

Diel schedules of locomotor, reproductive and feeding activity in wild populations of African annual killifish

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Diel patterns of different activities arise from adaptations to periodic cycling of environmental parameters and may involve trade-offs between acquiring benefits and minimizing associated costs. In this study, we provide fundamental baseline data on diel activity of natural populations of *Nothobranchius* fishes, model organisms in laboratory studies, including links between diurnal rhythms and ageing. Initially, we quantified the diel change in activity in wild populations of three African killifish species (*Nothobranchius furzeri*, *Nothobranchius orthonotus* and *Nothobranchius pienaari*) and compared average activity between sexes. In all species, males were more active than females, probably as a result of their active pursuit of females. Swimming activity peaked at midday. In *N. furzeri*, the only species occurring at all sites, oocytes were ovulated in the early morning, and most spawning events had occurred by the early afternoon. Gut fullness and diet richness increased before spawning activity and peaked in the morning. Daytime diet was dominated by chironomid larvae, whereas notonectid bugs were the dominant prey at night, perhaps as a result of different prey detectability over the diel cycle. Finally, no loyalty to any particular pool section was detected in *N. furzeri*. Collectively, these data provide the first empirical description of diel activity in three wild populations of African killifish.

ADDITIONAL KEYWORDS: activity pattern – circadian activity – *Nothobranchius*.

INTRODUCTION

Organisms face periodic environmental changes, ranging from seasonal to diel cycles (Beale *et al.*, 2016). In animals, adaptation to the periodicity of 24 h cycles (diurnal rhythms) involves conspicuous diel patterns of particular activities, including individual locomotor activity (Davis, 1964), feeding regime (Metcalf *et al.*, 1999) and reproductive behaviour (Lema *et al.*, 2010). Physiologically, diurnal rhythms are synchronized endogenously (Ekström & Meissl, 1997). From an ecological and evolutionary perspective, diurnal rhythms have evolved to match particular activities with the most suitable abiotic (e.g. light intensity, temperature, oxygen level) and biotic (food availability, reproduction, intraspecific competition or predation risk) parameters (Daan, 1981; Helfman, 1986). For example, patterns of locomotor activity are optimized to allocate energy effectively for finding food, finding mating partners and rest (Werner *et al.*, 1993; Smith

& Blumstein, 2008) and represent a compromise between particular costs (e.g. predation risk, energy expenditure) and benefits (e.g. finding food or a partner) (Werner *et al.*, 1993; Metcalfe *et al.*, 1999).

The cost–benefit trade-offs behind temporal dynamics in activity patterns are often sex specific (Perrin & Mazalov, 2000; Croft *et al.*, 2003). Females allocate more energy to gamete production (Trivers, 1972; Graf *et al.*, 2010), whereas males are often the more active sex, being involved in searching for and competing for access to mates (Haas, 1976a; Croft *et al.*, 2003). Behavioural trade-offs constrain the concurrent incidence of two behaviours (Cuthill & Houston, 1997) and result in diel periodicity of specific behavioural repertoires, particularly in organisms with daily reproductive activities. For example, in Amargosa pupfish, *Cyprinodon nevadensis amargosae* Miller, 1948, feeding activity peaks in the morning, whereas courtship and spawning activity peaks in the afternoon (Lema *et al.*, 2010). Laboratory experiments have demonstrated that reproductive cycles are largely controlled endogenously and are not directly

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dependent on timing of the feeding regime (Weber & Spieler, 1987).

The main goal of this study was to characterize the diel change of locomotor activity, reproduction and feeding in wild populations of African annual killifishes of the genus *Nothobranchius*. In controlled laboratory conditions, *Nothobranchius* fishes are primarily active during daylight (Lucas-Sánchez *et al.*, 2013; Almáida-Pagán *et al.*, 2018), and light conditions are probably important in mate searching. Males are larger and brightly coloured (Sedláček *et al.*, 2014) and actively pursue females (Polačik & Reichard, 2011). Active mate searching indicates the importance of locomotory activity in male reproductive success. However, their higher activity and bright coloration expose males to higher predation risk from ambush predators (Haas, 1976b), which might explain why wild populations tend to be female biased (Reichard *et al.*, 2014). Light conditions might affect the feeding activity because *Nothobranchius* spp. are visual micropredators; their brain anatomy is characterized by a prominent optic tectum with small olfactory bulbs (D'Angelo, 2013), and their diet contains a large proportion of small, actively moving aquatic invertebrates (Polačik & Reichard, 2010). Thus, light conditions might have a profound effect on locomotory activity.

In addition to the main goal, we tested their loyalty to particular sites, suggesting potential territoriality. Territorial behaviour could affect the activity of an individual owing to its spatial restriction (Hanson *et al.*, 2008). Male territoriality is common in fishes, especially in sexually dimorphic species where males are larger (Gross & Sargent, 1985; Kodric-Brown, 1988). In laboratory conditions, male *Nothobranchius* have been observed to defend a spot with a spawning substrate (Polačik *et al.*, 2016). Although this has been implicated indirectly for wild populations (Reichard *et al.*, 2009), no study has yet examined territoriality of *Nothobranchius* males in the wild.

Nothobranchius fishes have an extremely rapid life history adapted to life in ephemeral pools (Reichard & Polačik, 2019). The eggs survive desiccation in the substrate, and fish hatch after heavy seasonal rains that briefly inundate their habitat. Upon hatching, they grow rapidly and mature within 2 weeks (Vrtílek *et al.*, 2018b). Sexually mature females ovulate a batch of 20–120 eggs every day (Vrtílek & Reichard, 2016). This represents high energetic demands that need to be covered by effective feeding. Under strong pressure of habitat desiccation (Grégoir *et al.*, 2018), they devote a significant proportion of time to reproduction. This might lead to behavioural conflict between feeding and reproduction, potentially resulting in temporally synchronized schedules of feeding and reproductive activity to maximize their efficiency.

We estimated the diel change of locomotor activity in wild populations of three species of African annual killifish. *Nothobranchius furzeri* Jubb, 1971, *Nothobranchius orthonotus* (Peters, 1844) and *Nothobranchius pienaar* Shidlovskiy *et al.*, 2010, in their natural habitat in southern Mozambique. We predicted high locomotor activity throughout the day, associated with feeding in the morning, when fish have to replenish energy reserves after the nocturnal period, and reproductive activity prevailing later in day after egg ovulation (Haas, 1976a; Lema *et al.*, 2010). This would be indicated by temporal dynamics in gut fullness, gonad mass and the number of ovulated oocytes in female ovaries. We further predicted males to be more active than females on average, driven by their reproductive strategy of active mate searching. Finally, we investigated territoriality in wild *Nothobranchius* by examining the fidelity of individual fish to a particular section of the pool using a spatially informed capture–mark–recapture study. Overall, this study provides the first evidence of diel change of locomotor activity, feeding regime and reproduction in wild *Nothobranchius* populations. This is important because African annual killifish are an established laboratory model in biomedical and ecological research (Cellerino *et al.*, 2016; Hu & Brunet, 2018), including circadian rhythmicity with regard to senescence (Valenzano *et al.*, 2006; Terzibaszi Tozzini *et al.*, 2017).

MATERIAL AND METHODS

The study was conducted at four sites (ephemeral pools in southern Mozambique) between March and May 2016. An overview and details of the sampling sites and procedures are given in Table 1, and detailed information on sample sizes is presented in the Supporting Information (Table S1).

DIET CHANGE OF ACTIVITY

To estimate diel change of fish activity, a 24 h snapshot study at three sites (sites 1–3) was performed (Table 1). Fish activity was estimated using non-baited minnow traps (260 mm × 260 mm × 450 mm, with two 55 mm funnel openings, mesh size 4 mm; Zebco Europe, Tostedt, Germany), a passive sampling method that required fish movement for capture (Diaz Pauli *et al.*, 2015; Žák *et al.*, 2018a). A pilot study showed that baited and non-baited traps were equally efficient for killifish, and non-baited traps did not attract lungfish, a potential killifish predator (Reichard *et al.*, 2014). No other fish species occurred at the study sites. Four (site 1) or six (sites 2 and 3) traps were used, depending on pool size and fish density. The traps were

Table 1. Overview of sampling sites

Site	GPS	Analyses	Trapping	Reference sampling	Fish age (days)	Pool area (m ²)	Maximal depth (cm)	Number of traps	CPUE (individuals per haul) of species ratio F:O:P and sex ratio F:M F:M F:M
Site 1	22°30'28"S 32°34'54"E	Activity	22 March	22 March	56	220	20	4	12.5:54.0:0.5
Site 2	23°30'15"S	Activity, dispersal, diet and reproductive parameters	30 April	1 May	50	1265	110	6	9.5:3.0 36.0:18.0 NA 3.0:0.8:0.0
Site 3	32°34'21"E 24°18'15"S	Activity	16 March	17 March	78	760	35	6	2.4:0.6 NA NA 2.0:2.0:5.8
Site 4	32°36'55"E 23°38'42"S	Reproductive parameters	NA	6 May	53	1110	45	-	NA NA 5.8:0.0 5.0:0.2:0.4
	32°36'07"E								3.8:1.2 NA NA

Catch per unit effort (CPUE) of species and sex ratio represent the number of fish per one seine net haul. Species and sex ratios were estimated from seine net samples (site 1, two seine net hauls; site 2, five seine net hauls; site 3, five seine net hauls). Owing to dense vegetation at site 3, additional dip net samples were taken to obtain more appropriate species and sex ratios, which differ slightly from the presented values from seining. Fish age is taken from [Vrtílek et al., \(2018a\)](#) and was estimated from otolith readings ([Reichard et al., 2017](#)) and recalculated for the date of reference sampling at a particular site. Abbreviations: F, *Nothobranchius furzeri*; F:M, female-to-male ratio, which is presented only for species and sites where sex-specific activity was analysed (sample size greater than five); O, *Nothobranchius orthonotus*; P, *Nothobranchius ptenaari*.

spaced (55–211 m² per trap) to minimize interference. All available pool habitats (deep water, surface water, vegetated sections and open sections) were sampled to capture potential non-random movement of fish in the pool. Trap openings were always fully submerged. Trapping over 24 h yielded 234 fish of three species across all three study sites (Supporting Information, Table S1).

During the day, traps were checked every hour to prevent trap saturation (Prchalová *et al.*, 2011; Bacheler *et al.*, 2013). At night, traps were checked at 3 h intervals because there was a lower risk of trap saturation; laboratory studies have demonstrated that *Nothobranchius* fishes are more active during the day (Lucas-Sánchez *et al.*, 2011). Fish removed from traps were immediately identified to species. Males possess species-specific colour patterns, and females can be readily separated by body shape and mouth position (Wildekamp, 2004). Then, fish were immediately released at the place of capture. The probability of recapturing the same individual was likely to be very low; a trap sampling at site 2 where 237 colour-marked *N. furzeri* were released (in one of four colours; see 'Home range in *N. furzeri*' section), 15 colour-marked individuals were recaptured. With that set of four colours on 237 individuals, no fish with the same colour was recaptured in the same trap in two consecutive time periods. Five identical combinations (colour/sex/trap) across two (of six) traps used at that site were captured during 24 h trap sampling, but it is not likely that they represented the same individual fish.

Each trap was equipped with a waterproof temperature and light datalogger (HOBO Onset Computer, UA-002-64, Bourne, MA, USA). Data were recorded every 1 min. In each pool, surface and bottom temperatures were recorded (Supporting Information, Fig. S1). Light intensity (in lux) was collected from the water surface datalogger (Supporting Information, Fig. S2). Oxygen concentration (in milligrams per litre) was measured in close proximity to the traps with a YSI ProODO hand-held meter (YSI Inc./Xylem Inc, USA) at site 3. No oxygen meter was available for sampling at the other sites. One surface (5 cm below the water surface) and one bottom (10 cm above the substrate) measurement was completed near each trap (Supporting Information, Fig. S3). Oxygen measurements were completed before every second checking of each trap during the day and every 3 h overnight. The mean values calculated from three surface and three bottom measurements of oxygen concentration at each 2 h sampling interval were used for analysis (at night, they were estimated as the mean value from the two closest sampling points).

For statistical analysis, fish activity was expressed as the cumulative number of fish collected per 2 h of trapping relative to the total catch (sampling unit,

altogether from three sites; $N = 36$ sampling units). A 2 h trapping interval was chosen to reduce the probability of zero captures during the period of fish activity. The three species were pooled in the analysis, given variations in environmental (e.g. size, turbidity, habitat structure) and biotic (species and sex ratio, fish density) conditions that confound a species-specific interpretation of activity. In addition, we obtained more adequate values for each sampling point by species pooling ($N = 234$ fish) and a better model fit indicated by the Akaike information criterion (AIC) (AIC pooled = 102; AIC with species identity = 216). All three coexisting species are also ecologically similar (Žák *et al.*, 2018b; Lambert *et al.*, 2019), which makes data pooling ecologically relevant. The data were square-root transformed, enabling the use of a generalized additive model (GAM) with a Gaussian error distribution. A GAM with a binomial error structure was originally used, but it yielded a skewed distribution of residuals and low model predictability.

All recorded explanatory variables were strongly associated and non-independent (concurrency 0.62–0.99). Therefore, separate models were fitted for each explanatory variable (always with and without an interaction with site), and their relative fits were compared using the AIC (Table 2) (Wood *et al.*, 2016). Given superior fits of models with interactions, the effect of predictor on site-specific activity was interpreted from interaction terms. All continuous variables (except for the response variable and time of day, given that sampling times were the same at all sites) were standardized (zero-mean centred and scaled to 1 SD) before analysis to enable interpretation of models with an interaction term (Schielzeth, 2010).

SEX-SPECIFIC ACTIVITY

Reference estimates of sex ratio were obtained using a seine net (2.7 m long, 0.7 m deep, mesh size 4 mm) and a dip net (triangular 0.45 m × 0.45 m × 0.45 m, attached to a 1.5 m wooden pole) within 1 day before or after trapping (Table 1). Seine and dip net collection provides unbiased estimates of killifish sex and species ratios (Diaz Pauli *et al.*, 2015; Vrřilek *et al.*, 2018a). Estimates were based on five seine net hauls at sites 2 and 3; site 1 accommodated only two hauls owing to its small surface area (Table 1). Site 3 was densely vegetated, making seine netting suboptimal; therefore, ratios were estimated from an additional 100 dip net sweeps only. Overall, reference sampling yielded 187 fish (34 *N. furzeri*, 117 *N. orthonotus* and 36 *N. pienaar*).

The hypothesis of higher male activity was tested by comparing sex ratios from traps with those from reference samples. For this analysis, samples were pooled across 24 h of trapping within each site.

Table 2. Quantitative comparison of candidate models (generalized additive models with Gaussian error) of fish activity that included different environmental explanatory variables

Model name	Model structure	d.f.	ΔAIC
Light × Site	relative_catch~s(light,by=site)+site	13.009	0
Time × Site	relative_catch~s(time,by=site)+site	14.462	3.27
Light + Site	relative_catch~s(light)+site	12.839	15.3
Surface temperature × Site	relative_catch~s(surface_temperature,by=site)+site	9.081	34.42
Time + Site	relative_catch~s(time)+site	7.809	36.66
Bottom temperature × Site	relative_catch~s(bottom_temperature,by=site)+site	9.783	46.48
Surface temperature + Site	relative_catch~s(surface_temperature)+site	5	49.6
Bottom temperature + Site	relative_catch~s(bottom_temperature)+site	5	55.09

Degrees of freedom and difference in Akaike information criterion (ΔAIC) from the best model are shown for models with time of the day, light intensity, surface temperature and bottom temperature as explanatory variables, with and without an interaction between environmental predictor and site.

Only samples greater than five (species/sex/method combination) were used in the analysis. A generalized mixed-effect model (GLMM) with a binomial error distribution was used, with sex ratio as the response variable and sampling method (traps, reference/nets) as the explanatory variable. Species and site were included as random factors. A second model was fitted to test for a potential effect of species on sex-specific activity, with species as a fixed factor in an interaction with sampling method. Non-significant terms were removed from the model on the basis of AIC.

HOME RANGE IN *N. FURZERI*

One week before the activity experiment (Table 1), we estimated how far killifish disperse within site 2. We divided the pool area into four equal sections. In each section, killifish were collected using nine seine net hauls (5–10 m long). A total of 237 *N. furzeri* individuals were captured across all sections. Fish were anaesthetized with clove oil, measured for body size and marked subcutaneously on their upper flank with a section-specific VIE (visible implant elastomer; Northwest Marine Technology, USA) colour mark (33 green, 40 orange, 33 white and 131 yellow). After full recovery from anaesthesia, the fish were released back to the section of their origin. All sections were resampled after 24 h using the same method and effort. The VIE mark, sex and body size were recorded for all recaptured fish. We assumed that 1 week was sufficient for full recovery and did not affect the activity measurements (trapping) 1 week later.

Fidelity of *N. furzeri* to a particular section of the pool was estimated using a set of section-specific multinomial regressions. The proportion of individuals with a particular colour mark recaptured in a section were compared with 1000 sets of individuals randomly selected from all marked fish. The number of individuals in the random sample was equal to

the number of individuals recaptured in the section. Predictors were origin (recaptured or randomly selected individuals), sex and body size. Confidence intervals (95%) of χ^2 , *P*-values and likelihood of *P*-value being > 0.05, derived from 1000 permutations, were estimated for each section separately.

DIEL PATTERN IN FEEDING

Gut fullness, diet richness and diet composition were estimated from a sample of 100 *N. furzeri* (50 males and 50 females) collected at site 2. Five samples were taken at 05.00 (1 h before onset of activity), 10.30 (increasing activity), 14.30 (culminating activity), 17.45 (decreasing activity) and 00.00 h (zero activity) using a 2.7 m seine net until ten females and ten males were collected. Captured fish were euthanized with an overdose of clove oil and stored in 4% formaldehyde solution for later dissection. The fish were dissected in the laboratory at the Institute of Vertebrate Biology in Brno, Czech Republic. Their gut length was measured to the nearest millimetre. Gut fullness was estimated as the difference in mass between the full and rinsed gut (weighed to the nearest 0.001 g). Analysis of diet was conducted semi-quantitatively, by visual estimation of the relative biomass proportions of diet categories (De Crespín De Billy *et al.*, 2000). Fourteen categories were distinguished (Chironomidae, Notonectidae, Cladocera, Ostracoda, Copepoda, Odonata, Ephemeroptera, terrestrial insects, Coleoptera, Isopoda, tadpoles, killifish eggs, mineral matter and plant detritus). Diet richness was estimated as the number of different diet categories in the gut of individual fish.

The temporal pattern in gut fullness was analysed using GAM with a Gaussian error distribution. Gut fullness (mass of gut content) was the response variable, and time of day was a continuous explanatory variable (five sampling points) with a set basis dimension, *k* (*k* = 4 was selected over *k* = 5 on the basis of model

parsimony based on equal AIC). Sex was added as a fixed factor, and gut length was included as a covariate ($k = 7$) because there was temporal change in gut length (ANOVA, $F_{4,94} = 5.24$, $P < 0.001$). Temporal dynamics in diet richness was tested using a GAM with a Poisson error (with unknown scale parameter = -1). Diet richness was the response variable, time of day was a continuous explanatory variable ($k = 4$), and sex was a fixed factor. Gut fullness was included as a covariate ($k = 3$).

Temporal dynamics in the probability of dominance of a specific diet category (i.e. diet category with the highest relative biomass in a particular individual) was examined using multinomial regression. Dominant diet category was the response variable, and time of day (a factor with five levels; factor was selected over continuous variable to observe a potential non-linear pattern, i.e. diel cyclicity), sex and their interaction were included as explanatory variables. Body size was added as a covariate. Multiple pairwise comparisons were performed using Tukey's HSD test.

DIEL PATTERN IN REPRODUCTION

Reproductive parameters (ovarian mass and the number of ovulated eggs in females and testicular mass in males) were estimated from the same individuals (i.e. 50 males and 50 females from site 2) as for dietary parameters and, additionally, from another 50 *N. furzeri* females from nearby site 4. Site 4 was sampled at five time points: 08.00, 10.00, 12.00, 14.00 and 17.00 h. Both sites were sampled on the same day (and were of comparable age; Table 1), 1 week after the diel activity change was estimated at site 2 (Table 1). The eviscerated body mass and gonad mass of each fish were weighed to 0.001 g. The number of ovulated oocytes (discernible by their orange translucent appearance) in ovaries was counted under a stereomicroscope.

The temporal pattern in gonad mass was analysed using sex and site (owing to site-specific sampling times) specific GAMs with a Gaussian error distribution. Changes in ovarian mass were tested using time of day as a continuous explanatory variable (both sites, $k = 5$) and eviscerated body mass as a covariate (site 2, $k = 4$; site 4, $k = 3$) given a superior estimate of model validity. Diel change in testicular mass at site 2 was analysed by linear regression (given the linear relationship), with time of day as a continuous explanatory variable and eviscerated body mass as a covariate. The site-specific change in the number of mature oocytes during the day was tested using zero-inflated Poisson GAM, with time of day as a continuous explanatory variable (site 2, $k = 5$; site 4, $k = 3$). Eviscerated body mass was not included as a covariate, because it did not vary temporally across

sampling period (site 2, one-way ANOVA, $F_{4,45} = 0.57$, $P = 0.683$; site 4, $F_{4,45} = 2.12$, $P = 0.094$), and the number of oocytes was more affected by time of day than by female body mass (see Results). Note that adding eviscerated body mass to the model provided concordant conclusions.

All data analyses were conducted in the R v.3.4.3. environment (R Core Team, 2017), using *mgcv* (Wood, 2017), *MuMIn* (Bartoń, 2016), *lsmmeans* (Lenth, 2016), *lme4* (Bates et al., 2015) and *nnet* (Venables & Ripley, 2002) packages.

All work was carried out in accordance with relevant guidelines and regulations. Sample collection complied with the legal regulations of Mozambique (collection licence: ADNAP-170/7.10/16), and research procedures and experimental protocols were approved by the ethical committee of the Institute of Vertebrate Biology (79-17-UBO), in accordance with legal regulations of the Czech Republic.

RESULTS

DIEL CHANGE IN ACTIVITY

A clear unimodal activity pattern was detected at two sites (sites 2 and 3), with the peak of activity at midday. No temporal change in diel activity was apparent at site 1 (Fig. 1; Table 3A). This variation among sites resulted in significant interactions between environmental predictors and site for all parameters measured (time of day, light level and water temperature; Table 2). Light level and time of day were the best predictors of diel activity change at sites 2 and 3 (Table 2), followed by water temperature. At site 3, where oxygen measurements were performed, killifish activity was also related to surface oxygen concentrations (Supporting Information, Table S1). Predictor-specific models are provided in Table 2.

Males were more active than females. The proportion of males in traps was 25% higher than in the reference sample (GLMM: $\chi^2 = 23.52$, d.f. = 1, $P < 0.001$; Fig. 2). Male-biased activity was consistent among all three species (GLMM: species, $\chi^2 = 3.43$, d.f. = 2, $P = 0.180$; species \times method interaction, $\chi^2 = 2.54$, d.f. = 2, $P = 0.280$).

HOME RANGE

A total of 127 out of 237 marked *N. furzeri* were recaptured (54%). Recaptured fish were distributed randomly throughout the pool in relationship to their initial capture location, suggesting large home ranges. There was no effect of tag colour, sex or body size on the probability of recapture in the same pool section (Table 4).

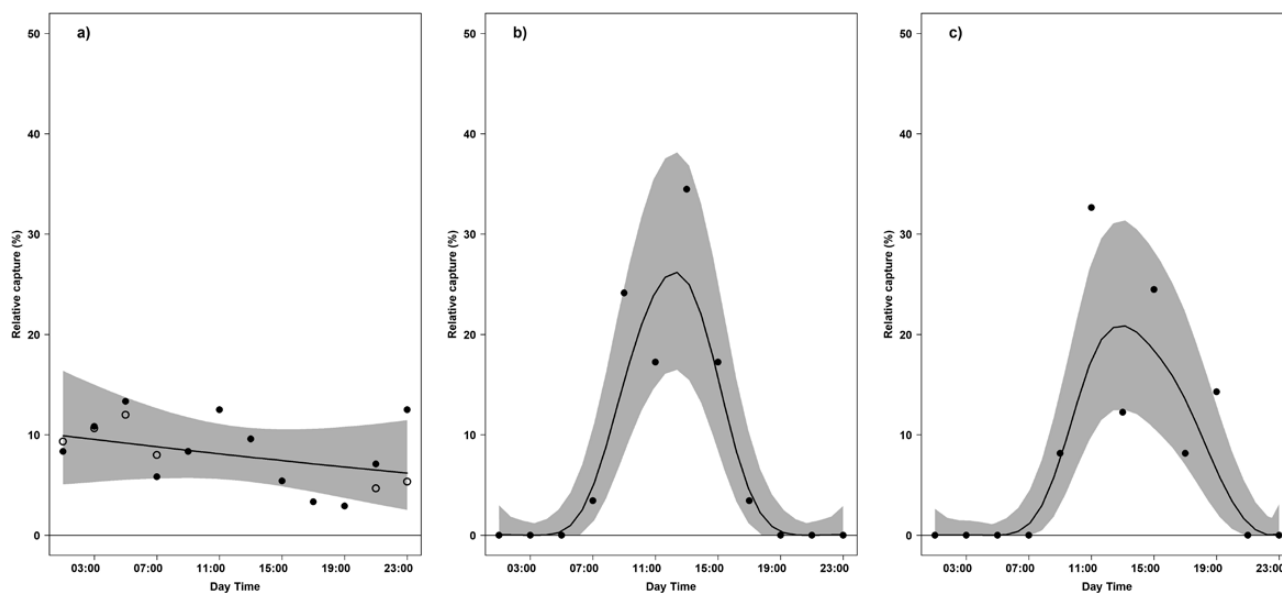


Figure 1. Diel change of locomotor activity. Diel dynamics of locomotor activity were estimated from the fit of generalized additive models at site 1 (A), with no peak in activity, and at sites 2 (B) and 3 (C), with a clear peak of activity. Filled circles represent relative catch per each sampling point calculated from the total catch. Open circles represent additional observations at site 1 (trapping continued after 24 h) that were not included in the analysis. Grey area indicates 95% confidence intervals.

REPRODUCTIVE PARAMETERS

Ovarian mass declined significantly during the day at both sites [GAM: site 2, time $F = 6.50$, estimated degrees of freedom (e.d.f.) = 3.96, $P < 0.001$; site 4, time $F = 6.03$, e.d.f. = 3.751, $P < 0.001$], with a gradual decrease from noon (12.00 h) at site 4 and a mid-afternoon (14.30 h) decrease at site 2 (Fig. 3A). Eviscerated body mass was a significant covariate (site 2, $F = 5.82$, e.d.f. = 2.62, $P = 0.005$; site 4, $F = 17.26$, e.d.f. = 1.33, $P < 0.001$).

The number of ovulated oocytes had a clear unimodal pattern at both sites (GAM: site 2, $\chi^2 = 474.40$, e.d.f. = 3.95, $P < 0.001$; site 4, $\chi^2 = 113.5$, e.d.f. = 1.89, $P < 0.001$; Fig. 3B). Interestingly, peaks in the number of ovulated oocytes differed between the sites. At site 4 (sampled at 08.00, 10.00, 12.00, 14.00 and 17.00 h), the highest number of ovulated oocytes was recorded at 08.00 h, with all ovulated oocytes spent by 14.00 h. At site 2 (sampled at 05.00, 10.30, 14.30, 17.45 and 00.00 h), females had not ovulated oocytes 1 h before sunrise (05.00 h), and the model suggested that their number peaked between 10.30 and 14.30 h, followed by a sharp decline (Fig. 3B).

Testicular mass did not vary over the diel cycle ($F = 0.01$, d.f. = 1, $P = 0.944$), and eviscerated body mass was a significant covariate of testicular mass ($F = 25.84$, e.d.f. = 1.00, $P < 0.001$).

DIET AND FEEDING PATTERNS

Gut fullness varied significantly over time (GAM: time, $F = 6.83$, e.d.f. = 2.90, $P < 0.001$), being lowest

before sunrise (05.00 h), peaking at 10.30 h and then gradually decreasing (Fig. 4A). Gut fullness did not differ between the sexes ($F = 2.14$, d.f. = 1, $P = 0.147$). Gut length was a significant covariate of gut fullness ($F = 7.91$, e.d.f. = 5.23, $P < 0.001$). The time \times sex interaction was omitted from the final model ($\Delta\text{AIC} +4.28$).

Diet richness also exhibited temporal dynamics (GAM: time, $F = 3.17$, e.d.f. = 2.86, $P = 0.030$), with a peak in mid-morning (10.30 h; Fig. 4B). Gut fullness ($F = 5.11$, e.d.f. = 1.34, $P = 0.021$) was a significant covariate. The sexes did not differ in diet richness ($F = 0.85$, d.f. = 1, $P = 0.359$). The time \times sex interaction did not improve model fit ($\Delta\text{AIC} = -1.06$) and was omitted from the final model. Within individuals, diet was dominated by chironomid larvae (40% of individuals), notonectids (35%), non-aquatic insects (17%) and Odonata larvae (5%). Copepoda, tadpoles and Ephemeroptera were rarely dominant (< 5% individuals), hence they were omitted from analysis of the diel pattern along with all minor diet categories (Cladocera, Ostracoda, Coleoptera, Isopoda, killifish eggs, mineral and plant detritus). The dominant diet varied over the day (multinomial regression: $\chi^2 = 88.2$, d.f. = 12, $P < 0.001$) and was significantly affected by fish body size ($\chi^2 = 11.3$, d.f. = 3, $P = 0.010$). Chironomid larvae were the dominant dietary item during the day (Fig. 5A), whereas notonectids (mainly nymphs) were dominant at night (Fig. 5B). The dominance of terrestrial insects increased from afternoon to

Table 3. Results of generalized additive models with continuous predictor in interaction with site: A, time of the day; B, light intensity; and C, results of two separate models of surface and bottom oxygen concentration at site 3

A, time of the day				
Parametric terms		d.f.	<i>F</i>	<i>P</i> -value
Site		2	4.397	0.0244
Approximate significance of smooth terms	e.d.f.	Ref.d.f.		
Time:Site 1	1	1	0.727	0.403
Time:Site 2	4.881	5.96	11.825	< 0.001
Time:Site 3	4.581	5.616	10.483	< 0.001
B, light intensity				
Parametric terms		d.f.	<i>F</i>	<i>P</i> -value
Site		2	0.43	0.655
Approximate significance of smooth terms	e.d.f.	Ref.d.f.		
Light:Site 1	1	1	0	0.983
Light:Site 2	2.256	2.674	29.31	< 0.001
Light:Site 3	5.753	5.946	12.94	< 0.001
C, oxygen concentration				
Predictor	e.d.f.	Ref.d.f.	<i>F</i>	<i>P</i> -value
Surface oxygen concentration	1.798	2.191	4.082	0.516
Bottom oxygen concentration	1.934	2.404	4.713	0.037

Statistically significant predictors are indicated in bold.
Abbreviations: e.d.f., effective degrees of freedom; Ref.d.f., reference degrees of freedom.

midnight (Fig. 5C), and the dominance of Odonata larvae showed no temporal pattern (Fig. 5D).

DISCUSSION

Diel changes in activity are shaped by ecological and evolutionary constraints (Fry, 1947; Daan, 1981). In accordance with our predictions, we demonstrated that *Nothobranchius* annual killifish were generally active during daytime, with a single peak of activity around noon, although at the site with an exceptionally high population density (Vrřílek *et al.*, 2018a) no diel changes in activity were detected. Time, light intensity, surface temperature and oxygen concentration near the surface were all significantly associated with activity levels. Males were more active than females of all species. Unexpectedly, analysis of the spatial distribution of marked *N. furzeri* suggests that individuals move across the whole pool and lack permanent territories. Females ovulated their eggs only after sunrise, and most eggs were oviposited between late morning and mid-afternoon, with high feeding activity before oviposition of all oocytes. Chironomid larvae were the dominant diet during the day, whereas notonectids dominated at night.

Diel variation in locomotor activity is shaped by various requirements (such as feeding, social and reproductive activities) and constraints (such as environmental

conditions or predator avoidance) (Noakes, 1992; Fraser *et al.*, 2004; Claireaux & Lefrançois, 2007). African annual killifishes maintained strong diurnal activity at two out of three sites (Fig. 1), consistent with variation in their activity in laboratory conditions (Lucas-Sánchez *et al.*, 2011; Almáida-Pagán *et al.*, 2018). A strong peak in activity at midday, during the period of highest light intensity, corresponded to the period of the day when visibility in the habitat was most favourable (Supporting Information, Fig. S2). We suppose that high visibility is important for effective foraging and for mate searching in these visually oriented micropredators. We speculate that a unimodal activity pattern in killifish is not suppressed by their potentially increased detectability from visually orienting avian predators (Haas, 1976b; Reichard *et al.*, 2018). In addition to light levels, water temperature and oxygen concentration were also strongly associated with the diel change in killifish locomotor activity, but they peaked and dropped later than activity and light intensity. This light–activity association is in accordance with observations from stable temperature and oxygen conditions in captivity, where light manipulation (turned on/off) has a clear impact on killifish activity (Lucas-Sánchez *et al.*, 2011; Almáida-Pagán *et al.*, 2018). In addition, oxygen concentration acts as a limiting factor on activity (Fry, 1947; McKenzie & Claireaux, 2010), especially when fish experience oxygen concentrations as low as 0.25 mg L⁻¹, as observed in our study (Supporting Information, Fig.

S3). These low oxygen concentrations are characteristic for African (J. Žák and M. Vrtílek, personal observation) and South American ephemeral pools (Podrabsky *et al.*, 1998; Podrabsky & Wilson, 2016). Hence, low oxygen concentration can have an additional effect on diel activity changes in African killifish.

We acknowledge that the propensity to enter a trap is not necessarily associated only with locomotor activity but can be related to variation in personality traits (Biro & Dingemanse, 2009). For example, traps might be used by killifish as a shelter during the period of highest visibility to visually oriented predators. However, this explanation is at odds with the assumption of decreased antipredator response

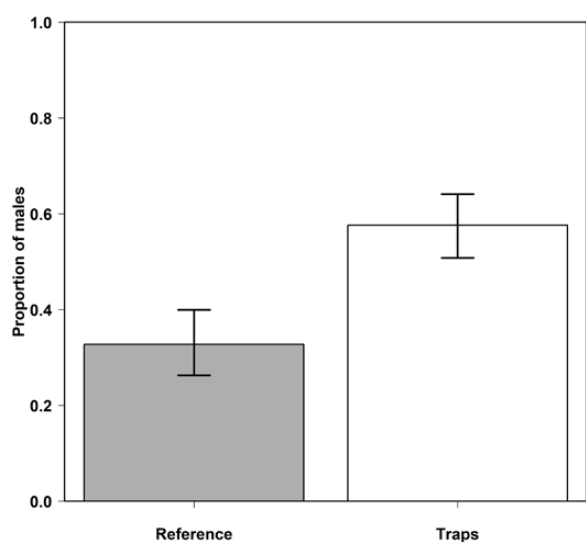


Figure 2. Males are the more active sex. The proportion of males in reference samples and trapping indicates their overrepresentation in traps. Data were pooled across species and sites (for sites 1–3). Whiskers represent 95% confidence intervals.

of African killifish in unpredictable environments (Polačik & Janáč, 2017). In addition, the increase in female bias over the season (Reichard *et al.*, 2014) does not indicate substantial antipredator behaviour among males. Across several species, traps were more frequently visited by bolder individuals (Wilson *et al.*, 1993; Biro & Dingemanse, 2009), which does not suggest that traps were used as shelters. Hence, we conclude that the entry of killifish into traps is most probably related to their activity or, potentially, to greater boldness in males. We accept, however, that the proximate cause of entrance into traps can only be disentangled in a dedicated laboratory experiment.

Fish activity is a plastic trait and responds flexibly to environmental cues (Reebs, 2002; Claireaux & Lefrançois, 2007) and physiological state (Metcalf & Steele, 2001) despite strong constraints and trade-offs for the best timing of particular activities. The absence of any change in *Nothobranchius* activity at site 1 is likely to be related to the unusually high fish density [44 fish per standardized haul; compared with six to 13 fish per standardized haul from pools of comparable age (Vrtílek *et al.*, 2018a)], perhaps leading to physical interference between conspecifics, resulting in increased activity at night (Fingerle *et al.*, 2016). High fish density also reduces resources such as food and, consequently, slows growth (Post *et al.*, 1999). Indeed, fish at site 1 were smaller than usual for their age (Vrtílek *et al.*, 2019). Hungry fish are more active as they search for food (Metcalf & Steele, 2001), and this may also have contributed to the observed modification of diel activity. Another possibility is that site 1 was strongly dominated by *N. orthonotus*, which might indicate that this species is not strictly diurnal. However, we do not believe this to be the case, because all 18 trapped *N. orthonotus* individuals at sites with normal fish density (sites 2 and 3; compare values in Table 1 with those of Vrtílek *et al.* 2018a) were captured during the day. In addition to mechanistic explanations (interference and persistent

Table 4. Section-specific analyses of *Nothobranchius furzeri* within-pool dispersal

Variable	χ^2	<i>P</i> -value	Prob > 0.05	χ^2	<i>P</i> -value	Prob > 0.05
	Section 1			Section 2		
Capture	6.17–6.69	0.214–0.245	0.685	3.24–3.53	0.415–0.447	0.949
Sex	6.01–6.52	0.218–0.248	0.707	4.97–5.32	0.246–0.274	0.836
Body size	7.45–7.95	0.132–0.155	0.554	2.74–2.96	0.475–0.508	0.979
	Section 3			Section 4		
Capture	2.35–2.61	0.547–0.581	0.968	1.60–1.75	0.660–0.688	0.996
Sex	2.31–2.53	0.542–0.575	0.985	3.74–4.09	0.385–0.424	0.902
Body size	2.55–2.79	0.506–0.539	0.980	7.72–8.28	0.143–0.169	0.515

Results are based on 1000 replicates of multinomial regression comparing the proportions of specific colour marks in the sample of recaptured fish with simulated datasets from repeated random sampling of all marked individuals. The 95% confidence intervals of χ^2 values and *P*-values from 1000 simulated tests are presented. The probability of the *P*-value from 1000 simulated tests being > 0.05 (Prob > 0.05) provides the likelihood of the *P*-value becoming non-significant. All tests are with three degrees of freedom.

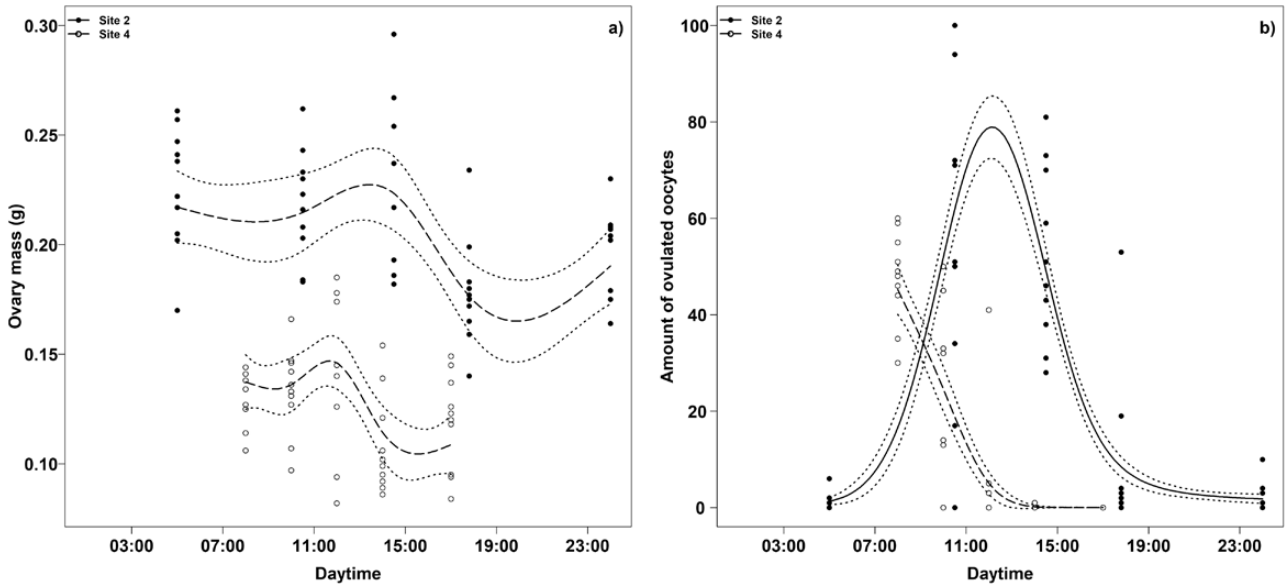


Figure 3. Reproductive parameters in *Nothobranchius furzeri* females. Generalized additive model (GAM) estimates from sites 2 and 4 demonstrate site-specific estimates of ovarian mass (GAM with a Gaussian error; A) and site-specific estimates of the number of mature oocytes (GAM with a zero-inflated Poisson error; B). Filled circles and continuous line represent estimates for site 2; open circles and dashed line represent estimates for site 4. Dotted lines are 95% confidence intervals.

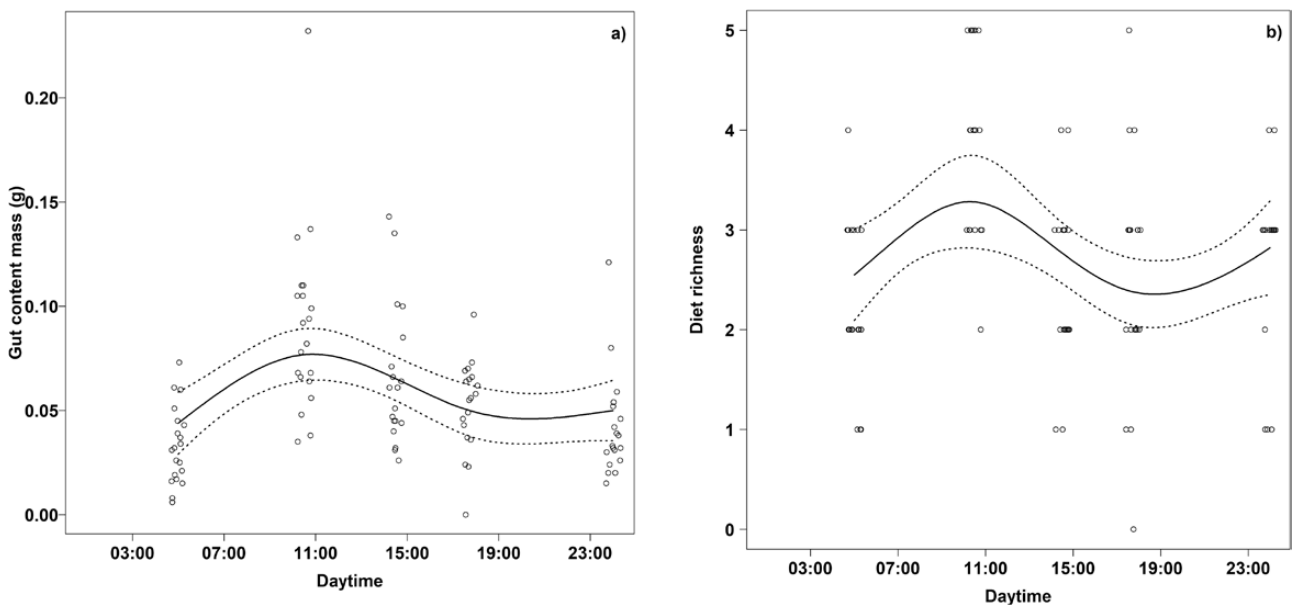


Figure 4. Diel dynamic of gut content mass and dietary richness in *Nothobranchius furzeri*. Model estimates of diel dynamic of gut content mass in both sexes of *N. furzeri* [generalized additive model (GAM) with a Gaussian error structure; A] and dietary richness (GAM with a quasi-Poisson error; B). Estimates of gut content mass are corrected for gut length. Estimates of dietary richness are corrected on gut content mass. Dashed lines represent 95% confidence intervals.

search for food), high population density and lack of food are major stressors, and activity patterns become aphasical in chronically stressed fish (MacFarlane & Livingston, 1983).

Sexual differences in locomotor activity are contingent on the mating system (Perrin & Mazalov, 2000; Croft *et al.*, 2003) and result in increased risk of sex-specific predation (Haas, 1976b). We found that males were

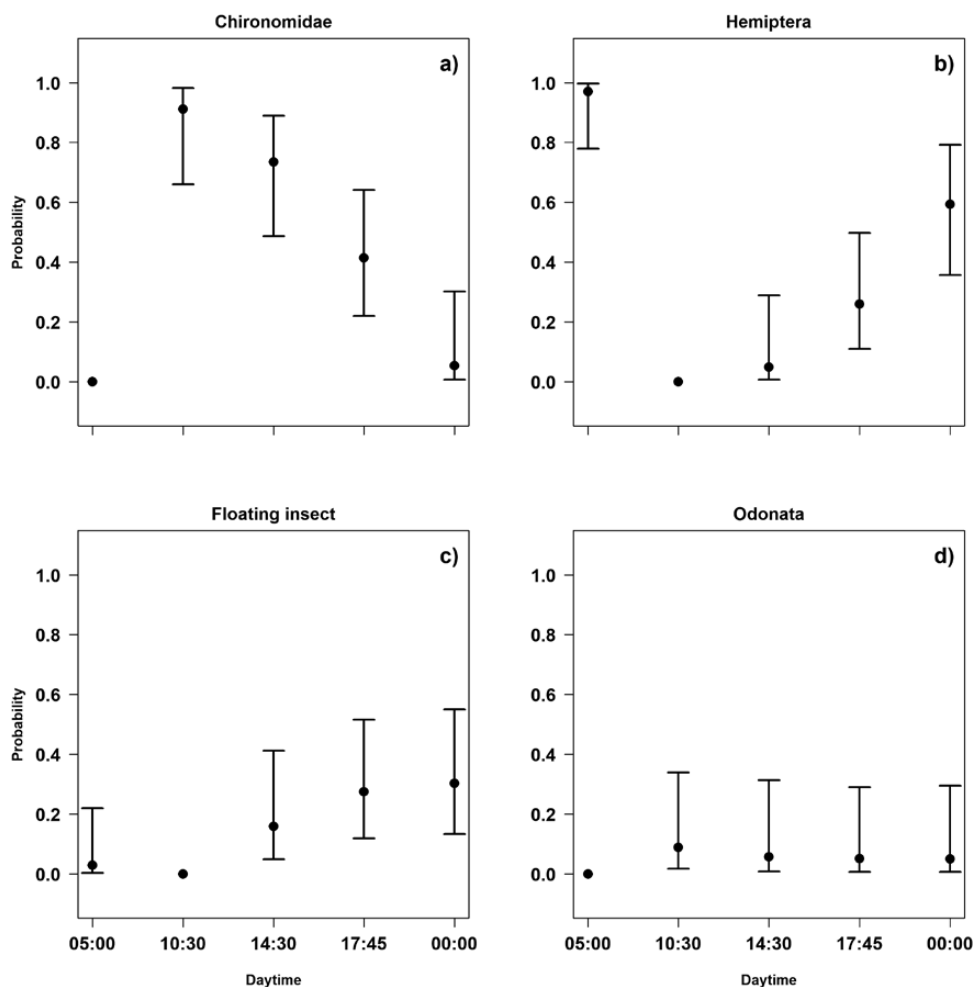


Figure 5. Diel change in the probability of dominance in the gut of *Nothobranchius furzeri* from site 2 in the four most frequently dominant dietary categories. Results are derived from multinomial regression, with time of day as a fixed factor. Error bars represent 95% confidence intervals.

more active than females across all three species. Spawning in African killifishes involves males actively pursuing females (Polačik & Reichard, 2011). High male activity conceivably increases their reproductive success, in a similar manner to Trinidadian guppies (*Poecilia reticulata*), in which active males enjoy higher mating success (Croft *et al.*, 2003). Higher male activity exposes them to a higher risk from ambush predators, such as giant *Belostoma* bugs (Heteroptera) and piscivorous birds (Haas, 1976b; Reichard *et al.*, 2014), thus female-biased natural populations are common in the wild (including the sites examined in this study) despite equal sex ratios at maturation (Reichard *et al.*, 2014; Vrřilek *et al.*, 2018a).

Male territories in the form of spawning arenas have been suggested in wild *N. furzeri* (Reichard *et al.*, 2009). Our data on dispersal of *N. furzeri* within the entire pool over a 24 h period contradict the suggestion of permanent territories. Marked fish

dispersed across the entire pool over a short period, with no difference between males and females. It is possible that *Nothobranchius* males possess short-term spawning arenas that are dissolved at night and resettled the next day (Lucas *et al.*, 2001), with no individual connection to the arena occupied the previous day. We speculate that the mating behaviour of *Nothobranchius* males involves active pursuit of females and opportunistic use of spawning sites rather than signalling and oviposition in permanent territories. Nevertheless, the possibility that random redistribution of *N. furzeri* is a consequence of disturbance caused by our capture, release and recapture efforts cannot be excluded entirely. Although our data suggest that the African killifish mating strategy does not include permanent territories or spawning arenas, we acknowledge that only direct observation of male behaviour in natural conditions might ultimately resolve the potential existence of

short-term male-defended locations that are observed in the laboratory, where the oviposition substrate is spatially constrained (Polačik *et al.*, 2016).

One of the behavioural conflicts is between individual maintenance and reproduction (Cuthill & Houston, 1997; Heino & Kaitala, 1999). Activities directly associated with individual maintenance, such as feeding, are usually prioritized over reproduction, and when nutritional requirements are not met, reproduction can cease (Tyler & Dunn, 1976). Such behavioural conflict is especially critical in short-lived income breeders, such as annual killifish. The highest scores of gut fullness and dietary richness in morning samples demonstrate that feeding is prioritized over reproduction early in the morning in *N. furzeri*. This is similar to the temporal partition of those behaviours (morning peak in feeding activity, afternoon peak in reproduction) in the Amargosa pupfish (Lema *et al.*, 2010).

Annual killifish are income breeders and expend energy on reproduction on a daily basis (Blažek *et al.*, 2013; Vrtilek & Reichard, 2015), resulting in diel periodicity of gonad status (Vrtilek & Reichard, 2016). We recorded the highest ovarian mass in the morning, with a decline from noon to evening. This decrease implies an average of 4% of eviscerated body mass (estimated as the average decrease in ovarian mass between morning and afternoon samples) spent daily on eggs production. Contrary to females, males displayed no diel change in gonad mass.

Light conditions appear to trigger ovulation in *Nothobranchius*. Several environmental cues (such as photoperiod or temperature) modulate egg ovulation (i.e. release of the mature egg from its follicle, making it ready for oviposition) in fishes (Wallace, 1978; Wang *et al.*, 2010). For example, the light–dark cycle governs oviposition in medaka (*Oryzias latipes*) (Ueda & Oishi, 1982; Weber & Spieler, 1987). It was believed that the eggs of *Nothobranchius* females are ovulated overnight (Vrtilek & Reichard, 2016) or shortly after sunrise (Haas, 1976a). We found that *Nothobranchius* females had almost no ovulated eggs at night, even as late as 1 h before sunrise (at 05.00 h), but ovaries were full of ovulated eggs shortly after sunrise (08.00 and 10.30 h). Although a sharp increase in light intensity over that period coincides with the changes in water temperature, laboratory studies report the same cyclicity of ovulation in constant temperature conditions (Blažek *et al.*, 2013; Vrtilek & Reichard, 2015). The duration of reproductive activity (i.e. the interval between the peak number of ovulated eggs in the ovaries and the time by which all eggs were spent) varied between populations. The different temporal estimates of peak egg abundance in ovaries, revealed in our analysis, could be related to slightly different sampling schedules between the two populations.

Variation in the time by which all eggs were spent was likely to be related to population density and availability of males, because females spent their ovaries sooner at site 4, where males were more abundant (Table 1). We suggest that sunrise ovulation might be a common trait for the *Nothobranchius* genus in the wild, because this has also been observed in *Nothobranchius guentheri* in a mesocosm (Haas, 1976a).

The extremely condensed life history of annual killifish (Vrtilek *et al.*, 2018b) and daily reproduction require a high energetic income. This requires substantial feeding activity, with diel patterns driven by trade-offs such as reproduction and/or prey detectability, which is related to light intensity (Maglio & Rosen, 1969; Lema *et al.*, 2010). Our data suggest that feeding is incessant; the guts of *N. furzeri* were never empty (except for a single male), including samples collected at night (00.00 and 05.00 h). Slow digestion and excretion time is an unlikely alternative explanation, because the diet differed qualitatively over the diel cycle. During daytime, the diet was dominated by chironomid larvae, coupled with an increase in the proportion of terrestrial insects in late afternoon. At night, notonectids (mainly nymphs) dominated the diet. Different digestion time among different prey is an unlikely explanation of the diel shift in diet, because most identified food items, whether recovered during the day or the night, had at least some indigestible and easily detectable hard chitinous structures, such as head capsules in chironomids, notonectid body or fragments of terrestrial insects.

Cyclical changes in dominant prey have been observed in other small-bodied fishes, such as mosquitofish, *Gambusia affinis* (Maglio & Rosen, 1969), and dwarf panchax, *Aplocheilichthys parvus* (Fernando *et al.*, 2015), and ascribed to variation in habitat use over the diel period (Maglio & Rosen, 1969). Owing to the strong spatial constraints of annual killifish habitats, we suggest that temporal differences in diet are related to variation in prey accessibility and detectability rather than to shifts in habitat use. Hence, we speculate that chironomid larvae are easily detectable visually during the daytime but not at night; notonectids might be detected at night via the fishes' lateral line or by contrast against the sky, and we suggest that the peak in terrestrial insects in late afternoon corresponds to the activity patterns of these insects (Reynoldy & Riley, 1979; Kasumyan, 2003).

We note that a very low proportion of notonectids was previously reported in the diet of *Nothobranchius* (Polačik & Reichard, 2010; but see Wildekamp, 1983), in contrast to our present study. In addition to differential prey availability among populations, we argue that differences in sampling time largely contributed to that difference. Samples

for the previous study on the diet of Mozambican *Nothobranchius* fishes (Polačik & Reichard, 2010) were collected at midday, before notonectids (and also terrestrial insects) started to form a substantial proportion of the diet.

In conclusion, we provide the first empirical description of diel activity in three wild populations of African killifish. All three *Nothobranchius* species studied are diurnal fishes with a single peak of locomotor activity around noon, with the exception of one densely populated site, where fish remained active throughout the 24 h cycle. Higher locomotor activity in males is likely to be associated with their active reproductive strategy, although their larger size might also intensify their activity. Individual *N. furzeri* move across the whole pool and do not occupy permanent territories. It appears that feeding is prioritized over reproduction in the morning, because gut fullness and dietary richness peak before all mature oocytes are spent. The composition of the diet varies over the diel cycle, primarily altering between dominance of chironomid larvae during the day and notonectid nymphs at night. Our results contribute to the understanding of the natural life history of African annual killifishes and provide baseline data for further laboratory studies of diel patterns in locomotor activity and their changes over the short *Nothobranchius* lifespan.

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REFERENCES

- Almaida-Pagán PF, Ortega-Sabater C, Lucas-Sánchez A, Martínez-Nicolas A, Espinosa C, Esteban MA, Madrid JA, Rol M, Mendiola P, de Costa J. 2018. Impact of a shift work-like lighting schedule on the functioning of the circadian system in the short-lived fish *Nothobranchius furzeri*. *Experimental Gerontology* **112**: 44–53.
- Bachelor NM, Schobernd ZH, Berrane DJ, Schobernd CM, Mitchell WA, Geraldi NR. 2013. When a trap is not a trap: converging entry and exit rates and their effect on trap saturation of black sea bass (*Centropristis striata*). *ICES Journal of Marine Science* **70**: 873–882.
- Bartoń K. 2016. *MuMIn: multi-model inference. R package version 1.15.6*. Available at: <https://cran.r-project.org/web/packages/MuMIn/index.html>.
- Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using {lme4}. *Journal of Statistical Software* **67**: 1–48.
- Beale AD, Whitmore D, Moran D. 2016. Life in a dark biosphere: a review of circadian physiology in “arrhythmic” environments. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* **186**: 947–968.
- Biro PA, Dingemans NJ. 2009. Sampling bias resulting from animal personality. *Trends in Ecology & Evolution* **24**: 66–67.
- Blažek R, Polačik M, Reichard M. 2013. Rapid growth, early maturation and short generation time in African annual fishes. *EvoDevo* **4**: 24.
- Cellerino A, Valenzano DR, Reichard M. 2016. From the bush to the bench: the annual *Nothobranchius* fishes as a new model system in biology. *Biological Reviews* **91**: 511–533.
- Claireaux G, Lefrançois C. 2007. Linking environmental variability and fish performance: integration through the concept of scope for activity. *Philosophical Transactions of the Royal Society B: Biological Sciences* **362**: 2031–2041.
- Croft DP, Albanese B, Arrowsmith BJ, Botham M. 2003. Sex-biased movement in the Guppy (*Poecilia reticulata*). *Oecologia* **137**: 62–68.
- Cuthill IC, Houston AI. 1997. Managing time and energy. In: Krebs JR, Davies NB, eds. *Behavioural ecology: an evolutionary approach*. Oxford: Blackwell Science, 97–120.
- D’Angelo L. 2013. Brain atlas of an emerging teleostean model: *Nothobranchius furzeri*. *Anatomical Record* **296**: 681–691.
- Daan S. 1981. Adaptive daily strategies in behavior. In: Aschoff J, ed. *Handbook of behavioral neurobiology, Vol 4. Biological rhythms*. New York: Plenum, 275–298.
- Davis RE. 1964. Daily ‘predawn’ peak of locomotion in fish. *Animal Behavior* **12**: 272–283.
- De Crespin De Billy V, Doledec S, Chessel D. 2000. Biplot presentation of diet composition data: an alternative for fish stomach contents analysis. *Journal of Fish Biology* **56**: 961–973.
- Diaz Pauli B, Wiech M, Heino M, Utne-Palm AC. 2015. Opposite selection on behavioural types by active and passive fishing gears in a simulated guppy *Poecilia reticulata* fishery. *Journal of Fish Biology* **86**: 1030–1045.
- Ekström P, Meissl H. 1997. The pineal organ of teleost fishes. *Reviews in Fish Biology and Fisheries* **7**: 199–284.
- Fernando GKAW, Jayakody S, Wijenayake WMHK, Galappaththy GNL, Yatawara M, Harishchandra RD, Wanninayake WMTB, Deniyage SL. 2015. Diurnal variation in the feeding patterns and food preferences of dwarf panchax (*Aplocheilichthys parvus*). *Sri Lanka Journal of Aquatic Sciences* **20**: 19–29.

- Fingerle A, Larranaga N, Steingrímsson SO. 2016.** Density-dependent diel activity in stream-dwelling Arctic charr *Salvelinus alpinus*. *Ecology and Evolution* **6**: 3965–3976.
- Fraser DF, Gilliam JF, Akkara JT, Albanese BW, Snider B. 2004.** Night feeding by guppies under predator release: effects on growth and daytime courtship. *Ecology* **85**: 312–319.
- Fry FEJ. 1947.** *Effects of the environment on animal activity*. Toronto: Publications of the Ontario Fisheries Research Laboratory.
- Graf M, Cellerino A, Englert C. 2010.** Gender separation increases somatic growth in females but does not affect lifespan in *Nothobranchius furzeri*. *PLoS ONE* **5**: e11958.
- Grégoir AF, Thoré ESJ, Philippe C, Pinceel T, Brendonck L, Vanschoenwinkel B. 2018.** Squeezing out the last egg—annual fish increase reproductive efforts in response to a predation threat. *Ecology and Evolution* **8**: 6390–6398.
- Gross MR, Sargent RC. 1985.** The evolution of male and female parental care in fishes. *American Zoologist* **25**: 807–822.
- Haas R. 1976a.** Behavioral biology of the annual killifish, *Nothobranchius guentheri*. *Copeia* **1976**: 80–91.
- Haas R. 1976b.** Sexual selection in *Nothobranchius guentheri* (Pisces: Cyprinodontidae). *Evolution* **30**: 614–622.
- Hanson KC, Hasler CT, Cooke SJ, Suski CD, Philipp DP. 2008.** Intersexual variation in the seasonal behaviour and depth distribution of a freshwater temperate fish, the largemouth bass. *Canadian Journal of Zoology* **86**: 801–811.
- Heino M, Kaitala V. 1999.** Evolution of resource allocation between growth and reproduction in animals with indeterminate growth. *Journal of Evolutionary Biology* **12**: 423–429.
- Helfman GS. 1986.** Fish behaviour by day, night and twilight. In: Pitcher TJ, eds. *Behaviour of teleost fishes*. London: Chapman and Hall, 366–387.
- Hu CK, Brunet A. 2018.** The African turquoise killifish: A research organism to study vertebrate aging and diapause. *Aging Cell* **17**: e12757.
- Kasumyan AO. 2003.** The lateral line in fish: structure, function, and role in behavior. *Journal of Ichthyology* **43**: 175–213.
- Kodric-Brown A. 1988.** Effect of population density, size of habitat and oviposition substrate on the breeding system of Pupfish. *Ethology* **43**: 28–43.
- Lambert JW, Reichard M, Pincheira-Donoso D. 2019.** Live fast, diversify non-adaptively: evolutionary diversification of exceptionally short-lived annual killifishes. *BMC Evolutionary Biology* **19**: 10.
- Lema SC, Wagstaff LJ, Gardner NM. 2010.** Diurnal rhythms of behavior and brain mRNA expression for arginine vasotocin, isotocin, and their receptors in wild amargosa pupfish (*Cyprinodon nevadensis amargosae*). *Marine and Freshwater Behaviour and Physiology* **43**: 257–281.
- Lenth RV. 2016.** Least-squares means: the R package lsmeans. *Journal of Statistical Software* **69**: 1–33.
- Lucas MC, Baras E, Thom TJ, Duncan A, Slavik O., (eds) 2001.** Types of migration. In: *Migration of freshwater fishes*. Oxford: John Wiley & Sons, 66–92.
- Lucas-Sánchez A, Almáida-Pagán PF, Madrid JA, de Costa J, Mendiola P. 2011.** Age-related changes in fatty acid profile and locomotor activity rhythms in *Nothobranchius korthausae*. *Experimental Gerontology* **46**: 970–978.
- Lucas-Sánchez A, Almáida-Pagán PF, Martínez-Nicolas A, Madrid JA, Mendiola P, de Costa J. 2013.** Rest-activity circadian rhythms in aged *Nothobranchius korthausae*. The effects of melatonin. *Experimental Gerontology* **48**: 507–516.
- MacFarlane RB, Livingston RJ. 1983.** Effects of acidified water on the locomotor behavior of the Gulf killifish, *Fundulus grandis*: a time series approach. *Archives of Environmental Contamination and Toxicology* **12**: 163–168.
- Maglio VJ, Rosen DE. 1969.** Changing preference for substrate color by reproductively active mosquitofish, *Gambusia affinis* (Baird and Girard) (Poeciliidae, Atheriniformes). *American Museum Novitates* **2379**: 1–37.
- McKenzie D, Claireaux G. 2010.** The effects of environmental factors on the physiology of aerobic exercise. In: Domenici P, Kapoor BG, eds. *Fish locomotion*. Boca Raton: CRC Press, 296–332.
- Metcalfe NB, Fraser NHC, Burns MD. 1999.** Food availability and the nocturnal vs. diurnal foraging trade-off in juvenile salmon. *Journal of Animal Ecology* **68**: 371–381.
- Metcalfe NB, Steele GI. 2001.** Changing nutritional status causes a shift in the balance of nocturnal to diurnal activity in European minnows. *Functional Ecology* **15**: 304–309.
- Noakes DLG. 1992.** Behavior and rhythms in fish. In: Ali MA, eds. *Rhythms in fish*. New York: Springer Science + Business Media, LLC, 39–50.
- Perrin N, Mazalov V. 2000.** Local competition, inbreeding, and the evolution of sex-biased dispersal. *The American Naturalist* **155**: 116–127.
- Podrabsky JE, Hrbek T, Hand SC. 1998.** Physical and chemical characteristics of ephemeral pond habitats in the Maracaibo basin and Llanos region of Venezuela. *Hydrobiologia* **362**: 67–78.
- Podrabsky JE, Wilson NE. 2016.** Hypoxia and anoxia tolerance in the annual killifish *Austrofundulus limnaeus*. *Integrative and Comparative Biology* **56**: 500–509.
- Polačik M, Blažek R, Reichard M. 2016.** Laboratory breeding of the short-lived annual killifish *Nothobranchius furzeri*. *Nature Protocols* **11**: 1396–1413.
- Polačik M, Janáč M. 2017.** Costly defense in a fluctuating environment—sensitivity of annual *Nothobranchius* fishes to predator kairomones. *Ecology and Evolution* **7**: 4289–4298.
- Polačik M, Reichard M. 2010.** Diet overlap among three sympatric African annual killifish species *Nothobranchius* spp. from Mozambique. *Journal of Fish Biology* **77**: 754–768.
- Polačik M, Reichard M. 2011.** Asymmetric reproductive isolation between two sympatric annual killifish with extremely short lifespans. *PLoS ONE* **6**: e22684.
- Post JR, Parkinson EA, Johnston NT. 1999.** Density-dependent processes in structured fish populations: interaction strengths in whole lake experiments. *Ecological Monographs* **69**: 155–175.
- Prehalová M, Mrkvička T, Peterka J, Čech M, Berec L, Kubečka J. 2011.** A model of gillnet catch in relation to the catchable biomass, saturation, soak time and sampling period. *Fisheries Research* **107**: 201–209.

- R Core Team. 2017.** *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.
- Reebs SG. 2002.** Plasticity of diel and circadian activity rhythms in fishes. *Reviews in Fish Biology and Fisheries* **12**: 349–371.
- Reichard M, Blažek R, Polačik M, Vrtílek M. 2017.** Hatching date variability in wild populations of four coexisting species of African annual fishes. *Developmental Dynamics* **246**: 827–837.
- Reichard M, Lanés LEK, Polačik M, Blažek R, Vrtílek M, Godoy RS, Maltchik L. 2018.** Avian predation mediates size-specific survival in a Neotropical annual fish: a field experiment. *Biological Journal of the Linnean Society* **124**: 56–66.
- Reichard M, Polačik M. 2019.** The Natural History of Model Organisms: *Nothobranchius furzeri*, an 'instant' fish from an ephemeral habitat. *eLife* **8**: e41548.
- Reichard M, Polačik M, Blažek R, Vrtílek M. 2014.** Female bias in the adult sex ratio of African annual fishes: interspecific differences, seasonal trends and environmental predictors. *Evolutionary Ecology* **28**: 1105–1120.
- Reichard M, Polačik M, Sedláček O. 2009.** Distribution, colour polymorphism and habitat use of the African killifish *Nothobranchius furzeri*, the vertebrate with the shortest life span. *Journal of Fish Biology* **74**: 198–212.
- Reynoldy DR, Riley JR. 1979.** Radar observations of concentrations of insects above a river in Mali, West Africa. *Ecological Entomology* **4**: 161–174.
- Schielzeth H. 2010.** Simple means to improve the interpretability of regression coefficients. *Methods in Ecology and Evolution* **1**: 103–113.
- Sedláček O, Baciaková B, Kratochvíl L. 2014.** Evolution of body colouration in killifishes (Cyprinodontiformes: Aplocheilidae, Nothobranchiidae, Rivulidae): is male ornamentation constrained by intersexual genetic correlation? *Zoologischer Anzeiger* **253**: 207–215.
- Shidlovskiy KM, Watters BR, Wildekamp RH. 2010.** Notes on the annual killifish species *Nothobranchius rachovii* (Cyprinodontiformes; Nothobranchiidae) with the description of two new species. *Zootaxa* **2724**: 37–57.
- Smith BR, Blumstein DT. 2008.** Fitness consequences of personality: a meta-analysis. *Behavioral Ecology* **19**: 448–455.
- Terzibasi Tozzini E, Martinez Nicolas A, Lucas Sánchez A. 2017.** The clock is ticking. Ageing of the circadian system: from physiology to cell cycle. *Seminars in Cell and Developmental Biology* **70**: 164–176.
- Trivers RL. 1972.** Parental investment and sexual selection. In: Campbell T, eds. *Sexual selection and the descent of man*. Chicago: Aldine Publishing Company, 136–179.
- Tyler AV, Dunn RS. 1976.** Ration, growth, and measures of somatic and organ condition in relation to meal frequency in Winter Flounder, *Pseudopleuronectes americanus*, with hypotheses regarding population homeostasis. *Journal of the Fisheries Research Board of Canada* **33**: 63–75.
- Ueda M, Oishi T. 1982.** Circadian oviposition rhythm and locomotor activity in the medaka, *Oryzias latipes*. *Journal of Interdisciplinary Cycle Research* **13**: 97–104.
- Valenzano DR, Terzibasi E, Cattaneo A, Domenici L, Cellerino A. 2006.** Temperature affects longevity and age-related locomotor and cognitive decay in the short-lived fish *Nothobranchius furzeri*. *Aging Cell* **5**: 275–278.
- Venables WN, Ripley BD. 2002.** *Modern applied statistics with S*. New York: Springer.
- Vrtílek M, Reichard M. 2015.** Highly plastic resource allocation to growth and reproduction in females of an African annual fish. *Ecology of Freshwater Fish* **24**: 616–628.
- Vrtílek M, Reichard M. 2016.** Female fecundity traits in wild populations of African annual fish: the role of the aridity gradient. *Ecology and Evolution* **6**: 5921–5931.
- Vrtílek M, Žák J, Polačik M, Blažek R, Reichard M. 2018a.** Longitudinal demographic study of wild populations of African annual killifish. *Scientific Reports* **8**: 4774.
- Vrtílek M, Žák J, Polačik M, Blažek R, Reichard M. 2019.** Rapid growth and large body size in annual fish populations are compromised by density-dependent regulation. *Journal of Fish Biology*. doi:10.1111/jfb.14052
- Vrtílek M, Žák J, Pšenička M, Reichard M. 2018b.** Extremely rapid maturation of a wild African annual fish. *Current Biology* **28**: R822–R824.
- Wallace RA. 1978.** Oogenesis in *Fundulus heteroclitus*: I. Preliminary observations on oocyte maturation *in vivo* and *in vitro*. *Developmental Biology* **62**: 354–369.
- Wang N, Teletchea F, Kestemont P, Milla S, Fontaine P. 2010.** Photothermal control of the reproductive cycle in temperate fishes. *Reviews in Aquaculture* **2**: 209–222.
- Weber DN, Spieler RE. 1987.** Effects of the light-dark cycle and scheduled feeding on behavioral and reproductive rhythms of the cyprinodont fish, Medaka, *Oryzias latipes*. *Experientia* **43**: 621–624.
- Werner EE, Anholtt BR, Arbor A. 1993.** Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *The American Naturalist* **142**: 242–272.
- Wildekamp RH. 1983.** *Assignment report. Preliminary study of the Somalian Nothobranchius species of larvivorous fishes*. EM/MAL/198 EM/VBC/41 SOM/MPD/001/RB World Health Organization.
- Wildekamp RH. 2004.** A world of killies: atlas of the oviparous cyprinodontiform fishes of the world. Volume IV. Elyria: American Killifish Association, 398 p. ISBN: 9781883494087.
- Wilson DS, Coleman K, Clark AB, Biederman L. 1993.** Shy–bold continuum in pumpkinseed sunfish (*Lepomis gibbosus*): an ecological study of a psychological trait. *Journal of Comparative Psychology* **107**: 250–260.
- Wood SN. 2017.** *Generalized additive models: an introduction with R*. New York: Chapman and Hall/CRC.
- Wood SN, Pya N, Säfken B. 2016.** Smoothing parameter and model selection for general smooth models. *Journal of the American Statistical Association* **111**: 1548–1563.
- Žák J, Jůza T, Blabolil P, Baran R, Bartoň D, Draštík V, Frouzová J, Holubová M, Ketelaars HAM, Kočvara L, Kubečka J, Mrkvička T, Muška M, Říha M, Sajdlová Z, Šmejkal M, Tušer M, Vašek M, Vejřík L, Vejříková I, Wagenvoort AJ. 2018a.** Invasive round goby *Neogobius melanostomus* has sex-dependent locomotor activity and is under-represented in catches from passive fishing gear compared with seine catches. *Journal of Fish Biology* **93**: 147–152.
- Žák J, Reichard M, Gvoždík L. 2018b.** Limited differentiation of fundamental thermal niches within the killifish assemblage from shallow temporary waters. *Journal of Thermal Biology* **78**: 257–362.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Surface and bottom water temperature (in degrees Celsius) over a 24 h period at sites 1–3 (A–C), measured during trapping. Black line shows temperature measured at pool surface (5 cm below water surface), and grey line represents bottom temperature (measured directly on the substrate). Logging interval was set to 1 min.

Figure S2. Light intensity (in lux) on water surface over a 24 h period at sites 1–3 (A–C) (measured during trapping). Logging interval was 1 min.

Figure S3. Oxygen concentration (in milligrams per litre) at site 3 over a 24 h period of fish trapping. Red line and points represent oxygen concentration measured at the pool surface (5 cm below the surface). Blue line and points represent values measured 10 cm above the pool substrate. Each point represents the mean value of three independent measurements performed at three different parts of the pool.

Table S1. Table of sample sizes used in analysis of activity, feeding and reproduction rhythmicity of the African annual killifish from genus *Nothobranchius*. Abbreviations: NF, *Nothobranchius furzeri*; NO, *Nothobranchius orthonotus*; NP, *Nothobranchius pienaar*.

Table S2. Quantitative comparison of candidate models (generalized additive models with Gaussian error) of fish activity at site 3 and their results. Degrees of freedom (d.f.) absolute Akaike information criterion (AIC), difference from the best model (Δ AIC) and their statistics, estimated degrees of freedom (e.d.f.), F -statistics and P -value, are shown for models, with time of day, light intensity, surface and bottom temperatures, surface and bottom oxygen concentrations as explanatory variables.

SHARED DATA

Original data are available at the Figshare repository (doi: 10.6084/m9.figshare.7326425).