



Individual-level pace-of-life syndromes in annual killifish are mediated by intersexual and interspecific differences

Caroline Methling¹ · Radim Blažek^{1,2} · Radomil Řežucha¹ · Martin Reichard^{1,2} 

Received: 14 January 2020 / Accepted: 16 June 2020
© Springer Nature Switzerland AG 2020

Abstract

Pace-of-life syndromes (POLS) describe covariations between life history (such as growth rate and age at maturity), behaviour (e.g. activity or boldness) and physiology (e.g. metabolic rate) along an axis from fast to slow lifestyles. This powerful concept can be applicable at a range of scales, from broad interspecific contrasts to the individual intra-population level, though its generality has recently been questioned. Using two species of African annual fishes with fast lifestyles, we tested how individual-level POLS covary between the sexes and contrasting social environments. Measuring three key metabolic parameters (standard metabolic rate, SMR; maximum metabolic rate, MMR; and aerobic scope, AS), we found extensive variation between species and sexes in the expression of POLS. Social environment affected individual metabolic traits, but not their covariation with behaviour and life history traits. In accordance with the POLS prediction, we observed a positive association between MMR, AS and boldness, and a negative association between MMR, AS and lifespan in *Nothobranchius orthonotus* males, although trait covariations were opposite in *N. orthonotus* females. In *Nothobranchius pienaari*, we confirmed the predicted negative correlation between SMR and lifespan which was not sex-specific. Contrary to POLS predictions, we observed a negative correlation between SMR and boldness in *N. pienaari*. Finally, there was no link between activity levels or size at maturity and metabolic traits in either species. Overall, we demonstrated limited support for POLS predictions, but found that specific pace-of-life trait associations were mediated by both interspecific and intersexual differences.

Keywords Behaviour · Life history · Metabolic rate · Slow-fast continuum

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10682-020-10059-9>) contains supplementary material, which is available to authorized users.

✉ Martin Reichard
reichard@ivb.cz

¹ Institute of Vertebrate Biology, Czech Academy of Sciences, Květná 8, 603 65 Brno, Czech Republic

² Department of Botany and Zoology, Faculty of Sciences, Masaryk University, Brno, Czech Republic

Introduction

Covariations between physiology, behaviour, and life-history traits have been integrated into the pace-of-life syndrome (POLS) framework that predicts animals to fall on a continuum from slow to fast lifestyles (Ricklefs and Wikelski 2002; Réale et al. 2010). At one end, a fast lifestyle is characterised by a high metabolic rate and early onset of reproduction, but at the cost of a short lifespan. Conversely, a slow lifestyle is characterised by a slow metabolic rate, later onset of reproduction, but a longer lifespan (Auer et al. 2018). Behavioural traits such as aggressiveness, boldness, and high activity are predicted for individuals with a fast lifestyle, whereas low levels of activity, aggression, and shyness are expected for individuals with a slow lifestyle (reviewed in Careau and Garland 2012; Réale et al. 2010).

The positive association between metabolic rate and behavioural traits linked to exploration, activity, aggressiveness, and boldness rests on the assumption that a high metabolic rate reflects the increased maintenance costs of a large metabolic capacity (termed “performance model”). To maintain body mass or achieve positive growth, individuals with a high maintenance metabolism must have correspondingly higher energy intake. Hence, these individuals must be more active to acquire resources and display behavioural traits that lead to higher resource acquisition (Careau et al. 2008). These behaviours can be costly and potentially increase mortality risk, by increasing the risk of predation (Biro et al. 2004) or risk of starvation when resources are limited due to high maintenance metabolic rate (Álvarez and Nicieza 2005; Finstad et al. 2004). Besides predation- and starvation-related mortality, the oxidative damage theory of ageing (Beckman and Ames 1998) suggests another link between metabolic rate and lifespan. This theory argues that generation of reactive oxygen species during ATP production is linked to accumulation of cellular damage and senescence. Nevertheless, evidence of a direct link between oxidative stress and lifespan is lacking (Speakman et al. 2015).

The minimal energy requirement for self-maintenance in fish is termed standard metabolic rate (SMR) and can vary two to three-fold even in similarly-sized conspecifics of the same age and sex, and which experienced the same thermal conditions (Millidine et al. 2009; Norin and Malte 2011; Auer et al. 2015). Some of this variation may be explained by genetic, epigenetic and physiological factors (Burton et al. 2011), but there may also be social (Grantner and Taborsky 1998; Sloman et al. 2000; Grobler and Wood 2013) and behavioural underpinnings. For example, in several salmonid fish species, individuals with a high standard metabolic rate tend to be more aggressive and more likely to establish dominance (Cutts et al. 1998; Sloat and Reeves 2014). Further, willingness to take risks has been linked to a higher metabolic rate in Atlantic salmon *Salmo salar* (Finstad et al. 2007; Robertsen et al. 2015) and common carp *Cyprinus carpio* (Huntingford et al. 2010). However, this pattern may not be general – some studies failed to detect a relationship between SMR and risk-taking behaviours (Binder et al. 2016; Polverino et al. 2016; White et al. 2016) or found them only under stressful conditions (Killen et al. 2012).

The overall empirical support for POLS is currently considered equivocal (Royauté et al. 2018), but in a series of theoretical studies and reviews, factors that might influence trait covariations have been identified, including resource availability and predation risks (Salzman et al. 2018), environmental stressors (Killen et al. 2013), and disparate life-history and physiology between males and females (Hämäläinen et al. 2018; Immonen et al. 2018). One possible reason for failure to record any association between metabolic rate and behavioural traits may be the use of standard metabolic rate. Behaviours that require high

levels of energy expenditure (e.g. burst swimming when chasing prey or escaping predators) may be more tightly linked to maximum metabolic rate (MMR) or aerobic scope (AS, i.e. the absolute difference between MMR and SMR, representing capacity to increase metabolic activity above maintenance levels) than to SMR (Killen et al. 2014; Binder et al. 2016). Indeed, individuals with a high MMR tend to have superior swimming performance (Reidy et al. 2000; Binning et al. 2015) and a high aerobic scope facilitates faster recovery from bouts of exhaustive exercise (Marras et al. 2010; Killen et al. 2014), traits that could prove advantageous for individuals prone to risk-taking. Hence, behaviours directly associated with foraging and expenditure, rather than behaviours related to general activity, may be more tightly linked to metabolic traits (Mathot et al. 2019).

We used two closely related species of African annual killifish, *Nothobranchius orthonotus* and *N. pienaari* to investigate covariation between metabolic rate and behaviour (boldness and exploratory activity) and life-history traits (size at maturity and lifespan) at the individual level. We compared males and females across both species and separated the effect of social environment on the expression of POLS. Several lineages of killifish have evolved fast lifestyles (rapid growth and maturation, high investment in reproduction) and thrive in ephemeral habitats that quickly desiccate (so-called annual species) (Furness et al. 2015; Furness 2016; Eckerström-Liedholm et al. 2017). Other killifish lineages live in permanent habitats (non-annual species). Recent work has demonstrated that at the broadest comparative level, annual killifish species display more rapid lifestyles than non-annual species (Sowersby et al. 2019). At the interpopulation level, however, no differences in metabolic or behavioural traits of four *Nothobranchius* species were detected between pairs of populations from contrasting wet and dry regions that differed predictably in the duration of habitat persistence (Blažek et al. 2017).

In the present study, we tested predictions of POLS at the individual level. Specifically, we predicted that SMR, MMR and AS are positively associated with risk-taking behaviours but negatively associated with size at maturity (as a proxy of juvenile growth rate) and lifespan. We also hypothesised that this effect may be mediated by sex differences and social environment. Given that our study species form male-dominated hierarchies, we kept fish in two contrasting social conditions – either housed singly or in communal tanks. We predicted that singly-housed fish will have a lower SMR due to the lack of social stress but may also have a lower MMR and AS due to lack of exercise. We hypothesised that the predicted associations are stronger for males than females (Smith and Blumstein 2008), as sexual selection causes higher variance in reproductive potential (and behaviour) among males compared to females (Bonduriansky et al. 2018). We predicted that more aggressive *N. orthonotus* will have higher SMR and MMR than *N. pienaari*, but we have not predicted *a priori* any differences in the strength of POLS between the species.

Materials and methods

Study species

Nothobranchius are small fishes from ephemeral savannah pools in East Africa. They have a naturally short lifespan which is driven by the seasonal desiccation of their habitat that lasts a few weeks to months (Vrřílek et al. 2018a). *Nothobranchius orthonotus* and *N. pienaari* are sympatric and often coexist in the same pools (Reichard et al. 2017). They have relatively similar morphology and ecology, but *N. orthonotus* is larger, more aggressive

and achieves sexual maturity earlier than *N. pienaar* (Blažek et al. 2017). While the two species share a part of their diet spectrum (Polačik et al. 2014), *N. orthonotus* feeds on larger prey items than *N. pienaar* (Polačik and Reichard 2010).

Nothobranchius fishes are extreme income breeders and females lay 20–100 single eggs sequentially every day (Vrtílek and Reichard 2015). Males are larger than females and form dominance hierarchies (Polačik and Reichard 2009), with dominant aggressive males potentially suppressing the activity and growth of subordinate males (Polačik et al. 2016). Dominant males may also stress females by coercive mating (Polačik and Reichard 2011).

Animals and husbandry

Experimental fish were F1 descendants of parents collected in southern and central Mozambique in March 2012 (Blažek et al. 2017). Experimental fish were hatched in May 2013 from eggs incubated at 23.5–24.5 °C for at least 16 weeks following standard methods (Polačik et al. 2016), to ensure that all embryos developed via diapause. Four days (*N. orthonotus*) or 10 days (*N. pienaar*) post hatching, fish were assigned either to single-housed fish treatment (2 L aquaria) or to group-housed treatment (24 L aquaria). In group-housed treatments, 10 (*N. orthonotus*) or 12 (*N. pienaar*) fish were housed in each tank, with equal sex ratios. In this setting, single-housed and group-housed fish lived at the same population density (i.e. at 2 L aquaria volume per fish irrespective of treatment). Species-specific fish density was contingent on body size differences between the species. At the age of 6 weeks, group-housed *N. orthonotus* were individually marked with a single elastomer tag (Northwestern Marine Technology, USA) implanted subcutaneously. The smaller size of *N. pienaar* precluded marking. The tanks were equipped with air-driven sponge filters and 25–30% of the water (aged tap water, conductivity 550 µS/cm², temperature 26 ± 2 °C) was exchanged 2–3 times a week. Once weaned from *Artemia* nauplii (fed 2–3 times a day), fish were fed chopped bloodworm (*Chironomus* larvae) and *Tubifex* (approximately 15% of tank biomass) once a day. Aquaria were monitored daily, and any dead fish were recorded and removed. Lifespan (in days) was measured as a difference between the dates of birth and death. Size at maturity (*N. orthonotus*: 4 weeks, *N. pienaar*: 6 weeks) was measured as Total length (TL, including caudal fin) from photographs of individual fish with a reference scale, using the *ImageJ* software and represented a proxy for juvenile growth. A subset of 124 experimental fish (with balanced sex ratio) was haphazardly chosen for behavioural and physiological measurements. Fish were returned to their home aquaria after the trials.

Behavioural tests were performed when fish were 16–20 weeks old. Exploration activity was tested on individual fish in a shallow opaque tank (450 × 350 mm, water depth 25 mm), with a ruler placed on the bottom to provide a scale for software calibration. Fish were transferred to the test tank and after 1 min acclimatization, movements were recorded by a camera mounted above the tank for 4 min. Light conditions were standardized by the use of an external dimmed lamp and fish were not fed before trials to standardize satiation and metabolic activity. Recordings were analysed using *Tracker 4.82* software (Brown 2009) and activity was quantified as absolute distance travelled during 4 min.

Boldness was scored as time taken to leave a refuge and enter a novel environment (Brown and Braithwaite 2004). The test arena consisted of an opaque plastic shelter (80 × 150 mm, water depth 80 mm) with a divider positioned to one side of the aquarium and equipped with a sliding door, positioned at one side of the test aquarium (550 × 490

mm). The three remaining sides were covered by opaque foil to prevent any external disturbance. All other experimental conditions were identical to those for scoring activity. Individual fish were transferred to the refuge section and after 2 min, the sliding door was raised up and the fish was allowed to leave the refuge. Two time-points were noted, Time 1: tip of snout outside refuge, and Time 2: entire body outside refuge. All fish left the refuge within 10 min. Analyses for Time 1 and Time 2 were congruent and only Time 2 is reported. We acknowledge that a proper protocol to measure boldness is subjective and depends on characteristics of the study species. While our experience with annual killifish suggests that we have indeed measured boldness using this set up, despite all fish left the refuge within 10 min and were not hesitant during emergence (limited variation between Time 1 and Time 2), an alternative view is that time to leave the refuge represented a stress response.

Respirometry

Respirometry measurements were performed in October and November 2013, when fish were 18–20 weeks old and population-level adult mortality <30% in all treatment groups. Standard metabolic rate (SMR) and maximum metabolic rate (MMR) were quantified by computerized intermittent flow-through respirometry (Steffensen 1989), using a static respirometry system with 55.0 or 90.0 mL cylindrical glass respirometers (Loligo Systems, Tjele, Denmark). Respirometers were submerged in an outer holding tank (50 L), supplied with aged tap water from a 100 L reservoir, continuously aerated and passed through a UV lamp to minimise microbial respiration. Water temperature was kept constant at 25 ± 0.1 °C which is in the middle of optimal temperature range for both species (Žák et al. 2018). The entire setup was shielded from outside disturbance and lighting followed a 12:12 light:dark regime. Fish were not fed for 24 h prior to measurements.

Each trial started in the afternoon by subjecting fish to a 3 min chasing + 30 s air exposure protocol to elicit MMR (Clark et al. 2013). The MMR was measured immediately after placing the fish in the respirometry chamber. Chamber oxygen partial pressure (pO_2) was measured with an OXY-4 mini (PreSens GmbH, Germany) fibre-optic O_2 transmitter and recorded using AutoResp4™ software (1 s^{-1}). Instantaneous mass-specific oxygen consumption rate (MO_2) was derived from the decrease in chamber pO_2 during a 3-minute measuring period ($d(pO_2)/dt$) according to the equation: $MO_2 = V(d(pO_2)/dt) \alpha M^{-1}$, where V is the volume of the chamber, α is specific oxygen solubility and M is wet mass. Chambers were then flushed for 3 min with water from the holding tank, followed by a closed 1 min wait period to reach steady state, before the next measurement period began. MO_2 measurements recorded during the following 24 h were used to estimate SMR. All measurements were corrected for microbial respiration by recording MO_2 in empty chambers before and after each measurement and subtracting the mean value from fish MO_2 .

Data analysis

Standard metabolic rate (SMR) was estimated according to Chabot et al. (2016) as the 20th percentile of all MO_2 values, where the regression coefficient of the slope $d(pO_2)/dt$ was $R^2 > 0.96$, using the R package *fishMO2*. Note that, by definition, SMR is measured in a resting, post-absorptive and non-reproducing state. However, *N. orthonotus* and *N. pien-aari* are extreme income breeders and reproduce continuously, so absolute SMR values

may have been overestimated. Maximum metabolic rate (MMR) was determined as the highest MO_2 of the first three measurements after the chasing protocol. Aerobic metabolic scope (AS) was calculated as the difference between MMR and SMR. To correct for size effects on mass-specific metabolic rate, metabolic variables were size-standardised using the residuals from the $\ln(\text{wet mass}) - \ln(\text{MO}_2)$ linear regressions. A linear relationship between \ln -transformed MO_2 and \ln -transformed wet mass was confirmed by significant regression coefficients for all metabolic variables (Supplementary Table 1).

We compared metabolic traits between *N. orthonotus* (N=57) and *N. pinnari* (N=67), females (N=64) and males (N=60) and individually housed (N=58) and group housed (N=66) using linear models (LM), with species, sex and housing as explanatory variables. We have not used mixed models, because only group housing (but not individual housing) would be subject to a random effect of home tank ID.

Model selection was performed by a backward elimination starting with full models including all variables and their interactions (Supplementary Table 2). Removal of variables and interactions was based on values of Akaike's information criterion (AIC), using the *stepAIC* function in R. The final model chosen was the model with the lowest AIC value that also retained the variable(s) central to our prediction.

Pace-of-life syndrome was investigated for each species separately. In *N. orthonotus*, we analysed data for grouped-housed and individually housed fish in single analyses, while this was not possible in *N. pinnari* as their small size precluded individual marking in social tanks. Associations between metabolic and behavioural, and metabolic and life-history traits were analysed using linear models (LM) with the trait of interest (activity, boldness, size at maturity and lifespan) as the dependent variable in the respective model, and sex (in *N. orthonotus* and *N. pinnari*) and housing (only in *N. orthonotus*) as fixed factors, including their interaction (Supplementary Tables 3–5). The assumptions of normal distribution and heteroscedasticity of residuals were checked by inspection of residual plots (scatterplots and QQ-plots) and presence of influential data points was evaluated using Cook's distance. All analyses were performed in the R statistical environment (R Core Team 2019).

Results

Metabolic rate varied between species, sex and social treatment

Overall, size-standardized standard metabolic rate (SMR) greatly varied between species, sex and social treatments (LM: species: $t_{110} = -8.29$, $P < 0.001$; sex: $t_{110} = -2.14$, $P = 0.035$; social treatment: $t_{110} = -4.35$, $P < 0.001$; $R_{\text{adj}}^2 = 0.44$; Fig. 1a–c), but there were no significant interactions between the variables (all $P > 0.05$ and removed from the final model during AIC-based stepwise backward selection). *Nothobranchius orthonotus* had, on average, 27.8% higher SMR on the arithmetic scale than *N. pinnari* [back-transformed estimated marginal mean: *N. orthonotus* = 189.25 (95% CI: 181–192) $\text{mgO}_2 \text{ kg}^{-1} \text{ h}^{-1}$, *N. pinnari* = 148.04 (95% CI: 142–154) $\text{mgO}_2 \text{ kg}^{-1} \text{ h}^{-1}$, for a 1.0 g fish]. Females had an average 6.5% higher SMR than males [females = 169.77 (95% CI: 163–177) $\text{mgO}_2 \text{ kg}^{-1} \text{ h}^{-1}$, males = 159.44 (95% CI: 153–166) $\text{mgO}_2 \text{ kg}^{-1} \text{ h}^{-1}$, for a 1.0 g fish]. Group-housed fish had an average 13.6% higher SMR than individually-housed fish [grouped = 174.58 (95% CI: 168–182) $\text{mgO}_2 \text{ kg}^{-1} \text{ h}^{-1}$, single = 153.66 (95% CI: 147–160) $\text{mgO}_2 \text{ kg}^{-1} \text{ h}^{-1}$, for a 1.0 g fish].

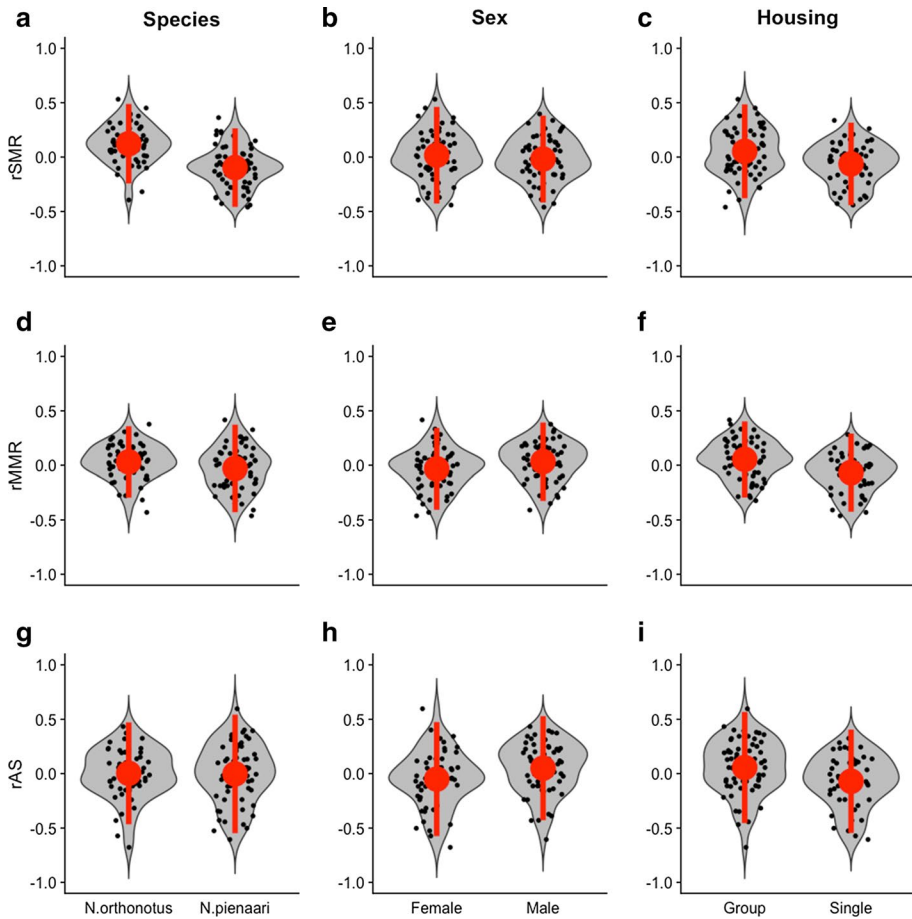


Fig. 1 Variation in metabolic traits between species, sex and social environment in *N. orthonotus* and *N. pienaar*. Data were size-standardized by using residuals from the allometric scaling relationship: $\ln(\text{wet mass}) - \ln(\text{metabolic rate})$. SMR: standard metabolic rate; MMR: maximum metabolic rate. AS, aerobic scope (MMR-SMR). Black dots represent individual data points and red line represents mean \pm SD

Size-standardized maximum metabolic rate (MMR) also varied between species, sex and social treatments (LM: species: $t_{110} = -2.09$, $P = 0.040$; sex: $t_{110} = 2.46$, $P = 0.016$; housing: $t_{110} = -4.13$, $P < 0.001$; Fig. 1d–f). *Nothobranchius orthonotus* had an average 6.9% higher MMR than *N. pienaar* [*N. orthonotus* = 626.96 (95% CI: 599–656) $\text{mgO}_2 \text{ kg}^{-1} \text{ h}^{-1}$, *N. pienaar* = 586.54 (95% CI: 562–613) $\text{mgO}_2 \text{ kg}^{-1} \text{ h}^{-1}$, for a 1.0 g fish]. Females had 8.2% lower MMR than males [females = 585.42 (95% CI: 557–609) $\text{mgO}_2 \text{ kg}^{-1} \text{ h}^{-1}$, males = 630.06 (95% CI: 602–659) $\text{mgO}_2 \text{ kg}^{-1} \text{ h}^{-1}$, for a 1.0 g fish]. Group-housed fish had a 14.3% higher MMR than individually-housed fish [grouped = 642.55 (95% CI: 616–670) $\text{mgO}_2 \text{ kg}^{-1} \text{ h}^{-1}$, single = 562.36 (95% CI: 536–590) $\text{mgO}_2 \text{ kg}^{-1} \text{ h}^{-1}$, for a 1.0 g fish].

There was no significant difference in aerobic scope (AS) between *N. orthonotus* and *N. pienaar* (LM: $t_{103} = -0.463$, $P = 0.644$; Fig. 1g), but AS was significantly lower in females (LM: $t_{103} = 2.43$, $P = 0.028$; Fig. 1h) and in individually housed fish (LM: $t_{103} =$

-3.30 , $P=0.002$; Fig. 1i). Summaries of full and final models are provided in Supplementary Table 2.

Relationship between behavioural and metabolic traits

The association between boldness and metabolic traits differed between the two species. In *N. orthonotus*, there was no significant relationship between boldness and SMR (LM: $t_{44}=1.23$, $P=0.230$; Fig. 2a), but a marginally significant association between boldness and MMR, mediated by sex (LM: boldness*sex interaction: $t_{43}=-2.08$, $P=0.044$, $R_{adj}^2=0.19$). Boldness was negatively associated with MMR in females but positively in males (Fig. 2b). This outcome translated into a negative relationship between aerobic scope and boldness in female and a positive relationship in male *N. orthonotus* (LM: boldness*sex: $t_{37}=-2.17$, $P=0.037$, $R_{adj}^2=0.11$; Fig. 2c). Social environment had no significant effect on associations between metabolic traits and boldness (i.e. there were no significant boldness*housing interactions and the terms were excluded during model selection (Supplementary Table 3).

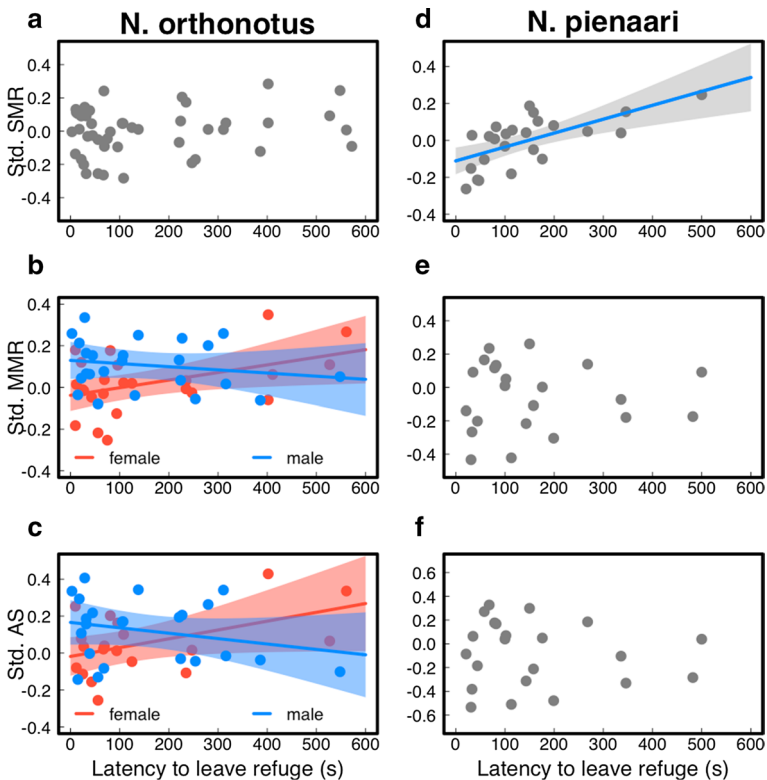


Fig. 2 Covariation between metabolic traits (standard metabolic rate, maximum metabolic rate and aerobic scope) and boldness (latency to leave refuge) in *N. orthonotus* (a–c) and *N. pienzaari* (d–f). Data was size-standardized by using residuals from the relationship: $\ln(\text{wet mass}) - \ln(\text{metabolic rate})$. Least squares regression lines are shown with 95% confidence intervals indicated by shaded areas

In *N. pienaari*, individuals with low SMR were bolder and left the refuge more readily (LM: $t_{22}=3.99$, $P<0.001$, $R_{adj}^2=0.39$; Fig. 2d). There was no association between boldness and MMR (LM: $t_{20}=0.10$, $P=0.925$, $R_{adj}^2=0.39$; Fig. 2e) or metabolic scope (LM: $t_{20}=-0.43$, $P=0.670$, $R_{adj}^2=0.39$; Fig. 2f). Model summaries are provided in Supplementary Table 3. Exploratory activity was not associated with any metabolic trait in either species (Supplementary Table 4).

Relationship between lifespan, maturation and metabolic traits

The associations between lifespan and metabolic traits also differed between species. In *N. orthonotus*, lifespan was not related to SMR (LM: $t_{26}=-0.08$, $P=0.94$, $R_{adj}^2=-0.02$; Fig. 3a). However, MMR was significantly associated with lifespan, with opposite effects in females and males (LM: lifespan*sex interaction: $t_{45}=2.28$, $P=0.028$, $R_{adj}^2=0.13$; Fig. 3b). Females with high MMR lived longer, while males with high MMR were shorter-lived. As with boldness, there was a positive association between lifespan and aerobic scope in female *N. orthonotus* and a negative one in males (LM, lifespan*sex interaction:

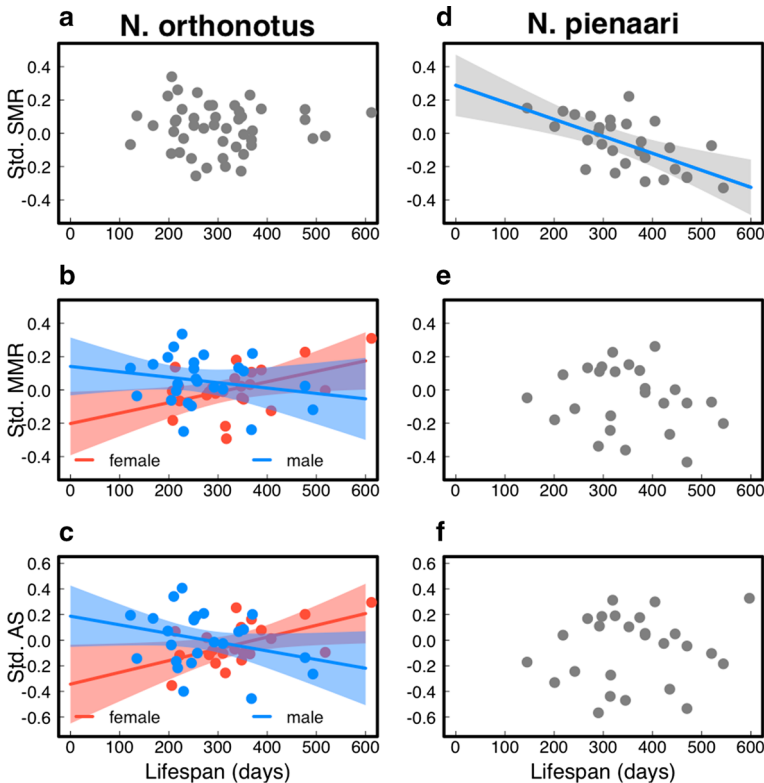


Fig. 3 Covariation between metabolic traits (standard metabolic rate, maximum metabolic rate and aerobic scope) and lifespan in *N. orthonotus* (a–c) and *N. pienaari* (d–f). Data was size-standardized by using residuals from the relationship: $\ln(\text{wet mass}) - \ln(\text{metabolic rate})$. Least squares regression lines are shown with 95% confidence intervals indicated by shaded areas

$t_{41}=2.68$, $P=0.011$, $R_{\text{adj}}^2=0.10$; Fig. 3c). Social environment had no significant effect on associations between metabolic traits and lifespan (i.e. there were no significant lifespan*housing interactions and terms were excluded during model selection; Supplementary Table 4).

In *N. pienaari*, fish with high SMR were shorter-lived (LM: $t_{26}=3.88$, $P=0.001$, $R_{\text{adj}}^2=0.34$; Fig. 3d), but no effects of MMR (LM: $t_{22}=-0.85$, $P=0.404$, $R_{\text{adj}}^2=-0.04$) or AS (LM: $t_{23}=0.577$, $P=0.570$, $R_{\text{adj}}^2=-0.06$) on lifespan were detected (Fig. 3e, f). Summaries of full and final models are provided in Supplementary Table 5.

There was no relationship between size at maturation and metabolic traits in either species (Supplementary Table 6).

Discussion

Pace-of-life syndromes (POLS) is powerful theoretical concept that links behaviour, physiology and life history along a single axis from fast to slow lifestyle, with covariation among traits centred on metabolic rates (Ricklefs and Wikelski 2002; Réale et al. 2010). The existence of POLS has been demonstrated across evolutionary scales—among taxa (Wiersma et al. 2007), among distinct populations within a species (Wikelski et al. 2003) and at inter-individual level within a population (Huntingford et al. 2010). Associations among POLS traits may be modified by social environment (Reid et al. 2012) and are often sex-specific (Hämäläinen et al. 2018). While the POLS concept received considerable support initially, more recently an increasing number of studies have questioned its generality (reviewed in Royauté et al. 2018).

Using two species of African annual fish, we show that metabolic traits vary between species, sex and social environment, but associations between metabolic rate, behaviour and life history are relatively weak and species- and sex-specific. We found that exploratory activity and size at maturity did not covary with metabolic traits. Individual boldness and lifespan demonstrated complex relationships to metabolic traits, with SMR linked to *N. pienaari* traits but MMR (and aerobic scope) to *N. orthonotus* traits. Individual *N. pienaari* with low SMR were bolder and longer-lived in both sexes and these associations were robust. In *N. orthonotus*, sexes differed in their covariation between the traits. As predicted, males with higher MMR (and aerobic scope) were bolder and shorter-lived, but the opposite was found in females; females with higher MMR were relatively shy and longer-lived.

Metabolic rate variation between species, sexes and social environment

Species with more active lifestyles and at higher trophic levels tend to have higher metabolic rates (Killen et al. 2010, 2017; Norin and Clark 2016). Our two study species have relatively similar morphology and ecology (Reichard et al. 2017), but *N. orthonotus* is larger, more aggressive and achieves sexual maturity earlier than *N. pienaari* (Blažek et al. 2017). The two species frequently co-occur in the wild (Reichard et al. 2017) and despite partial overlap in their diet spectrum (Polačik et al. 2014), *N. orthonotus* are more voracious than *N. pienaari* (Polačik and Reichard 2010). In accordance with our predictions, we demonstrated that the more aggressive and predatory *N. orthonotus* had higher SMR and MMR than more placid *N. pienaari*.

Life-history theory postulates that males and females diverge in their life histories (Stearns 1992; Roff 2002), with males exhibiting a faster life-history than females (Bonduriansky et al. 2008). Sex differences in pace-of-life traits related to behaviour (Smith and Blumenstein 2008) and metabolic rate (Arnqvist et al. 2017) are widely documented, but in fish, studies on intersexual differences in metabolic traits are surprisingly scarce. We observed a slightly higher SMR in females in both *Nothobranchius* species but propose this to be due to their reproductive status. *Nothobranchius* fishes are extreme income breeders (Vrtílek and Reichard 2015) and females therefore continuously undergo cycles of oocyte maturation. Given that gonad tissue has a higher mass-specific oxygen consumption than somatic tissue (Oikawa and Itazawa 2003), we predicted that to be reflected in higher SMR (Beamish 1964). In contrast, MMR (setting the upper limit for metabolic rate during elevated activity) was higher in males, in accordance with a faster pace-of-life that is typically associated with males. This result is similar to the situation reported in mosquitofish *Gambusia holbrooki* where males also had a higher MMR than females (Srean et al. 2017).

Social stress is known to affect several aspects of an individual's physiology with both short-term and long-term effects on SMR (Grantner and Taborsky 1998; Sloman et al. 2000; Dijkstra et al. 2016). Annual killifish form male-dominated hierarchies where dominant males suppress the activity and growth of subordinate males (Polačik and Reichard 2009; Passoss et al. 2015). Dominant males also exert strong reproductive coercion on females (Polačik and Reichard 2011). Harassment from dominant males therefore likely leads to social stress in subordinate fish. We found that individually-housed fish had consistently lower SMR, MMR and AS. We propose that the lower SMR in individually housed fish is the combined effect of absence of social stress and reduced reproductive allotment. Individually-housed fish grew considerably larger than group-housed fish, with an average 40% bigger body mass at the time of metabolic rate measurements. This indicates that the absence of social stress and lowered reproductive investment shifted individual energy budgets towards increased somatic growth. A comparable observation was made in the closely related *N. furzeri*, where individually-housed females reduced their reproductive effort relative to group-housed females, and this was accompanied by increased somatic growth (Graf et al. 2010).

The lower MMR (associated with a restricted aerobic scope) in individually-housed fish suggests that MMR is a plastic trait. Indeed, the within-individual repeatability of MMR estimates has been shown to decrease with time (Norin and Malte 2011). In our study, the lower MMR of individually-housed fish could perhaps be related to lack of exercise. Group-housed fish routinely engaged in high energy demanding exercise arising from social conflicts (such as to direct attacks or escape harassment), while individually-housed fish were comparatively less active and lived in a more confined space. Whether MMR is sensitive to training effects in fish is not well understood, but at least one study demonstrated that female guppies *Poecilia reticulata* exposed to male harassment had increased swimming efficiency and aerobic scope compared to non-harassed females (Killen et al. 2015).

Metabolic traits and behaviour

We predicted positive associations between metabolic rate and boldness and exploratory activity but did not find strong support for these predictions. The predicted positive association between boldness and metabolic rate was only observed in *N. orthonotus* males in

relation to MMR but not SMR, and the association was negative in *N. orthonotus* females. SMR was negatively associated with boldness in *N. pianaari*. Most studies on the relationship between risk-taking behaviour and metabolic rate in fish have focused on the SMR and often detected positive associations (Metcalf et al. 2016), though some studies failed to find any association (Binder et al. 2016; Polverino et al. 2016; White et al. 2016).

Our results suggest that other factors, such as stress, might influence the relationship between SMR and risk-taking. For example, in juvenile Atlantic salmon, a correlation between risk-taking and SMR was observed among individuals with access to cover, but not among individuals without access to cover (Finstad et al. 2007). Being confined to respirometry chambers may be perceived stressful and increase cortisol levels (Murray et al. 2017), with a concomitant increase in oxygen consumption (Morgan and Iwama 1996; O'Connor et al. 2010). Contrasting stress coping styles (proactive and reactive) are associated with boldness-shyness and activity of the hypothalamic-pituitary-adrenal axis (Koolhaas et al. 1999; Øverli et al. 2007). The lack of correlation (*N. orthonotus*) and negative correlation (*N. pianaari*) between SMR and boldness, could therefore be a consequence of individual variation in stress coping styles, as observed in the Senegalese sole, *Solea senegalensis*. Shy sole individuals (with reactive coping styles) had higher cortisol levels and increased SMR during respirometry measurements (Martins et al. 2011). Future studies should consider the possible confounding factors of stress-coping style when investigating correlations between behaviour and metabolic rate.

We also predicted that bold individuals would have higher MMR and AS, which we only observed in *N. orthonotus* males. Similar to our observation, where boldness was associated not with SMR but MMR and AS, bold juvenile bluegill sunfish, *Lepomis macrochirus* had higher MMR and AS compared to shy individuals, whereas no difference was observed in SMR (Binder et al. 2016). In juvenile Ambon damselfish, *Pomacentrus amboinensis*, aggression and dominance were positively associated with higher AS (Killen et al. 2014). Together, this suggests that MMR and AS may be closely related to behaviours that require high levels of energy expenditure. However, given that we observed no covariations between MMR, AS and boldness in *N. pianaari* and female *N. orthonotus*, this relationship appears potentially species- and sex-specific. The sex-specific association between MMR and boldness in *N. orthonotus* is supported by the outcome of a recent meta-analysis (Royauté et al. 2018). Across 42 studies on various taxa, females exhibited correlations between behaviour, physiology and life-history in the opposite direction to those observed in males and those predicted by the POLS hypothesis (Royauté et al. 2018). We speculate that stronger covariation between MMR and boldness in males is linked to higher variation in their reproductive potential (Bonduriansky et al. 2008). Especially in more aggressive *N. orthonotus*, this may enable coexistence of bolder dominant males and shyer subordinate males which approach reproductive opportunities by alternative tactics, with their behavioural traits perhaps regulated by neuroendocrine mechanisms (Tripp et al. 2018).

We did not find any support for the “performance model” (Careau et al. 2008) that predicts a positive association between SMR and activity. According to this model, a high individual SMR reflects larger metabolic machinery with a high capacity for energy output. However, to meet maintenance cost, individuals must be more active overall in the search for food (Careau et al. 2008). There was no correlation between exploratory activity and SMR or any other metabolic trait. Our results fit the predictions of the “independent model”, where SMR is considered to be independent of activity levels if skeletal muscle has a relatively low maintenance cost. Hence, being more active does not entail a higher energy expenditure at rest (Careau and Garland 2012). We note that these links may also be affected by fish motivation. In our standardised experimental conditions, fish could have

lacked the motivation to explore the environment, while measuring exploration activity in the wild would have produced a different outcome. Still, even under natural conditions, activity and SMR may not correlate (Farwell and McLaughlin 2009; Baktoft et al. 2016).

Metabolic traits and life history

The strongest evidence for a link between pace-of-life syndrome and life history was detected in *N. pienaar*, where SMR and lifespan were strongly negatively correlated. In *N. orthonotus*, this evidence was more ambiguous with a negative correlation between MMR (and AS) and lifespan observed only in males. The oxidative damage theory of ageing, a well-supported mechanistic theory of aging (Beckman and Ames 1998), provides a mechanistic link between metabolic rate and lifespan. The generation of reactive oxygen species (ROS) during oxidative phosphorylation (which is intrinsically positively linked to metabolic rate), causes cellular damage that accumulates and leads to functional senescence. Using the same cohort of fish as in the present study, Blažek et al. (2017) found a general increase in oxidative stress with age across tissues. This increase was accelerated in the populations of *N. orthonotus* and *N. pienaar* with intrinsically shorter lifespans. Yet, shorter lifespan was not associated with higher metabolic rate at the population level (Blažek et al. 2017). This makes oxidative stress from ATP production less likely as the underlying cause of the correlation between SMR and lifespan observed in the present study. Unfortunately, it was not possible to compare metabolic rate, oxidative stress and lifespan directly at the individual level, as individuals need to be sacrificed to obtain reliable estimates of oxidative stress in their tissues. Additionally, we note that whole-animal oxygen consumption rate does not accurately reflect ATP production, as the amount of ATP produced per unit of oxygen consumed can vary (Brand 2005). Given that we observed high SMR being strongly associated with shyness in *N. pienaar*, we propose this may be a manifestation of a reactive stress coping style. Glucocorticoids have been shown to modulate oxidative stress in vertebrates (Constantini et al. 2011), suggesting a link between stress-coping style and ageing. This appears an interesting subject for future studies.

Size at maturation was not associated with any metabolic trait, contrary to the positive association predicted by the POLS theory. It is possible that our metabolic measurements were taken too late, 12–14 weeks after maturation. In annual killifish sexual maturation is extremely rapid (Vrtílek et al. 2018b) and metabolic rate shows ontogenetic plasticity (Sowersby et al. 2019). Therefore, we could possibly have detected correlations between juvenile growth and metabolic traits in accordance with the POLS prediction, if metabolic traits were measured at an earlier age (Rosenfeld et al. 2015).

Conclusions

We report species- and sex-specific demonstrations of POLS in two species of African annual killifish. Overall, we found only limited support for relationships between metabolic rate, behaviour and life history at the level of individual fish. The predicted positive relationship between risk-taking behaviour and SMR was not demonstrated at all, and a negative association was actually present in *N. pienaar*. Instead, we report a positive association between MMR and risk-taking behaviour in male *N. orthonotus* but a negative one in females. There was robust support in *N. pienaar* (but not *N. orthonotus*) for our

prediction that low SMR is related to longer lifespan. In *N. orthonotus* there were clear differences between males and females in how MMR (and therefore capacity to perform aerobic activity) was related to individual boldness and lifespan, with associations conforming to our prediction in males rather than females. Exploratory activity and size at maturation were not associated with metabolic traits. Group-housed fish had higher respiration rates, but social environment did not mediate relationships between metabolism and behaviour or metabolism and life history. These findings provide limited support for POLS predictions but suggest, in line with a growing number of observations, that traits related to POLS form more complex associations mediated by sex and environmental factors.

Acknowledgements We thank Alexander Kotschal and two anonymous referees for constructive comments and Rowena Spence for comments and linguistic corrections.

Author contributions MR conceived the study and contributed to writing. CM, RB and RR performed experimental work. CM analyzed data and drafted the paper. All authors commented on the final version of the paper.

Funding Funding came from Czech Science Foundation (19-01781S).

Data availability Original data are deposited at Figshare (DOI: <https://doi.org/10.6084/m9.figshare.11605236>).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The research was conducted with the approval of ethical committees of the Institute of Vertebrate Biology (No. 163–12) and the Ministry of Agriculture (CZ 62760203), and complies with the legal regulations of the Czech Republic.

References

- Álvarez D, Nicieza AG (2005) Is metabolic rate a reliable predictor of growth and survival of brown trout (*Salmo trutta*) in the wild? *Can J Fish Aquat Sci* 62:643–649
- Arnqvist G, Stojković B, Rönn JL, Immonen E (2017) The pace-of-life: A sex-specific link between metabolic rate and life history in bean beetles. *Funct Ecol* 31:2299–2309
- Auer SK, Salin K, Rudolf AM, Anderson GJ, Metcalfe NB (2015) Flexibility in metabolic rate confers a growth advantage under changing food availability. *J Anim Ecol* 84:1405–1411
- Auer SK, Dick CA, Metcalfe NB, Reznick DN (2018) Metabolic rate evolves rapidly and in parallel with the pace of life history. *Nat Commun* 9:14
- Baktoft H, Jacobsen L, Skov C, Koed A, Jepsen N, Berg S, Boel M, Aarestrup K, Svendsen JC (2016) Phenotypic variation in metabolism and morphology correlating with animal swimming activity in the wild: relevance for the OCLTT (oxygen- and capacity-limitation of thermal tolerance), allocation and performance models. *Conserv Physiol* 4:cov055
- Beamish FWH (1964) Seasonal changes in the standard rate of oxygen consumption of fishes. *Can J Zool* 42:189–194
- Beckman KB, Ames B (1998) The free radical theory of aging matures. *Physiol Rev* 78:547–581
- Binder TR, Wilson ADM, Wilson SM, Suski CD, Godin JGJ, Cooke SJ (2016) Is there a pace-of-life syndrome linking boldness and metabolic capacity for locomotion in bluegill sunfish? *Anim Behav* 121:175–183
- Binning SA, Ros AFH, Nusbaumer D, Roche DG (2015) Physiological Plasticity to Water Flow Habitat in the Damselfish, *Acanthochromis polyacanthus*: Linking Phenotype to Performance. *PLoS One* 10:e0121983
- Biro PA, Abrahams MV, Post JR, Parkinson EA (2004) Predators select against high growth rates and risk-taking behaviour in domestic trout populations. *Proc R Soc B* 271:2233–2237

- Blažek R, Polačik M, Kačer P, Cellerino A, Řežucha R, Methling C, Tomášek O, Syslová K, Terzibasi Tozzini E, Albrecht T, Vrtílek M, Reichard M (2017) Repeated intraspecific divergence in life span and aging of African annual fishes along an aridity gradient. *Evolution* 71:386–402
- Bonduriansky R, Maklakov A, Zajitschek F, Brooks R (2008) Sexual selection, sexual conflict and the evolution of ageing and life span. *Funct Ecol* 22:443–453
- Brand MD (2005) The efficiency and plasticity of mitochondrial energy transduction. *Biochem Soc Trans* 33:897–904
- Brown D (2009) Tracker video analysis and modeling tool (version 4.82). Available at <https://physlets.org/tracker/index.html> Accessed 2 Sept 2019
- Brown C, Braithwaite VA (2004) Size matters: a test of boldness in eight populations of the poeciliid *Brachyraphis episcopi*. *Anim Behav* 68:1325–1329
- Burton T, Killen SS, Armstrong JD, Metcalfe NB (2011) What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proc R Soc B* 278:3465–3473
- Careau V, Thomas D, Humphries MM, Réale D (2008) Energy metabolism and animal personality. *Oikos* 117:641–653
- Careau V, Garland T (2012) Performance, personality, and energetics: correlation, causation, and mechanism. *Physiol Biochem Zool* 85:543–571
- Chabot D, Steffensen JF, Farrell AP (2016) The determination of standard metabolic rate in fishes. *J Fish Biol* 88:81–121
- Clark TD, Sandblom E, Jutfelt F (2013) Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *J Exp Biol* 216:2771–2782
- Costantini D, Marasco V, Møller AP (2011) A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. *J Comp Physiol B* 181:447–456
- Cutts CJ, Betcalfe NB, Caylor AC (1998) Aggression and growth depression in juvenile Atlantic salmon: the consequences of individual variation in standard metabolic rate. *J Fish Biol* 52:1026–1037
- Dijkstra PD, Pierotti ME, Seehausen O, Metcalfe NB (2016) Metabolism, oxidative stress and territorial behaviour in a female colour polymorphic cichlid fish. *Behav Ecol Sociobiol* 70:99–109
- Eckerström-Liedholm S, Sowersby W, Gonzalez-Voyer A, Rogell B (2017) Time-limited environments affect the evolution of egg–body size allometry. *Evolution* 71:1900–1910
- Farwell M, McLaughlin RL (2009) Alternative foraging tactics and risk taking in brook charr (*Salvelinus fontinalis*). *Behav Ecol* 20:913–921
- Finstad AG, Ugedal O, Forseth T, Næsje TF (2004) Energy-related juvenile winter mortality in a northern population of Atlantic salmon (*Salmo salar*). *Can J Fish Aquat Sci* 61:2358–2368
- Finstad AG, Forseth T, Ugedal O, Næsje T (2007) Metabolic rate, behaviour and winter performance in juvenile Atlantic salmon. *Funct Ecol* 21:905–912
- Furness AI (2016) The evolution of an annual life cycle in killifish: adaptation to ephemeral aquatic environments through embryonic diapause. *Biol Rev* 91:796–812
- Furness AI, Reznick DN, Springer MS, Meredith RW (2015) Convergent evolution of alternative developmental trajectories associated with diapause in African and South American killifish. *Proc R Soc B Biol Sci* 282:20142189
- 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
- Grantner A, Taborsky M (1998) The metabolic rates associated with resting, and with the performance of agonistic, submissive and digging behaviours in the cichlid fish *Neolamprologus pulcher* (Pisces: Cichlidae). *J Comp Physiol B* 168:427–433
- Grobler JMB, Wood CM (2013) The physiology of rainbow trout in social hierarchies: two ways of looking at the same data. *J Comp Physiol B* 183:787–799
- Huntingford FA, Andrew G, Mackenzie S, Morera D, Coyle SM, Pilarczyk M, Kadri S (2010) Coping strategies in a strongly schooling fish, the common carp *Cyprinus carpio*. *J Fish Biol* 76:1576–1591
- Hämäläinen A, Immonen E, Tarka M, Schuett W (2018) Evolution of sex-specific pace-of-life syndromes: causes and consequences. *Behav Ecol Sociobiol* 72:50
- Immonen E, Hämäläinen A, Schuett W, Tarka M (2018) Evolution of sex-specific pace-of-life syndromes: genetic architecture and physiological mechanisms. *Behav Ecol Sociobiol* 72:60
- Killen SS, Atkinson D, Glazier DG (2010) The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. *Ecol Lett* 13:184–193
- Killen SS, Marras S, Ryan MR, Domenici P, McKenzie DJ (2012) A relationship between metabolic rate and risk-taking behaviour is revealed during hypoxia in juvenile European sea bass. *Funct Ecol* 26:134–143
- Killen SS, Marras S, Metcalfe NB, McKenzie DJ, Domenici P (2013) Environmental stressors alter relationships between physiology and behavior. *Trends Ecol Evol* 28:651–658

- Killen SS, Mitchell MD, Rummer JL, Chivers DP, Ferrari MC, Meekan MG, McCormick MI (2014) Aerobic scope predicts dominance during early life in a tropical damselfish. *Funct Ecol* 28:1367–1376
- Killen SS, Croft DP, Salin K, Darden SK (2015) Male sexually coercive behaviour drives increased swimming efficiency in female guppies. *Funct Ecol* 30:576–583
- Killen SS, Norin T, Halsey LG (2017) Do method and species lifestyle affect measures of maximum metabolic rate in fish? *J Fish Biol* 90:1037–1046
- Koolhaas JM, Korte SM, De Boer SF, Van Der Vegt BJ, Van Reenen CG, Hopster H, De Jong IC, Ruis MAW, Blokhuis HJ (1999) Coping styles in animals: current status in behavior and stress-physiology. *Neurosci Biobehav Rev* 23:925–935
- Marras S, Claireaux G, McKenzie DJ, Nelson JA (2010) Individual variation and repeatability in aerobic and anaerobic swimming performance of European sea bass, *Dicentrarchus labrax* J Exp Biol 213:26–32
- Martins CIM, Castanheira MF, Engrola S, Costas B, Conceição LEC (2011) Individual differences in metabolism predict coping styles in fish. *Appl Anim Behav Sci* 130:135–143
- Mathot KJ, Dingemanse NJ, Nakagawa S (2019) The covariance between metabolic rate and behaviour varies across behaviours and thermal types: meta-analytic insights. *Biol Rev* 94:1056–1074
- Metcalf NB, Van Leeuwen TE, Killen SS (2016) Does individual variation in metabolic phenotype predict fish behaviour and performance? *J Fish Biol* 88:298–321
- Millidine KJ, Metcalfe NB, Armstrong JD (2009) Presence of a conspecific causes divergent changes in resting metabolism, depending on its relative size. *Proc R Soc B* 276:2103–2108
- Morgan JD, Iwama GK (1996) Cortisol-induced changes in oxygen consumption and ionic regulation in coastal cutthroat trout (*Oncorhynchus clarki clarki*) parr. *Fish Physiol Biochem* 15:385–394
- Murray L, Rennie MD, Svendsen JC, Enders EC (2017) Respirometry increases cortisol levels in rainbow trout *Oncorhynchus mykiss*: implications for measurements of metabolic rate. *J Fish Biol* 90:2206–2213
- Norin T, Clark TD (2016) Measurement and relevance of maximum metabolic rate in fishes. *J Fish Biol* 88:122–151
- Norin T, Malte H (2011) Repeatability of standard metabolic rate, active metabolic rate and aerobic scope in young brown trout during a period of moderate food availability. *J Exp Biol* 214:1668–1675
- O'Connor CM, Gilmour KM, Arlinghaus R, Matsumura S, Suski CD, Philipp DP, Cooke S (2010) The consequences of short-term cortisol elevation on individual physiology and growth rate in wild largemouth bass (*Micropterus salmoides*). *Can J Fish Aquat Sci* 68:693–705
- Oikawa S, Itazawa Y (2003) Relationship between summated tissue respiration and body size in a marine teleost, the porgy *Pagrus major* Fish Sci 69:687–694
- Øverli Ø, Sørensen C, Pulman KGT, Pottinger TG, Korzan W, Summers CH, Nilsson GE (2007) Evolutionary background for stress-coping styles: relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. *Neurosci Biobehav Rev* 31:396–412
- Passos C, Tassinio B, Rosenthal GG, Reichard M (2015) Reproductive behavior and sexual selection in annual fishes. In: Berois N, García G, de Sá RO (eds) *Annual Fishes: Life History Strategy, Diversity, and Evolution*. CRC Press, Boca Raton, pp 207–229
- Polačik M, Reichard M (2009) Indirect fitness benefits are not related to male dominance in a killifish. *Behav Ecol Sociobiol* 63:1427–1435
- Polačik M, Reichard M (2010) Diet overlap among three sympatric African annual killifish species *Nothobranchius* spp. from Mozambique. *J Fish Biol* 77:754–768
- Polačik M, Reichard M (2011) Asymmetric reproductive isolation between two sympatric annual killifish with extremely short lifespans. *PLoS One* 6:e22684
- Polačik M, Harrod C, Blažek R, Reichard M (2014) Trophic niche partitioning in communities of African annual fish: Evidence from stable isotopes. *Hydrobiologia* 721:99–106
- Polačik M, Blažek R, Reichard M (2016) Laboratory breeding of the short-lived annual killifish *Nothobranchius furzeri*. *Nat Protoc* 11:1396–1413
- Polverino G, Bierbach D, Killen SS, Uusi-Heikkilä S, Arlinghaus R (2016) Body length rather than routine metabolic rate and body condition correlates with activity and risk-taking in juvenile zebrafish *Danio rerio*. *J Fish Biol* 89:2251–2267
- R Core Team (2019) A language and environment for statistical computing. Retrieved from <https://www.R-project.org>
- Réale D, Garant D, Humphries MM, Bergeron P, Careau V, Montiglio PO (2010) Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philos Trans R Soc B* 365:4051–4063
- Reichard M, Janáč M, Polačik M, Blažek R, Vrtílek M (2017) Community assembly in *Nothobranchius* annual fishes: Nested patterns, environmental niche and biogeographic history. *Ecol Evol* 7:2294–2306
- Reid D, Armstrong JD, Metcalfe NB (2012) The performance advantage of a high resting metabolic rate in juvenile salmon is habitat dependent. *J Anim Ecol* 81:868–875
- Reidy SP, Kerr SR, Nelson JA (2000) Aerobic and anaerobic swimming performance of individual Atlantic cod. *J Exp Biol* 203:347–357

- Ricklefs RE, Wikelski M (2002) The physiology/life-history nexus. *Trends Ecol Evolut* 17:462–468
- Robertson G, Armstrong JD, Nislow KH, Herfindal I, McKelvey S, Einum S (2015) Spatial variation in the relationship between performance and metabolic rate in wild juvenile Atlantic salmon. *J Anim Ecol* 83:791–799
- Roff DA (2002) *Life History Evolution*. Sinauer Associates Inc, Massachusetts
- Rosenfeld J, Van Leeuwen T, Richards J, Allen D (2015) Relationship between growth and standard metabolic rate: measurement artefacts and implications for habitat use and life-history adaptation in salmonids. *J Anim Ecol* 84:4–20
- Royauté R, Berdal MA, Garrison CR, Dochtermann NA (2018) PACELESS life? A meta-analysis of the pace-of-life syndrome hypothesis. *Behav Ecol Sociobiol* 72:64
- Salzman TC, McLaughlin AL, Westneat DF, Crowley PH (2018) Energetic trade-offs and feedbacks between behavior and metabolism influence correlations between pace-of-life attributes. *Behav Ecol Sociobiol* 72:54
- Smith BR, Blumstein DT (2008) Fitness consequences of personality: a meta-analysis. *Behav Ecol* 19:448–455
- Sloat M, Reeves G (2014) Demographic and phenotypic responses of juvenile steelhead trout to spatial predictability of food resources. *Ecology* 95:2423–2433
- Sloman K, Motherwell G, O'Connor K, Taylor AC (2000) The effect of social stress on the Standard Metabolic Rate (SMR) of brown trout, *Salmo trutta*. *Fish Physiol Biochem* 23:49–53
- Sowersby W, Morozov S, Eckerström-Liedholm S, Lehmann P, Rowiński PK, Näslund J, Gonzalez-Voyer A, Rogell B (2019) Coevolution between life-history and metabolic rate depends on ontogenetic stage. *bioRxiv* 705707
- Speakman JR, Blount JD, Bronikowski AM, Buffenstein R, Isaksson C, Kirkwood TB, Monaghan P, Ozanne SE, Beaulieu M, Briga M, Carr SK, Christensen LL, Cochemé HM, Cram DL, Dantzer B, Harper JM, Jurk D, King A, Noguera JC, Salin K, Sild E, Simons MJ, Smith S, Stier A, Tobler M, Vitikainen E, Peaker M, Selman C (2015) Oxidative stress and life histories: unresolved issues and current needs. *Ecol Evolut* 5:5745–5757
- Srean P, Almeida D, Rubio-Gracia F, Luo Y, García-Berthou E (2017) Effects of size and sex on swimming performance and metabolism of invasive mosquitofish *Gambusia holbrooki*. *Ecol Freshw Fish* 26:424–433
- Stearns SC (1992) *The evolution of life histories*. Oxford University Press, Oxford
- Steffensen JF (1989) Some errors in respirometry of aquatic breathers: how to avoid and correct for them. *Fish Physiol Biochem* 6:49–59
- Tripp JA, Feng NY, Bass AH (2018) Behavioural tactic predicts preoptic-hypothalamic gene expression more strongly than developmental morph in fish with alternative reproductive tactics. *Proc R Soc B Biol Sci* 285:20172742
- Vrtílek M, Reichard M (2015) Highly plastic resource allocation to growth and reproduction in females of an African annual fish. *Ecol Freshw Fish* 24:616–628
- Vrtílek M, Žák J, Poláčik M, Blažek R, Reichard M (2018) Longitudinal demographic study of wild populations of African annual killifish. *Sci Rep* 8:4774
- Vrtílek M, Žák J, Pšenička M, Reichard M (2018) Extremely rapid maturation of a wild African annual fish. *Curr Biol* 28:R822–R824
- Wikelski M, Spinney L, Schelsky W, Scheuerlein A, Gwinner E (2003) Slow pace of life in tropical sedentary birds: a common-garden experiment on four stonechat populations from different latitudes. *Proc R Soc B* 270:2383–2388
- Wiersma P, Muñoz-García A, Walker A, Williams JB (2007) Tropical birds have a slow pace of life. *Proc Natl Acad Sci* 104:9340–9345
- White SJ, Kells TJ, Wilson AJ (2016) Metabolism, personality and pace of life in the Trinidadian guppy, *Poecilia reticulata*. *Behaviour* 153:1517–1543
- Žák J, Reichard M, Gvoždík L (2018) Limited differentiation of fundamental thermal niches within the killifish assemblage from shallow temporary waters. *J Therm Biol* 78:257–262