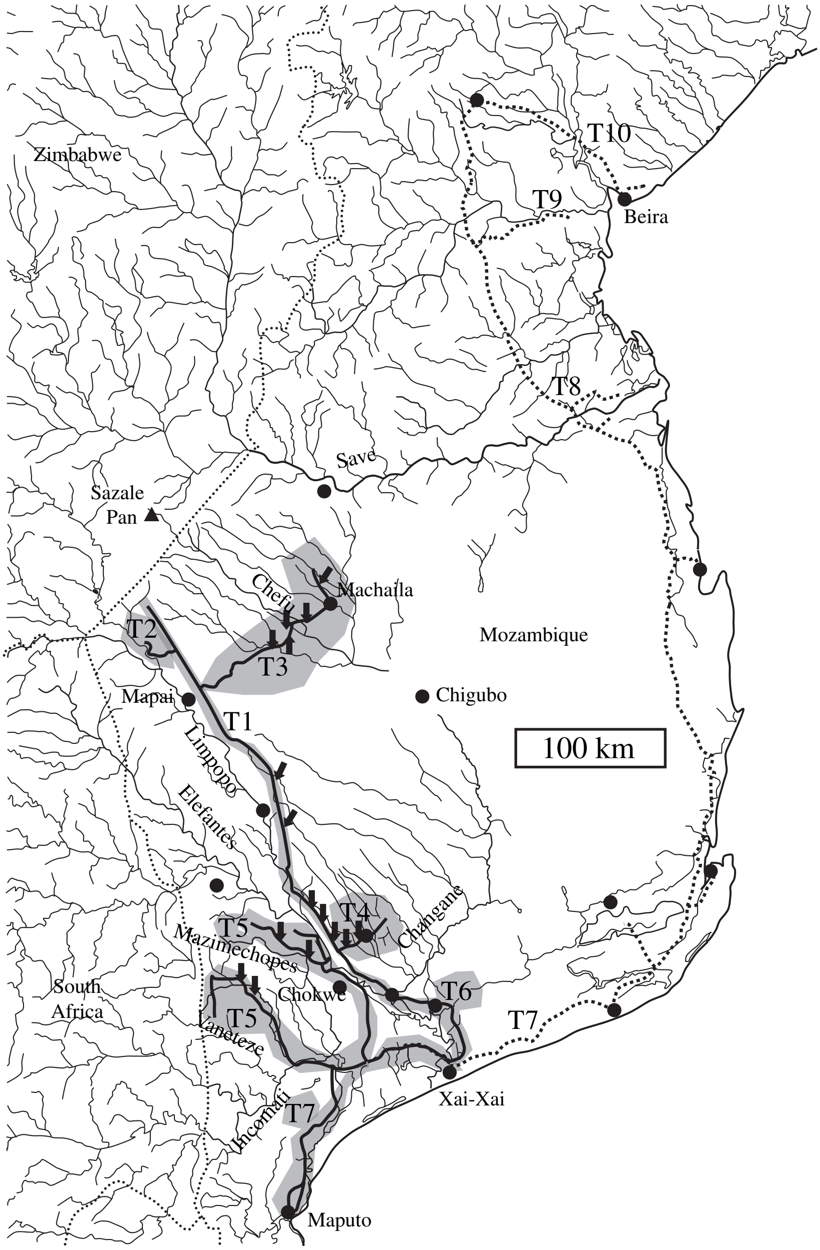

FIG. 1. Map of the southern Mozambique with river network (——), important towns (●) and collection transects (▬ highlighted by shaded areas with codes T1–T7) indicated. Transects outside the range of *Nothobranchius furzeri* (determined retrospectively) are marked (----, codes T7–T10). Pools containing *N. furzeri* populations are indicated (⬇) (one arrow may represent several adjacent populations). Note that many rivers are temporary and may not form flowing sections every rainy season. International borders (. . .).

as the straight distance), 10 sites were investigated. Finally, along transect 7 (T7, minor coastal basins) 17 sites were sampled along the main N1 road between the Maputo and the Save River of which four sites were considered within the range of *N. furzeri* retrospectively, and used in further habitat analysis (Fig. 1). At transects 8 (T8, the Save and Gorongosa River basins), T9 (the Buzi River basin) and T10 (the Pungue River basin) nine, five and eight sites were sampled, respectively. A summary of sampling sites is presented in Appendix.

Sampling was conducted between 8 and 22 February 2008 at the end of the rainy season. Pools encountered along the transect were sampled using a dip-net with a triangular metal frame (450 × 450 mm and mesh-size 5 mm) on a long (1·5 m) wooden pole. Typically, 15–40 hauls were performed at each site to allow semi-quantitative estimates of *Nothobranchius* spp. abundance. Additional hauls were performed, if more fishes were needed for a specific analysis (*e.g.* colour polymorphism and habitat use study). At some habitats, a beach seine (length 2·7 m, depth 0·7 m and mesh-size 4 mm) was used in addition to the dip-net. Fewer dip-net hauls were taken, if the habitat was too small to accommodate 15 hauls. The chosen mesh-size was sufficient to capture all *Nothobranchius* fishes present at sampling sites. At each site, GPS co-ordinates, altitude (Garmin eMaps GPS handset; Garmin Ltd, Olathe, KS, U.S.A.), water temperature (° C), conductivity (μS cm^{-1}) (Hanna Combo; Hanna Instruments, Woonsocket, RI, U.S.A.), maximum and typical water depth (to the nearest 50 mm), vegetation (submergent, emergent littoral, *Nymphaea* sp. as absent, rare or abundant), water turbidity (clear blackwater; transparent, bottom visible; turbid, visibility 10–100 m; very turbid, visibility $<$1 cm), substratum (sand, hard clay bottom, mud: a person standing on bottom sinks *c.* 30–150 mm, soft mud: a person standing on substratum sinks $>$150 mm), area (estimated to the nearest 10 m^2) and time of day were recorded. The distance to the nearest stream was measured to the nearest 0·1 km by superimposing GPS co-ordinates on fine-resolution satellite images using Google Earth (http://earth.google.com). Water temperature was not considered in statistical analyses because it fluctuated widely during the day. Waterproof dataloggers exposed at two representative pools of the study area at a depth of 100–150 mm below the surface (the middle of the water column) for 28 and 96 h, respectively, showed that water temperature reached minimum of 20·5° C before sunrise and maximum of 35·2° C at 1400 hours. All fishes encountered were identified to species (Cyprinidontiformes) or generic level (other orders) according to Skelton (2001) and Wildekamp (2004). A study of within-pool habitat use of *N. furzeri* was performed at site 55. A total of 20 dip-net points were sampled in each of the three habitat types present (*Nymphaea* sp. vegetation, submergent littoral vegetation, open water) and *N. furzeri* presence was compared using a generalized linear model (GLM) with binomial error, using water depth (measured at each sampling point) as a covariate.

For all analyses, a matrix of species presence and environmental variables was reduced by deletion of sites outside the range of *N. furzeri* (*i.e.* sites in basins, where *N. furzeri* had not been recorded), yielding a total of 79 sites in all analyses. A one-way ANOVA was used to compare sex and colour morph ratios among parts of the *N. furzeri* range (defined as transects). Sex and colour morph ratios were expressed as proportions of males and red morph, respectively. For these analyses, only populations with at least nine fish (sex ratio) or nine males (colour polymorphism) were included. Habitat characteristics at landscape scale (across individual pools) were visualized by detrended canonical correspondence analysis (dCCA) using CANOCO for Windows 4.5 (ter Braak & Šmilauer, 1997). The dCCA is an ordination technique that relates species presence to variation in environmental variables. For dCCA, the species matrix was coded for four columns; *N. furzeri*, *Nothobranchius orthonotus* (Peters), *Nothobranchius rachovii* Ahl and 'no *Nothobranchius*'. The dCCA is useful for providing insights into relationships between species and habitat since it reduces a multidimensional space of habitat variables and bivariate plots allowing a graphical illustration of species occurrence in relation to habitat variables. In brief, each habitat variable is illustrated by a vector; the length of each vector is proportional to the importance of the habitat variable in explaining the variability in the species matrix. The minimum adequate model was constructed by stepwise model simplification from a maximal model containing all explanatory habitat variables and quadratic terms of every continuous variable (Crawley, 2007) by Akaike information criterion (AIC) using GLM ANCOVA (a mixture of continuous and categorical variables) with a binary response (presence or absence) variable and log-link function in R 2.0.1 (R Development Core Team, 2006). Histograms were constructed for individual habitat variables to visualize their relationship to *N. furzeri* presence.

RESULTS

DISTRIBUTION

The presence of *N. furzeri* was recorded at 29 of 124 sampling sites investigated, of which 79 were retrospectively classified as within the range of *N. furzeri* (south of the River Save, excluding the Save and coastal plains of the Indian Ocean). A map of the *N. furzeri* range with sampling sites investigated is presented in Fig. 1. The findings presented here confirm previous records from the lower Limpopo River, Chefu and Mazimechopes River basins and show that pools with *N. furzeri* populations are not as sparse as believed previously (earlier collections identified only eight pools with *N. furzeri* populations). Further, two populations of *N. furzeri* in the Vaneteze River basin (part of the Incomati River basin) were recorded, which extend *N. furzeri* distribution in a south-eastern direction.

The recorded range of *N. furzeri* is 282 km across its longest axis, which increases to 333 km if the type locality is included. The distance between the furthest upstream and furthest downstream populations along the Limpopo River is 125 km, with an altitudinal range from 45 to 144 masl. The furthest site from the left bank of the Limpopo River was 117 km at the Chefu River basin and 52 km from the right bank of the Limpopo River in the Vaneteze River basin.

SEX RATIO AND COLOUR POLYMORPHISM

Females dominated most populations with a mean proportion of 72% across 19 populations, where at least 25 *N. furzeri* were collected. Of those 19 populations, only one had a male-biased sex ratio (53 males and 34 females), while site 124 had the most female-biased sex ratio (nine males and 105 females) (Table I). There was a less female-biased sex ratio in populations along T3 than along T4 and T5 (ANOVA, $F_{3,13}$, $P = 0{\cdot}01$; Tukey HSD tests for T3 *v.* T4: $P < 0{\cdot}05$ and for T3 *v.* T5: $P < 0{\cdot}05$, all other pair-wise comparisons $P > 0{\cdot}05$). Mean ± S.E. sex ratios were 0·41 ± 0·05 in T3 populations, 0·32 ± 0·04 in T1, 0·21 ± 0·04 in T4 and 0·17 ± 0·06 in T5.

A total of 14 sites with pure red populations, three sites with pure yellow populations (though they contained only two, three and nine males captured) and 12 mixed populations were recorded. Pure red populations occurred at transect 1 (Limpopo River basin) and transect 4 (Changane River basin). Mixed populations were found at high-altitude sites in transect 1 (two sites at 129 and 146 masl), along transect 3 (five sites between 124 and 128 masl), at three sites in transect 4 (between 24 and 34 masl) and two sites in transect 5 (30 and 56 masl). Exclusively yellow males were found on the right bank of

TABLE I. Sex ratio (proportion of males) and colour morph ratio (proportion of red morph) of 24 populations of *Nothobranchius furzeri* across its range. Note that in five populations (sites 3, 4, 7, 9 and 54), sex and colour morph ratios were not estimated due to small sample size, non-quantitative sampling or logistic reasons. All these populations contained red males only

		Sex ratio				Male morphs			
Site	Transect	Males	Females	Total	Sex ratio	Red	Yellow	Proportion red	Note
1	T1	12	31	43	0·28	12	0	1·00	
2	T1	9	22	31	0·29	9	0	1·00	
8	T1	11	16	27	0·41	11	0	1·00	
13	T1	11	19	30	0·37	108	3	0·97	
23	T3	53	34	87	0·61	32	21	0·60	
28	T3	56	100	156	0·36	48	8	0·86	
29	T3	Not estimated				6	3	0·67	
33	T3	61	86	147	0·41	11	50	0·18	
34	T3	12	33	45	0·27	10	2	0·83	
43	T1	Not estimated				6	10	0·38	
50	T1	8	23	31	0·26	8	0	1·00	
51	T4	6	23	29	0·21	6	0	1·00	
53	T4	12	45	57	0·21	11	1	0·92	
55	T4	11	95	106	0·10	10	1	0·91	
56	T4	5	20	25	0·20	4	1	0·80	
120	T5	10	31	41	0·24	1	9	0·10	
121	T4	81	153	234	0·35	72	9	0·89	
122	T4	Not estimated				40	0	1·00	
124	T5	9	105	114	0·08	0	9	0·00	
119	T5	2	8	10	0·20	0	2	0·00	#
123	T5	3	4	7	0·43	0	3	0·00	#
40	T3	1	3	4	0·25	0	1	0·00	#
59	T4	0	3	3	0·00				#
61	T4	2	0	2	1·00	2	0	1·00	#
Total		375	854	1229	0·28				

#, <9 males captured; population was not considered in statistical analysis.

the Limpopo River at two sites in the Vaneteze River basin (transect 5, with three and nine males captured at 88–96 masl) and one site in the Mazimechopes basin (transect 5, only two males collected, 48 masl). In mixed populations, red males typically (but not always) dominated (Table I). There was a significant difference in the proportion of red males among transects (ANOVA, $F_{3,15}$, $P = 0·001$), with significantly fewer red males in populations along T5 (mean ± S.E. 0·05 ± 0·15) than along T1 (0·89 ± 0·09), T3 (0·63 ± 0·09) and T4 (0·92 ± 0·09) (Tukey HSD tests for T5 *v.* all other transects $P < 0·05$).

COEXISTENCE WITH OTHER FISH SPECIES

Up to three *Nothobranchius* species inhabited a single temporary pool. Within the *N. furzeri* range (79 sites), *N. furzeri* was recorded with *N. orthonotus* at 10 sites (35% from 29 sites, where *N. furzeri* was found), with *N. orthonotus* and *N. rachovii* at five sites (17%) and with *N. rachovii* exclusively at three sites (10%). At 16 sites, *N. furzeri* occurred without any other *Nothobranchius* species (55%).

Other fishes recorded sympatric with *N. furzeri* were small cyprinids, *Barbus* sp. (three sites), lungfish *Protopterus annectens brieni* Poll (four sites) and catfish, *Clarias gariepinus* (Burchell) (two sites). *Nothobranchius furzeri* was never recorded sympatric with tilapias [*Tilapia s. l.* juveniles including *Oreochromis mossambicus* (Peters) and *Tilapia rendalli* (Boulenger) for which determination was confirmed on adult individuals] that occurred at 11 sampled sites within the *N. furzeri* range.

Habitat segregation among *Nothobranchius* spp. is presented in Fig. 2. The first two axes in the CCA accounted for 92·1% of variation in species-habitat data (55·2% for the first axis) (test for all canonical axes, $F_{11,499}$, $P < 0{\cdot}05$). For the first three axes, eigenvalues were 0·338, 0·073 and 0·024. The highest interspecific segregation was across the gradients of water turbidity, bottom composition and water conductivity (Fig. 2).

HABITAT USE

The dCCA revealed that *N. furzeri* inhabited sites with soft substratum and high turbidity (Fig. 2). The minimal adequate model (GLM with binomial

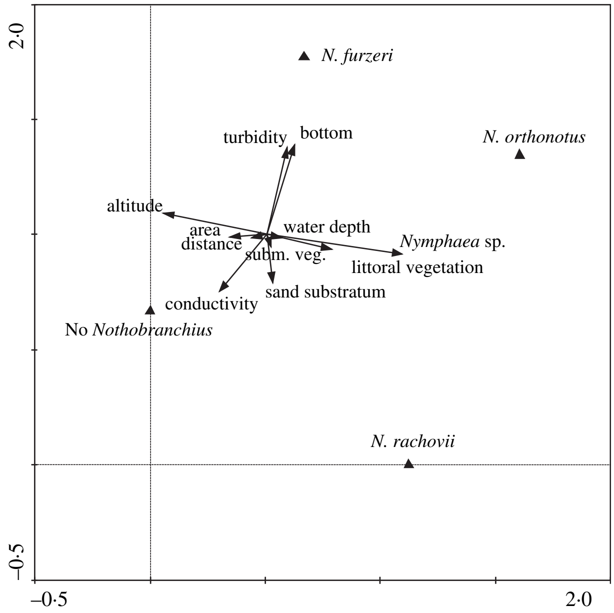

Fig. 2. Bivariate plot of detrended canonical correspondence analysis showing the position of *Nothobranchius* species (▲) and habitat variables (—▶) along the first two canonical axes. Data on altitude, area (estimated area of water surface), bottom (along the gradient of hard to soft), conductivity, distance from the nearest (temporary) stream, littoral vegetation, *Nymphaea* sp., submergent vegetation (subm. veg.) occurrence of sand substratum and water depth are superimposed over species presence data.

error) retained conductivity, substratum, altitude, distance from the nearest river and surface area as significant factors affecting the presence of *N. furzeri* populations (Table II). *Nothobranchius furzeri* was found at altitudes between 18 and 140 masl, at sites with conductivity from 50 to 625 μS cm^{-1}, soft or very soft substrata and surface area typically between 50 and 625 m^2 (6–70 000 m^2 including outliers). The relationship between *N. furzeri* presence and distance from the nearest river was complex (Fig. 3).

Nothobranchius furzeri often inhabited simple shallow pools without any vegetation. Within pools with vegetation, *N. furzeri* typically occurred in *Nymphaea* sp. in close proximity to open water or at the interface between vegetation and open water. At site 55 where quantitative sampling was performed, *N. furzeri* preferred *Nymphaea* sp. vegetation (present at 12 out of 20 points) over littoral vegetation (three of 20) and open water (zero of 20) and the difference in the use of *Nymphaea* sp. vegetation compared to the other two habitats was significant (GLM with binomial error, $P < 0{\cdot}05$ for habitat type). Water depth was not a significant covariate ($P > 0{\cdot}05$). Water temperature was not significant predictor of *N. furzeri* presence and was higher in flooded littoral vegetation than in open water and in *Nymphaea* sp. vegetation (ANOVA, $F_{2,16}$, $P < 0{\cdot}001$). Mean ± s.e. water temperature in littoral vegetation was 30·77 ± 0·08° C, 29·50 ± 0·08° C in *Nymphaea* sp. vegetation and 29·39 ± 0·07° C in open water.

DISCUSSION

The distribution of *N. furzeri* extends over the Incomati, Limpopo and Chefu River basins in a relatively small part of southern Mozambique. Additionally, at least one *N. furzeri* population occurs in the Zimbabwean part of the Chefu River basin (Jubb, 1971). The type locality in Sazale pan is situated at 422 masl, while all other known populations inhabit pools between 18 and 140 masl. It is likely that more *N. furzeri* populations occur in the upper Chefu River basin, between Sazale pan and the collection sites along transect 3 at

Table II. Minimal adequate model with binary response variable (*Nothobranchius furzeri* presence or absence) describing habitat preferences of *N. furzeri* at landscape scale

	Estimate	s.e.	*t*	*P*
Intercept	1·196	0·502	2·384	0·020*
Conductivity	−0·346	0·123	−2·811	0·007**
Area	0·152	0·088	1·723	0·090
Littoral vegetation	0·171	0·108	1·587	0·117
Mud bottom	−0·462	0·164	−2·818	0·006**
Hard clay bottom	−0·539	0·200	−2·692	0·009**
Sand bottom	−0·647	0·209	−3·120	0·003**
Altitude	0·006	0·003	2·097	0·040*
Distance from river	−0·072	0·042	−1·704	0·093
Area quadratic	−0·015	0·007	−2·181	0·034*
Altitude quadratic	−0·00004	0·00001	−3·024	0·004**
Distance quadratic	0·007	0·003	2·142	0·036*

*, $P > 0{\cdot}01$; **, $P > 0{\cdot}001$.

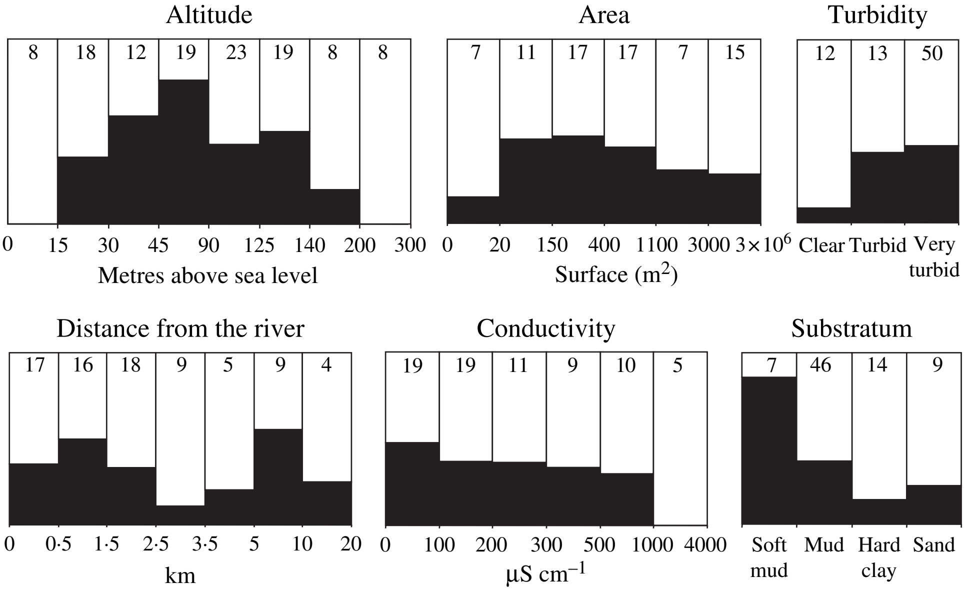

FIG. 3. The association between *Nothobranchius furzeri* occurrence and habitat variables: the proportion of habitats with *N. furzeri* populations in a given interval of the habitat variable (■). The number of habitats within the intervals is indicated for each histogram. For water turbidity, the category 'clear' sums blackwater ($n = 2$) and transparent water ($n = 10$).

132 and 128 masl (sites 33 and 40), respectively. Unfortunately, the area in Zimbabwe and near the border between Zimbabwe and Mozambique is inaccessible to research for security reasons, and there is little prospect of obtaining permits to sample that part of the *N. furzeri* range. There is a report of a collection of *N. furzeri* from the upper Chefu River basin at the Mozambican–Zimbabwean border, though no details on sampling are available (B. Watters, pers. comm.). The present study included sampling at high altitude area of the Limpopo River basin (up to 363 masl), but no freshwater pools were encountered higher than 256 masl, and no *N. furzeri* populations were found higher than 140 masl in the Limpopo River basin. Therefore, it is possible that high altitude populations occur solely in the upper Chefu River basin. To confirm this speculation, a larger area in the upper Limpopo River basin needs to be investigated, but the savannah further from the roads is inaccessible for logistical reasons (presence of land mines). Ultimately, the internal structure of the *N. furzeri* range can be determined using genetic markers, but current knowledge and the assumption that *Nothobranchius* spp. are weak dispersers upstream (Wildekamp, 2004) lend support to a putative hypothesis that the upper Chefu River basin contains the source populations of *N. furzeri* and that *N. furzeri* are dispersed by occasional extensive floods resulting from cyclones forming off Mozambique in the Indian Ocean. Such rare events may connect isolated pools and allow downstream migration and colonization. How *N. furzeri* colonized the high altitude sites and its spreading downstream and into adjacent basins is the subject of ongoing phylogeographic investigations that include *N. furzeri* and its sister, yet undescribed, species from the lower Save basin.

The two colour morphs that are expressed in male *N. furzeri* have largely overlapping ranges. In the type population in the upper Chefu River basin, only yellow males were found (Jubb, 1971). In the lower Chefu River basin, all populations were composed of a mixture of yellow and red males, while only seven of 13 populations in the Limpopo River basin contained any yellow males. The yellow morph also dominated in the Incomati River basin in the south-western part of the *N. furzeri* range, with exclusively yellow males found in the Vaneteze River basin and yellow-dominated populations in the Mazimechopes River basin. In contrast, the red morph dominated in most populations in the Limpopo River basin that includes the centre and the largest part of the *N. furzeri* range. Colour morphs are often associated with different life-histories and behavioural tactics and their coexistence may be enabled by fluctuation in environmental and population factors (Sinervo *et al.*, 2000). There are some preliminary data suggesting that *N. furzeri* populations from more humid areas have a longer life span and later commencement of physiological changes linked to senescence (Terzibasi *et al.*, in press), but nothing is currently known about the association between differences in colouration and life history.

The sex ratio was almost exclusively female biased, but the degree of female bias varied among transects. In fishes, there is a great variability in sex determination and genetic and environmental determination of sex has evolved multiple times (Mank *et al.*, 2006). In a closely related species, *Nothobranchius guentheri* (Pfeffer), sex is determined by a complex combination of sex chromosomes (Ewulonu *et al.*, 1985), and it is likely that sex determination in *N. furzeri* is also genetic. No sex related differences in mortality rate or biases in the adult sex ratio were reported in laboratory studies of life expectancy (Valdesalici & Cellerino, 2003; Genade *et al.*, 2005; Valenzano *et al.*, 2006*a*, *b*) suggesting increased male extrinsic mortality in natural populations. Male colouration in *Nothobranchius* spp. is sexually selected (Haas, 1976*b*) and showy sexual displays may be associated with increased mortality risk (Hunt *et al.*, 2004). Another plausible explanation is that high male mortality stems from severe male–male disputes over occupancy of superior positions in spawning arenas that are located at particular places within the habitat (Haas, 1976*a*) and where females probably prefer to lay their eggs. Male–male competition is intense in *Nothobranchius* species and often involves serious injuries to subordinate males (Huber, 2000).

The habitat of all *Nothobranchius* species is characterized by vertisol soils on alluvial deposits (Wildekamp, 2004) and *Nothobranchius* species populations do not occur in pools developed on laterite soils, because only vertisols provide suitable soil structure for survival of dormant eggs during the dry season (Wildekamp, 2004). The present study revealed that other habitat factors are also significantly associated with the presence of *N. furzeri* populations. The best predictors of *N. furzeri* presence were a soft muddy substratum and very turbid water (Figs 2 and 3). The water turbidity was probably associated with disturbance by domestic cattle. The cattle, their hoofprints and dung were often encountered at the *N. furzeri* sites, but no quantitative data were collected to substantiate this observation. *Nothobranchius furzeri* co-occurred with two other *Nothobranchius* species at several sites. Multivariate analysis of habitat associations revealed that *N. orthonotus* is intermediate in its habitat requirements between *N. rachovii*, a coastal plain species (Wildekamp, 2004), and *N. furzeri*. *Polypterus annectens brieni* and *C. gariepinus* sometimes co-occur with *N. furzeri* and are their potential predators. In contrast, *N. furzeri* was not recorded in sympatry with tilapias, which are otherwise abundant in many pools in the study area. This is likely to be a consequence of the different habitat requirements of tilapias, such as connection to permanent habitats.

It is believed that the present data on the distribution and ecology of *N. furzeri* will contribute to the understanding of the association between environmental conditions, life expectancy and the evolution of rapid onset of senescence. The latest advance of research on *N. furzeri* ageing revealed that a laboratory line derived from a population from a high altitude and low precipitation site (transect 3 in the present study) showed a shorter life span and more rapid onset of cognitive decline than a laboratory line derived from a population inhabiting a low altitude and high precipitation site (low altitude site at transect 1 in the present study) (Terzibasi *et al.*, in press). Ageing in those laboratory strains also differed from an established laboratory strain originated in the Gona Re Zhou (Valdesalici & Cellerino, 2003; Terzibasi *et al.*, 2007). While such results make *N. furzeri* an ideal model species for studies on genetic control of life-history traits (Genade *et al.*, 2005; Terzibasi *et al.*, 2007), data presented here show that a larger scale study using a series of natural populations along clines of altitude and environmental variables is possible and can bring stronger insights into the evolution of ageing. In particular, geographic variation in habitat duration is expected to be reflected in variation in developmental rates, ageing and life span. Preliminary data on six *N. furzeri* populations showed high inter-population genetic distances at a neutral genetic marker (a fragment of cox1 gene in the mitochondrial genome) (Terzibasi *et al.*, in press). This deep genetic structuring makes the system highly amenable to research into the genetic basis of the ageing process, including the identification of critical genes (and their expression) affecting the ageing process. Experimental gerontology has been so far largely limited to mutant strains of laboratory organisms (Terzibasi *et al.*, 2007), but recent progress in genomic procedures in natural populations of non-model species should enable synthesis of ecological, evolutionary and molecular approaches (Ellegren & Sheldon, 2008). Unlike the genes linked to the short life span in laboratory organisms, which are often coupled with serious developmental deficiencies (Terzibasi *et al.*, 2007), the genes responsible for rapid ageing in *N. furzeri* have been selected in the context of life-history evolution under natural conditions and are expected to result from adaptive trade-offs between costs and benefits associated with their functions. Hence, it is hoped that the present study constitutes an important step to the understanding of ageing in natural populations.

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APPENDIX. List of sampled pools within the range of *Nothobranchius furzeri*, with their position in the transect, latitude and longitude and presence of *Nothobranchius* spp. indicated

Site code	Transet	Latitude (S)	Longitude (E)	*N. furzeri*	*Nothobranchius orthonotus*	*Nothobranchius rachovii*
MZCS08-1	T1	24° 09·6	32° 48·1	1	1	1
MZCS08-2	T1	24° 03·8	32° 43·9	1	1	1
MZCS08-3	T1	24° 03·8	32° 43·9	1	0	1
MZCS08-4	T1	24° 03·8	32° 43·9	1	0	1
MZCS08-5	T1	24° 02·1	32° 42·1	0	0	0
MZCS08-6	T1	24° 02·1	32° 42·1	0	0	0
MZCS08-7	T1	23° 41·6	32° 36·6	1	0	0
MZCS08-8	T1	23° 41·6	32° 36·6	1	1	0
MZCS08-9	T1	23° 41·6	32° 36·6	1	1	0
MZCS08-10	T1	23° 37·4	32° 35·8	0	0	0
MZCS08-11	T1	23° 37·4	32° 35·8	0	0	0
MZCS08-12	T1	23° 28·4	32° 34·0	0	0	0
MZCS08-13	T1	23° 27·5	32° 33·8	1	0	0
MZCS08-14	T1	23° 08·1	32° 24·4	0	0	0
MZCS08-15	T1	23° 06·0	32° 21·7	0	0	0
MZCS08-16	T1	23° 05·1	32° 20·2	0	0	0
MZCS08-17	T1	23° 05·0	32° 19·8	0	0	0
MZCS08-18	T1	23° 00·8	32° 14·2	0	0	0
MZCS08-19	T1	22° 41·5	32° 02·0	0	0	0
MZCS08-20	T3	22° 44·7	32° 05·4	0	0	0
MZCS08-21	T3	22° 39·0	32° 16·3	0	0	0
MZCS08-22	T3	22° 32·3	32° 28·5	0	0	0
MZCS08-23	T3	22° 30·5	32° 33·0	1	0	0
MZCS08-24	T3	22° 30·5	32° 33·0	0	0	0
MZCS08-26	T3	22° 30·2	32° 34·2	0	0	0
MZCS08-27	T3	22° 28·9	32° 37·2	0	0	0
MZCS08-28	T3	22° 28·9	32° 37·2	1	0	0
MZCS08-29	T3	22° 27·0	32° 38·8	1	0	0
MZCS08-30	T3	22° 23·3	32° 40·1	0	0	0
MZCS08-31	T3	22° 23·3	32° 40·1	0	0	0
MZCS08-32	T3	22° 23·3	32° 40·1	0	0	0
MZCS08-33	T3	22° 21·8	32° 41·9	1	0	0
MZCS08-34	T3	22° 08·8	32° 49·5	1	1	1
MZCS08-35	T3	22° 10·9	32° 52·0	0	0	0
MZCS08-36	T3	22° 14·4	32° 54·9	0	0	0
MZCS08-37	T3	22° 20·7	32° 48·1	0	0	0
MZCS08-38	T3	22° 20·8	32° 47·8	0	0	0
MZCS08-39	T3	22° 21·8	32° 44·4	0	0	0
MZCS08-40	T3	22° 21·8	32° 43·5	1	0	0
MZCS08-41	T3	22° 31·8	32° 29·4	0	0	0
MZCS08-42	T3	22° 32·8	32° 27·9	0	0	0

APPENDIX. Continued

Site code	Transet	Latitude (S)	Longitude (E)	*N. furzeri*	*Nothobranchius orthonotus*	*Nothobranchius rachovii*
MZCS08-43	T1	23° 18·4	32° 32·1	1	1	1
MZCS08-44	T1	23° 26·9	32° 33·7	0	0	0
MZCS08-49	T1	24° 12·9	32° 50·0	0	0	0
MZCS08-50	T1	24° 12·9	32° 50·0	1	0	0
MZCS08-51	T4	24° 23·4	32° 53·7	1	0	0
MZCS08-52	T4	24° 22·8	32° 55·4	0	0	0
MZCS08-53	T4	24° 22·2	32° 57·0	1	0	0
MZCS08-54	T4	24° 22·2	32° 57·0	1	0	0
MZCS08-55	T4	24° 21·8	32° 57·7	1	0	0
MZCS08-56	T4	24° 21·8	32° 57·7	1	0	0
MZCS08-57	T4	24° 19·2	33° 01·9	0	0	0
MZCS08-58	T4	24° 17·8	33° 03·3	0	0	0
MZCS08-59	T4	24° 17·8	33° 03·4	1	1	1
MZCS08-60	T4	24° 17·8	33° 03·4	0	0	0
MZCS08-61	T4	24° 14·4	33° 09·8	1	1	0
MZCS08-62	T6	24° 27·5	33° 00·9	0	0	0
MZCS08-63	T6	24° 32·8	33° 07·7	0	0	0
MZCS08-64	T6	24° 32·9	33° 07·8	0	0	0
MZCS08-65	T6	24° 37·0	33° 16·7	0	0	0
MZCS08-66	T6	24° 39·9	33° 21·2	0	0	0
MZCS08-67	T6	24° 40·4	33° 24·6	0	1	1
MZCS08-68	T6	24° 48·6	33° 30·5	0	1	1
MZCS08-69	T7	25° 3·7	33° 60·0	0	0	0
MZCS08-70	T7	25° 07·1	33° 48·9	0	0	0
MZCS08-71	T7	25° 46·4	33° 40·5	0	0	0
MZCS08-72	T7	25° 47·6	33° 40·4	0	0	0
MZCS08-73	T6	24° 59·7	33° 34·0	0	0	0
MZCS08-74	T6	25° 00·0	33° 34·9	0	0	1
MZCS08-115	T6	24° 59·9	33° 34·3	0	0	0
MZCS08-116	T5	24° 44·8	33° 07·1	0	0	0
MZCS08-117	T5	24° 41·0	33° 05·5	0	0	0
MZCS08-118	T5	24° 29·0	32° 54·3	0	0	0
MZCS08-119	T5	24° 25·1	32° 46·7	1	0	1
MZCS08-120	T5	24° 19·5	32° 43·2	1	1	0
MZCS08-121	T4	24° 21·5	32° 58·4	1	1	0
MZCS08-122	T4	24° 18·2	33° 02·8	1	0	0
MZCS08-123	T5	24° 38·8	32° 26·7	1	0	0
MZCS08-124	T5	24° 35·6	32° 24·3	1	0	0