Open access • Journal Article • DOI:10.1111/JFB.12933

Behaviour in a standardized assay, but not metabolic or growth rate, predicts behavioural variation in an adult aquatic top predator Esox lucius in the wild

Kate L. Laskowski, Christopher T. Monk, Giovanni Polverino, Josep Alós ...+6 more authors

Institutions: Leibniz Association, Humboldt University of Berlin

Published on: 01 Apr 2016 - Journal of Fish Biology (J Fish Biol)

PERSONALITY AND PACE-OF-LIFE SYNDROME IN FISHES: NEW PERSPECTIVES

DISSERTATION

19 Dezember 2016

zur Erlangung des akademischen Grades
Doctor of Philosophy (Ph.D)

eingereicht an der
Lebenswissenschaftlichen Fakultät der Humboldt-Universität zu Berlin

von

M. Sc., Giovanni Polverino

Präsident der Humboldt-Universität zu Berlin
Prof. Dr.-Ing. Dr. Sabine Kunst

Dekan der Lebenswissenschaftlichen Fakultät der Humboldt-Universität zu Berlin
Prof. Dr. Bernhard Grimm

Vorsitzender:	Prof. Dr. Dieter Kirschke
Gutachter:	1. PD Dr. Thomas Mehner 2. Prof. Dr. Jens Krause 3. Prof. Mag. Dr. Barbara Taborsky
Weiteres Mitglied:	Dr. Max Wolf

Faculty of Life Sciences

IGB
Leibniz-Institute of
Freshwater Ecology and Inland Fisheries

Department of Biology and Ecology of Fishes

B-Types
Ecological, Evolutionary and Managerial Consequences of Behavioral Types

B-Type group

Giovanni Polverino

Personality and pace-of-life syndrome in fishes: new perspectives

Humboldt-Universität zu Berlin, Germany 2016

Contents

Summary

Among-individual differences in behavior (i.e., animal personality) are assumed to be consistent over time and contexts. In theory, they are often explained by individual variations in energy costs of self-maintenance as well as life history among animals (i.e., state variables), commonly expressed as the pace-of-life syndrome hypothesis. Yet, recent theories have disputed the consistency of personality types over lifetime and their rigid state-dependency. This thesis aims to offer novel insights on the mechanisms behind the emergence and development of personality over lifetime of fishes, its context dependency, and its link to individual variation in state variables. In a sequence of five independent yet interconnected studies, I tested the assumptions above and observed that (1) personality differences increased during lifetime as a function of consistent declines in the behavioral plasticity with increasing age of animals; (2) personality estimates in young animals were weaker and thus more vulnerable to experimental biases compared to adults; (3) personality variation did not depend upon individual differences in energy costs of self-maintenance and life-history traits among individuals, under both laboratory and natural settings; and (4) the relationship between behavioral, metabolic, and life-history traits was manifested only in fish populations with slow rather than fast life-history strategies. This thesis suggests that personality variation in animals might be the inevitable outcome of development, raising questions about the reliability of personality estimates in juvenile individuals. Furthermore, individual variation in personality and "states" may act independently (i.e., phenotypes are uncorrelated) under environmental conditions and evolutionary contexts that mask or select against their trade-offs.

Zusammenfassung

Individuelle Verhaltensunterschiede (sog. „Animal personality“) werden oft als konsistent über die Zeit und situationsunabhängig angenommen. Vielfach werden solche Persönlichkeitsunterschiede zwischen Tieren einer Art durch individuelle Unterschiede im Energiehaushalt sowie Lebenszyklusvariablen (sog. ‚state variables‘, dt. Zustandsgrößen) erklärt. Dies ist in der „pace-of-life“ Hypothese zusammengefasst. In neueren Arbeiten wurde jedoch die Konsistenz von Persönlichkeitsmerkmalen über den Lebensverlauf und deren strikte Abhängigkeit von Zustandsgrößen in Frage gestellt. Die vorliegende Dissertation soll neue Einblicke in die Mechanismen, die zur Entstehung von Persönlichkeitsunterschieden im Verlauf des Lebens von Fischen, deren Situationsabhängigkeit sowie ihre Verbindung zu individuellen Unterschieden in Zustandsgrößen liefern. In einer Abfolge von fünf unabhängigen Studien untersuchte ich die genannten Annahmen und fand, dass (1) Persönlichkeitsunterschiede sich im Laufe des Lebens von Tieren vergrößern; (2) Persönlichkeitsabschätzungen bei jungen Tieren oft stärker vom experimentellen Aufbau beeinflusst werden als bei Erwachsenen; (3) der Energiehaushalt und Lebenszyklusvariablen Persönlichkeitsunterschiede sowohl unter Laborbedingungen als auch im Freiland nicht hinreichend erklären können; (4) Beziehungen zwischen Persönlichkeitsunterschieden und Unterschieden im Energiehaushalt und in Lebenszyklusvariablen fanden sich nur bei Fischen einer Population mit langsamer Lebenszyklusstrategie nicht jedoch in einer Population mit schnellem Lebenszyklus. Die vorliegende Arbeit suggeriert daher, dass sich erst im Verlauf des Lebens eines Tieres Persönlichkeitsunterschiede unvermeidbar entwickeln. Dies stellt Persönlichkeitsmessungen bei juvenilen Tieren

grundsätzlich in Frage. Weiterhin scheinen Persönlichkeitsunterschiede und Zustandsgrößen unter bestimmten Umweltbedingungen und evolutiven Szenarien voneinander unabhängig zu sein.

Chapter 1

General introduction

ANIMAL PERSONALITY

The idea that individuals behave differently from each other in a predictable and consistent manner is relatively recent in the field of behavioral ecology. It originates instead in the human-psychology literature, whereby five main personality axes (i.e., extraversion, neuroticism, agreeableness, conscientiousness, and openness to experience) were found to capture approximatively 50 percent of the behavioral variation among individuals (Goldberg 1990; Costa and McCrae 1992).

Increasing evidence, however, suggests that the personality concept can be extended to the whole animal kingdom. Animals within the same species and/or population thus differ in their behavior among each other and those differences are maintained over time and contexts (for reviews see (Gosling and John 1999; Gosling 2001; Carere and Eens 2005; Réale et al. 2007; Dingemanse et al. 2010)) in both wild and laboratory settings (Herborn et al. 2010; Laskowski et al. 2016). Such individual differences in behavior, traditionally ignored by the classic behavioral ecology research, are attracting increasing interest in the scientific community (Sih et al. 2012; Wolf and Weissing 2012). In fact, differences in behavior among individuals have been found to resemble or even exceed those attributed to differences among species (Palkovacs and Dalton 2012).

Causes of animal personality The evolution of animal personality is mainly linked to genetic, parental, and environmental factors (Réale et al. 2007). Personality has indeed a heritable component and approximately 52 percent of the variation in animal personality is caused by additive genetic variation (Dochtermann et al. 2015). Parental signals (e.g., hormones, olfactory cues, parental behavior, etc.) also represent an informative inheritance capable of preparing offspring to face environmental challenges after birth (Taborsky 2006; Bell et al. 2016). Nevertheless, environmental challenges experienced during life can overwrite prenatal factors (Gorski et al. 2006; Stratmann and Taborsky 2014) and drive different behavioral strategies among individuals as a result of varying coping strategies (Wolf et al. 2007). For example, individuals might adapt their risk-taking strategies later on in life based on their own perception of predation risk from the environment in which they grew up (Stratmann and Taborsky 2014).

A corollary of the animal personality theory is that personality differences should not be present in the first place when genetic, parental, and environmental source of behavioral variation are absent. However, this critical assumption has received very little empirical attention. In this thesis, I explore this aspect by testing whether personality types were present when differences in genetic, maternal, and environmental factors among animals were experimentally minimized.

Personality over lifetime A key assumption in the animal personality theory is that behavioral differences among individuals are consistent over time and contexts (Réale et al. 2007). However, recent evidence suggest that personality types might not be as consistent as previously thought, especially when considering relatively long time intervals (reviewed by (Biro and Stamps 2015)). Indeed, most studies do not follow individuals over their lifetime (Trillmich et al. 2015), even though this is crucial for the concept of animal personality.

Contrary to general predictions from the animal personality theory, recent longitudinal studies have detected increases in the repeatability of among-individual differences in behavior over the lifetime of field crickets (Fisher et al. 2015), mangrove killifish (Edenbrow and Croft 2013), Eastern mosquitofish (Polverino et al. 2016a), red junglefowls (Favati et al. 2015), and laboratory mice (Freund et al. 2013). These findings imply that either individual behavior becomes more consistent and/or that behavioral differences among individuals increase over time. With respect to the first scenario, recent theoretical predictions suggest that natural selection favors high plasticity (and hence weak personality) in the behavior of young individuals, which face uncertain environmental conditions and variable selection pressures (Fischer et al. 2014; Fawcett and Frankenhuis 2015; Nettle and Bateson 2015). However, behavioral plasticity is expected to decline with age once the environmental uncertainty is reduced (Stamps and Krishnan 2014; Fawcett and Frankenhuis 2015) and benefits of plasticity in older animals no longer outweigh its costs (Fischer et al. 2014). Under this perspective, it can be assumed that personality differences might be more pronounced in adults than in juvenile individuals. As a consequence, personality estimates on young animals might be more vulnerable to experimental biases compared to adults. To the best of my knowledge, however, no empirical studies have ever tested those assumptions.

In the second chapter of this thesis, I tested whether the decline in behavioral plasticity over lifetime represents a potential mechanism for the behavioral differentiation of animals. The main assumption of the study is that consistent declines in behavioral plasticity over lifetime can magnify small initial differences in the behavior of conspecifics that are, in turn, associated to inevitable and elusive discrepancies in the early development of animals. In the third chapter, I tested whether personality estimates on young individuals (potentially weaker compared to adults) were more vulnerable to experimental biases compared to personality estimates on adult animals. This last research question was inspired by the surprising lack of empirical evidence on the context dependency of personality in adult animals, which have instead been well documented on juveniles (Biro et al. 2010; Näslund et al. 2015).

PACE-OF-LIFE SYNDROME HYPOTHESIS

The pace-of-life syndrome (POLS) hypothesis suggests that consistent among-individual differences in behavior (i.e., personality) and metabolic rate mediate, and are in turn mediated by, life-history trade-offs (Stamps 2007; Wolf et al. 2007; Réale et al. 2010). Life-history trade-offs thus explain why behavioral and metabolic variations are maintained within populations. For instance, higher metabolic rates are expected to allow bold individuals to sustain a greater muscular activity (White and Kearney 2013) to be more successful in competing for resources and thus grow faster (Biro and Stamps 2008) and produce more offspring (Boon et al. 2007) than their conspecifics. The cost of those high performances is a less efficient immune system (Woodhams et al. 2016) and a shorter life span (Careau et al. 2010). Fluctuating ecological conditions should then favor the coexistence of different strategies within populations (Wolf and McNamara 2012). The POLS thus expands predictions from the classic life-history theory (Stearns 1992; Roff 1993) by including behavior and metabolism into the evolutionary framework, in which individuals differ from each other in allocating resources favoring either survival or reproduction (Stearns 1989; Stearns 2000).

However, previous studies have found mixed support for the POLS (see the review by (Hille and Cooper 2015)) and approximately 50 percent of the studies have rejected its predictions (Dammhahn et al. in prep). Methodological issues when testing the POLS could potentially explain the substantial discrepancy between predictions and observations (Mathot and Dingemanse 2015). In particular, artificial environments might not be suitable for studying adaptive polymorphisms in animals, such as personality. For example, personality types measured under standardized settings might not match those expressed under natural conditions (Niemelä and Dingemanse 2014). As a consequence, spurious personality estimates could impede testing the POLS assumptions under laboratory settings. To further investigate this hypothesis, I have tested the assumptions of the POLS in both wild and laboratory contexts through two independent studies included in this thesis.

Evolutionary implications on the POLS It has been suggested that the generality of the POLS hypothesis may not allow for precise predictions on the strength and sign of phenotypic correlations across different ecological and evolutionary contexts (Réale et al. 2010). For example, high metabolic rates should allow animals to spend more energy in activity-related behaviors only when those traits do not compete with each other for energy resources (i.e., when resources are unlimited). On the contrary, negative or no correlations between metabolic and behavioral traits should be respectively expected under fixed energy budgets (i.e., when high metabolic rates obligate individuals to decrease energetically costly behaviors) and when high metabolic rates do not preclude energetic investments on costly behaviors (Mathot and Dingemanse 2015). As a result, the relationship between behavioral, metabolic, and life-history traits may diverge from POLS predictions under evolutionary scenarios in which selection alters or suppresses trade-offs between specific traits (Hille and Cooper 2015).

Certain selection pressures might disfavor the evolution of phenotypic correlations and thus reject the POLS assumptions, as illustrated in the following example. In a given water body, natural selection acts against a specific phenotype (e.g., large body size of fishes) that is strongly correlated with another phenotype not under selection (e.g., boldness of fishes). Consequently, the ability of individuals to rapidly adapt to one selective pressure (e.g., size-selected fishing; (Enberg et al. 2012)) would reduce substantially, since both size and boldness should evolve simultaneously. Lowered abilities of individuals to adapt to strong selection pressures might ultimately cause the loss of both phenotypes within the population (i.e., large body size and boldness), as suggested by (Arlinghaus et al. 2016). Alternatively, deleterious correlations should be disfavored by selection and traits are expected to evolve freely when those selective pressures are maintained over time (Sih et al. 2004). Recent evidence shows that chronic differences in predator regimes can give rise to diverse correlation patterns between behavioral traits in animal populations (Heinen-Kay et al. 2016). Those correlation patterns are genetically established and thus maintained even outside of their ecological context (Heinen-Kay et al. 2016).

In the last chapter of this thesis I explored those evolutionary implications on predictions from the POLS in a longitudinal experiment. In particular, I tested predictions from the POLS at both the between-population and among-individual level in two separate fish populations adapted to diverse environmental conditions (e.g., different predator regimes).

STUDY SPECIES

There is an increasing utilization of fishes as animal models for behavioral studies because of their relatively high reproductive rate and short inter-generation time compared to mammals, with the possibility of maintaining fishes at high densities. Accordingly, a florid literature on fishes behavior has supported applications to technological (Krause et al. 2011), biomedical (Gerlai 2010), ecological (Lienart et al. 2014), and evolutionary (Evans et al. 2011) research questions.

Moreover, fishes typically go through substantial increments in size during life that can cause substantial shifts in their ecological niches (Lundvall et al. 1999), resulting in significant variations in their mean behaviors (Dial et al. 2008). For example, variable optimal strategies with respect to foraging, risk-taking, and social interactions over lifetime can cause substantial changes in the mean behavior of fishes (Clark 1994; Biro et al. 2005; Stratmann and Taborsky 2014). Variable behavioral strategies over the lifetime of fishes make them excellent models for investigating how personality traits develop and are potentially maintained over time at the individual level (reviewed by (Toms et al. 2010)). Poeciliids are good candidates for ontogenetic studies, since these live bearing fishes require low maintenance compared to other fish taxa and their development can be visually determined based on the morphogenesis of their sexual organs during maturation (Pyke 2005; Greven 2011). Among poeciliids, the Eastern mosquitofish (*Gambusia holbrooki*) is a model species that I used in previous laboratory studies on fish behavior. Being familiar with behavioral tests, husbandry procedures, and maintenance of a model species is a technical skill that can be remarkably relevant when approaching long-term studies. Therefore, I choose the Eastern mosquitofish as a model species for all ontogenetic studies described in this thesis. Moreover, the Eastern mosquitofish is receiving increasing interest from the scientific community for its remarkable diffusion outside its native range, causing negative impacts on both the economy and biodiversity of those recently colonized regions (Lowe et al. 2000). Hence, studying the behavioral differentiation of Eastern mosquitofish over their development could provide new insights for the management of this invasive species.

With respect to the non-ontogenetic studies included in this thesis, I have instead selected two distinct fish species that could well fit the requirements for testing POLS under both laboratory and natural settings. Zebrafish (*Danio rerio*) represents the most diffused aquatic vertebrate utilized for behavioral studies (Miklósi and Andrew 2006). Its behavioral, metabolic, and life-history variation dominates the fish literature under laboratory settings and, by favoring results' validation across studies, zebrafish was selected for testing the POLS in my laboratory study. Similarly, the Northern pike (*Esox lucius*) was chosen to test POLS assumptions under natural conditions. This freshwater predator species is well distributed across the Northern hemisphere and its behavioral, metabolic, and life-history polymorphisms are well documented in the literature (Raat 1988).

OUTLINE OF THE THESIS

The two main goals of this thesis were to further investigate the development of animal personality over lifetime and the relation between personality, metabolic, and life-history traits. My main hypotheses were that personality differences strengthened over lifetime and were systematically associated with individual differences in energy demand for self-maintenance (i.e., metabolic rate) and life history (i.e., growth, size at maturation, morphology, and fecundity).

This thesis includes five independent scientific manuscripts, four of which have been published on peer-reviewed journals and one that is being reviewed by coauthors before submission. For copyright reasons, I retained the original formatting styles of the scientific journals in which they were published. For the general introduction (**chapter 1**) and discussion (**chapter 6**) I used the American spelling and the first person, although each manuscript (**chapters 2-5**) includes my own contribution to the ones from several coauthors, as indicated in the author list (and the acknowledgement section) of each manuscript. However, at the beginning of each chapter I highlighted my contribution to each manuscript. I used the word fish as the plural form of fish, while I used the term fishes when referring to different species only.

Since manuscripts build on each other, I present them in the following order with increasing levels of complexity. In **chapter 2**, I tested whether behavioral plasticity of animals declines with increasing age (i.e., individual behavior becomes more predictable with advancing age). The main goal of this chapter is to test whether the decline in behavioral plasticity over lifetime of fish could magnify small initial differences in the behavior of animals, thus representing a relevant mechanism for their behavioral differentiation. If this is true, personality differences among individuals may develop over ontogeny, contrasting the general prediction that animal personalities are stable and consistent over time. In addition, I have experimentally controlled for genetic, maternal, and environmental differences among individuals, which represent the common causes for personality differences in animals. As a result, I also tested here whether personality is present in the first place once the main sources of behavioral differentiation are minimized.

In **chapter 3**, I tested whether personality estimates on young individuals are more vulnerable to experimental biases compared to adults. In fact, the literature suggests that laboratory settings may bias personality estimates, but empirical evidence mainly concerns immature individuals. To shed light on this aspect, I tested the behavioral response in newly born, immature, and mature fish across several experimental tanks that varied in size. By doing this, I tested whether personality ranks were maintained across treatments independently by an animal's age. This study also allowed me to test whether mean behaviors varied as a function of the tank size and whether behaviors differed on average between newly born, immature, and mature individuals. In fact, the effect of the test tank size on the behavioral response of fishes remains a controversial topic, while results on the way in which behavior should change during the ontogeny of fishes are contradictory (e.g., increases or declines in risk taking over fish ontogeny).

In **chapter 4** I tested whether personality variation is linked to individual differences in metabolic rate and growth rate or size at a given age, as predicted by the POLS hypothesis. To determine whether laboratory or wild settings are more suitable for testing the POLS predictions, I tested the POLS in two independent studies in which laboratory and wild animals were utilized respectively. Since the main criticism against standardized settings is that they might bias the outcome of personality estimates, I have also compared personality estimates of wild individuals in their natural contexts against personality estimates of the same individuals within a standardized assay.

Chapter 5 investigates how evolutionary adaptations to different selection pressures may favor (or act against) links between personality, metabolic rate, and life history in animals. In fact, relationships between those phenotypes may diverge from POLS predictions under ecological and evolutionary scenarios in which selection alters or suppresses trade-offs between specific traits. For this study, I have utilized first-generation offspring from different fish populations whose native environments differed from each other in abiotic and biotic factors. One goal of this study was to test, in a standardized way, whether different environments shape different life-history trajectories in animals and whether fast and slow life-history trajectories reflect fast and slow trajectories in behavior and metabolic demands. As the second goal of the study, I tested whether populations with different life histories exhibit similar phenotypic correlations, as expected by the POLS. In short, I tested the predictions from POLS initially at the population level (i.e., mean comparisons between populations) and then at the individual level (i.e., differences among individuals) for each population separately. The scientific effort of this chapter aims to offer novel insights in the closely-related fields of animal personality and pace-of-life syndrome (POLS).

Finally, in the last chapter of this thesis (**chapter 6**) I discuss the implications of the results from chapters 2-5. In detail, in this final chapter I summarize the current state of knowledge, I synthesize the thesis findings, I state their contribution to the current knowledge, and I suggest new perspectives for future research.

.

Chapter 2

Emergence and development of personality over the ontogeny of fish in absence of environmental stress factors

This chapter consists of the following publication:

G Polverino, C Cigliano, S Nakayama, T Mehner, 2016. Emergence and development of personality over the ontogeny of fish in absence of environmental stress factors. *Behavioral Ecology and Sociobiology* 0:1-11.

Authors' contributions (*i.e., entire, large, predominant, partial):*

GP (**large**) and TM developed the concept; **GP (entire)** did the literature research; **GP (entire)** developed the methods; **GP (predominant)** and CC collected the data; **GP (large)**, CC, and SN analyzed the data; **GP (large)**, SN, and TM discussed the results; **GP (entire)** wrote the manuscript; **GP (predominant)**, SN, and TM revised the manuscript.

Behav Ecol Sociobiol
DOI 10.1007/s00265-016-2206-z

ORIGINAL ARTICLE

Emergence and development of personality over the ontogeny of fish in absence of environmental stress factors

Giovanni Polverino[1] · **Claudia Cigliano**[1] · **Shinnosuke Nakayama**[1,2] · **Thomas Mehner**[1]

Received: 7 June 2016 / Revised: 19 August 2016 / Accepted: 24 August 2016

Abstract
Animals typically display among-individual differences in behavior that are consistent over time (i.e., personality). These differences are often triggered by variable individual responses to environmental stress factors experienced during life, such as competition for resources and risk of predation. While the causes underlying animal personality are considered to be an issue of prime importance, it is still unknown whether personality emerges and develops over ontogeny if the main sources of behavioral differentiation are absent. Here, we tested whether personality emerged and was strengthened during the lifetime of Eastern mosquitofish (*Gambusia holbrooki*), once intraspecific competition and risk of predation were completely removed and genetic and maternal differences minimized. We found that individual differences in behavior were overall repeatable over ontogeny (i.e., personality was manifested). Personality was, however, not detectable in juvenile individuals but emerged during and after their sexual maturation. The emergence of personality was triggered by the decline in behavioral plasticity of individuals over ontogeny, while differences in behavior among individuals did not vary with age. Our results suggest that animal personality might be inevitable and emerge in fish under laboratory-controlled conditions even in absence of extrinsic factors that typically lead to behavioral differentiation. The decline of behavioral plasticity over lifetime might be a relevant mechanism for the development of personality in animals.

Significance statement
Increasing evidence suggests that animals have personality, that is, individuals consistently differ in behavior among each other (e.g., bold and shy or social and non-social individuals). Personality differences among animals should be, by definition, consistent over time and often caused by environmental challenges experienced early in life. In this study, we observed that personality differences were not present at juvenile age in social fish but emerged later in their life, despite the fact that environmental challenges (i.e., predation risk and competition for space, food, and mates) were absent. Personality differences strengthened over lifetime, resulting from declines in individual behavioral plasticity. Our results suggest that the decline in behavioral plasticity with age may represent a relevant mechanism for behavioral differentiation in animals, in agreement with evidences from the human literature on age-related loss in behavioral plasticity.

Keywords Behavioral type · Developmental plasticity · Lifetime · Gambusia · Repeatability · Temperament

---

Communicated by J. Lindström

Electronic supplementary material The online version of this article (doi:10.1007/s00265-016-2206-z) contains supplementary material, which is available to authorized users.

✉ Giovanni Polverino
polverino@igb-berlin.de

[1] Department of Biology and Ecology of Fishes, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, 12587 Berlin, Germany

[2] Division of Integrative Fisheries Management, Faculty of Life Sciences, Humboldt Universität zu Berlin, Berlin, Germany

Introduction

Consistent among-individual differences in behavior (i.e., personality or behavioral types) have been found across numerous animal taxa (Gosling 2001; Bell et al. 2009) in both wild and laboratory contexts (Herborn et al. 2010; van Overveld and Matthysen 2010). Several theoretical studies have explored the potential causes of animal personality (Wolf et al. 2007; Biro and Stamps 2010; Wolf and Weissing 2010; Wolf and McNamara 2012; Sih et al. 2015), whereas empirical studies on their emergence and persistence over the lifetime of animals are surprisingly scarce (Trillmich and Hudson 2011; DiRienzo et al. 2012).

The hallmark of animal personality variation is that behavioral differences among individuals are consistent over time (Réale et al. 2007). Thus, if personality is present, current traits like activity levels and risk-taking tendencies (i.e., boldness) of animals should allow predictions on the future behavior of those individuals. Nevertheless, recent studies have challenged the stability of personality types (reviewed by Biro and Stamps 2015) over the lifetime of animals. These studies suggest that natural selection may favor high plasticity (and hence weak personality) in the behavior of young individuals, which face uncertain environmental conditions and fluctuating selection pressures (Stearns 1989; West-Eberhard 1989; Fischer et al. 2014; Fawcett and Frankenhuis 2015; Nettle and Bateson 2015; Trillmich et al. 2015; Polverino et al. 2016). Thus, adaptive developmental plasticity (Nettle and Bateson 2015) is expected to delay the development of personality in animals until sufficient information on the state of the environment has been acquired (Fischer et al. 2014; Fawcett and Frankenhuis 2015). In turn, behavioral plasticity is expected to decline toward the end of life (age-dependent plasticity (Baltes 1997; Fischer et al. 2014; Fawcett and Frankenhuis 2015)) once the environmental uncertainty is reduced (Stamps and Krishnan 2014; Fawcett and Frankenhuis 2015; Nettle and Bateson 2015) and costs of phenotypic adjustments exceed their benefits as a function of the limited time that the animal has for profiting before it dies (Fischer et al. 2014).

A second possible mechanism that can explain the development of behavioral differences among animals with increasing age is that stochastic events may accrue over lifetime and cause small changes in the behavior of individuals (Freund et al. 2013). These behavioral changes can, in turn, activate internal processes that accelerate the phenotypic differentiation among individuals (somatic state-based developmental plasticity (Nettle and Bateson 2015)). Under this perspective, Freund and collaborators (2013) observed that activity and exploration diverged over time in genetically similar mice, with a positive correlation between exploration rates and neurogenesis detected in mice at advanced age (Freund et al. 2013).

Evidence from theoretical and empirical studies supports the idea that the evolution of animal personality is mainly linked to genetic, maternal, and environmental factors (Carere et al. 2005; van Oers et al. 2005; Reddon 2011; Kain et al. 2012). Indeed, personalities are partially inherited (Drent et al. 2003) and studies suggest that approximately 52 % of the variation in animal personality is caused by additive genetic variation (Dochtermann et al. 2015). Maternal signals also represent an informative inheritance on the environmental conditions that offspring will face after birth and typically drive the behavioral strategies in newly born animals (Bestion et al. 2014). Nevertheless, environmental stressors experienced during life (e.g., risk of predation, competition for resources, etc.) can overwrite prenatal factors (Gorski et al. 2006) and favor behavioral differentiation among individuals in response to different coping strategies (i.e., feed in presence of predation risk or delay feeding with risk of starvation (Carere et al. 2005; Killen et al. 2013; Urszán et al. 2015)). The first longitudinal studies have recently confirmed increases in the behavioral repeatability during the lifetime of several animal taxa (Edenbrow and Croft 2013; Freund et al. 2013; Favati et al. 2015; Fisher et al. 2015), probably related to social factors, in addition to increasing age. Indeed, environmental challenges associated with social contexts can contribute substantially to differentiate the behavioral strategies among individuals (Edenbrow and Croft 2013; Rittschof et al. 2014; Wright et al. 2014). This implies that bold and aggressive individuals have higher access to food resources and mates and increasingly outcompete smaller animals over time via a positive feedback mechanism (Biro and Stamps 2008; Wong and Balshine 2011; Matthews and Wong 2015). Accordingly, manipulations of the group composition were found to decrease the repeatability of behavior in social animals (Laskowski and Pruitt 2014), while stable social environments strengthened individual personalities (Laskowski et al. 2016). Therefore, longitudinal studies on the development of behavior in animals whose prenatal differences and environmental stressors (including agonistic interactions among conspecifics) are minimized are expected to offer an innovative and critical approach to explore whether personality would still develop over time in absence of the main sources of behavioral differentiation.

Here, we tested whether (a) risk-taking and activity-related behaviors increased, on average, with fish age; (b) personality still emerged early in life of animals whose genetic, maternal, and environmental differences were experimentally minimized; and (c) personality strengthened during the ontogeny of mosquitofish in absence of competition for space, food, and mates. To do this, we used a housing system that allowed highly inbred siblings of Eastern mosquitofish (*Gambusia holbrooki*) to develop throughout several ontogenetic stages in the absence of predation risk, competition for resources, and physical interactions with conspecifics, which may, otherwise, cause behavioral differentiation in these social fish (Polverino et al. 2016). However, to prevent the development of atypical behaviors caused by complete social isolation (Halperin et al. 1992; Earley et al. 2006), individuals could establish and maintain intraspecific social interactions based on visual and chemical cues (Ward and Mehner 2010; Polverino et al. 2013). We predicted that (i) risk-taking and activity-related behaviors increase, on average, with age (Sogard 1997; Nilsson and Brönmark 2000); (ii) personality is not present in the early-life stages of animals when differences in genetic, maternal, and environmental factors are low or absent; and (iii) information about the environmental state and/or stochastic events may, however, accrue over the lifetime, triggering decreases in behavioral plasticity and the subsequent emergence of personality later in the animal's life, as proven within the human personality literature (reviewed by Baltes and Baltes 1993; Baltes 1997).

Materials and methods

To minimize observer bias, blinded methods were used when all behavioral data were recorded and/or analyzed. All experiments were performed at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) in Berlin, Germany.

Study organism and maintenance

Fish utilized in this study were first-generation progeny of wild-caught individuals from Torre Castiglione (Italy, +40° 17′ 20.44″, +17° 49′ 24.30″ (Ariani et al. 2000)). Notably, fish from this isolated population are characterized by an extremely low heterozygosis compared to other wild populations of mosquitofish (J. Jourdan et al. unpublished data). Since genetic diversity among these fish is naturally low, laboratory-reared first-generation siblings represent an excellent model for longitudinal studies intending to explore the development of personality during the ontogeny of animals (Senner et al. 2015).

Wild-caught fish were housed in 50-l aquaria with a maximum density of 0.4 fish/l and acclimatized for a minimum of 5 months after capture to the laboratory conditions as described in Polverino et al. (2016). After the acclimatization period, pregnant females were removed from their housing tanks and individually transferred into 10-l aquaria until they gave birth. The 10-l aquaria were located in a climate chamber (Feutron Temperaturzelle, Feutron Klimasimulation GmbH, Germany) to minimize external disturbances, while maintaining a constant water temperature of 23 °C, in agreement with the water temperature set for the 50-l tanks. Each 10-l aquarium was equipped with an air filter (Quick-Draw Corner Aquarium Filter, Penn Plax, NY), plastic plants, and plastic material to provide refuge for juvenile fish after birth. Illumination was provided via fluorescent light bulbs on a 12-h cycle in accordance with the circadian rhythm of the species (Pyke 2005). Each 10-l aquarium was checked twice a day until newly born fish were found. Soon after giving birth, females were transferred back into their original housing tank, while clutches of newly born fish (on average, 20 individuals each) were kept in the 10-l aquaria for the first 15 days after birth. Notably, mosquitofish do not show social hierarchy and aggression shortly after birth (Pyke 2005). Juvenile fish were fed to satiation twice a day with live and frozen *Artemia* nauplii.

Fifteen days after birth, experimental fish ($N = 40$) were randomly selected from the pools of juveniles available by following a priori criteria: (i) no malformation or physical anomalies were visible and (ii) all experimental fish had to be born on the same calendar day. The prerequisite of this study that all experimental fish had to be born on the same day prevented aggressive manifestations among individuals in their housing tanks (albeit reduced to only visual or chemical signals). In fact, dominance hierarchies in poeciliids are typically favored by size differences between individuals that are associated with different ages (Bisazza 1993). Siblings born from three mothers, strictly kept under identical experimental conditions, were used for experiments to minimize potential confounding effects on the behavior of juvenile fish (i.e., maternal effects) caused by maternal exposures to different stress-related conditions. Then, experimental fish were randomly assigned to two identical experimental housing tanks (25 cm high, 25 cm wide, and 120 cm long each; Fig. 1a). Each of these tanks was arranged with an external filtering system (EHEIM Professionel 2222, EHEIM GmbH & Co KG, Germany) and a heater to maintain water condition and water temperature as described above. Each experimental housing tank hosted two parallel rows of ten transparent Plexiglas cylinders (18 cm high and 10-cm diameter each) fitted with a stainless steel net on the bottom and submerged in 10 cm of water (Fig. 1a). Experimental fish were housed individually in the Plexiglas cylinders, which allowed the individual identification even at the juvenile stage, when common marking procedures are not possible or may result in very high mortality rates (Frederick 1997). Furthermore, the system also allowed to minimize environmental stress factors that are common when mosquitofish are housed together (Dadda et al. 2005; Pyke 2005), while eluding social isolation that is known to negatively affect health and longevity of social fish (Seeman and McEwen 1996) and alter their hormonal levels

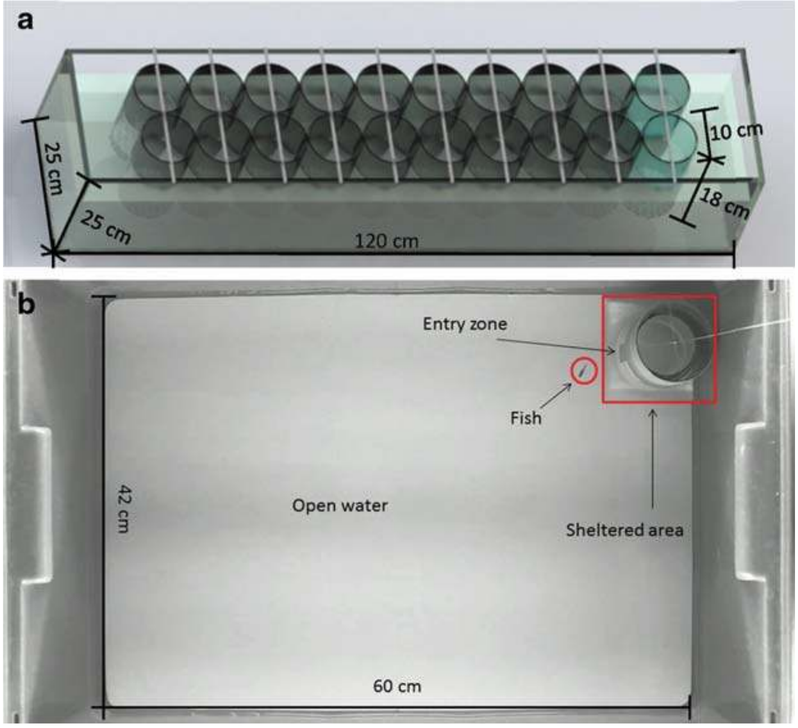


Fig. 1 Scheme of an experimental housing tank (**a**) and snapshot of the experimental arena (**b**). Each of the two experimental housing tanks (**a**) hosted two parallel arrays of ten transparent Plexiglas cylinders. Sibling mosquitofish were housed individually in the cylinders (i.e., 20 fish per tank), facilitating the exchange of visual and chemical cues among individuals but preventing competition and aggression. The experimental arena (**b**) was virtually divided into open-water area, sheltered area, and entry zone (consisting of a white plastic cylinder and its movable door)

(Hannes and Franck 1983; Oliveira et al. 1996) or behavioral outcomes (Halperin et al. 1992; Earley et al. 2006). Since social mosquitofish interact mostly by visual and chemical clues (Ward and Mehner 2010; Polverino et al. 2013), visual and chemical interactions among individuals were facilitated by the transparent cylinders and the stainless steel net sealed on their bottom surface, respectively (Fig. 1a). Focal fish were maintained in these cylinders during the entire study and the position of each cylinder was randomized each week within a given experimental housing tank to homogenize social stimuli among fish (i.e., all fish experienced different neighbor compositions).

Fish were acclimatized for a total of 15 days in the transparent Plexiglas cylinders before behavioral tests started. Thus, fish were approximately 1 month old when tested at their juvenile stage, while behavior at subadult and adult stages was measured approximately 2.5 and 4.5 months after birth, respectively (Fig. 2). Notably, the subadult stage corresponded to the morphogenesis of the anal fin observed in mosquitofish males (Turner 1941), which started concurrently in all male fish after 2 months since birth. In this study, we considered fish as “subadults” when a semiformed gonopodium was visible (Pyke 2005). Since male and female mosquitofish do not differ in time to reach sexual maturity

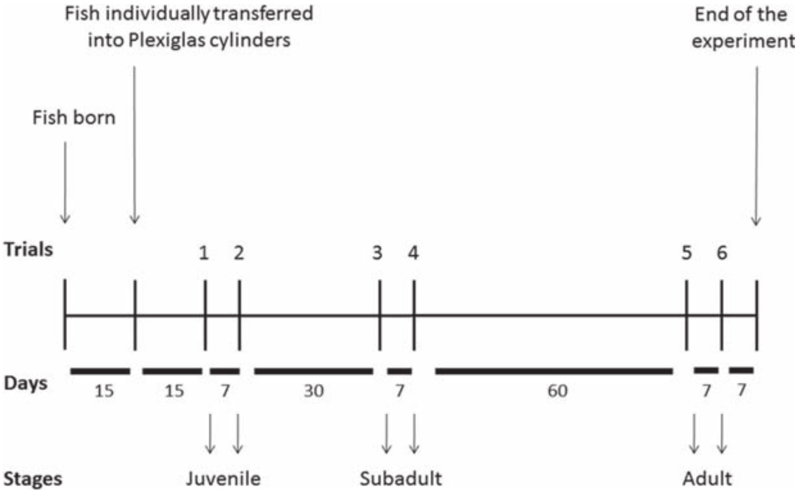


Fig. 2 Experimental timeline. Each fish was tested twice during the juvenile (trials 1 and 2), subadult (3 and 4), and adult (5 and 6) ontogenetic stages, with a break of 7 days within each pair of trials. The whole experimental campaign covered a period of approximately 5 months

(Pyke 2005), females were considered as subadult when the formation of the gonopodium was observed in their male siblings (Fig. S1 in the Electronic Supplementary Material). Sexual dimorphism was fully manifested 4.5 months after birth, with females showing the characteristic periproctal spot on their ventral area and males displaying the fully mature gonopodium (Fig. S1 in the Electronic Supplementary Material (Pyke 2005)). Under natural conditions, mosquitofish have a relatively short lifespan (i.e., less than a year), as observed in populations from North America (Haake and Dean 1983) and Europe (Fernández-Delgado and Rossomanno 1997).

Fish were fed to satiation twice a day with *Artemia* nauplii and flake food during both juvenile and subadult stages, while *Artemia* nauplii, blood worms, and flake food were used for adults.

Open-field test

Behavioral tests described below were performed in a rectangular experimental arena (30 cm high, 42 cm wide, and 60 cm long), with a capacity of 75 l (Fig. 1b). A white plastic cylinder (10 cm high and 5 cm of diameter), with a squared entry zone (2 × 2 cm) at its base, was used as experimental refuge for fish acclimatization before the initiation of the test. A larger concentric cylinder (10 cm high and 6 cm of diameter) was used as a movable "door" to ensure that fish could not escape from the refuge during the acclimatization period or re-enter into the refuge once out. The squared zone delimited by the experimental refuge represented the only sheltered area available to the fish, while the remaining part of the arena represented homogenous open-water conditions (Fig. 1b). The experimental arena was filled with 7 cm of conditioned water to guarantee natural shallow-water conditions to the experimental fish (Pyke 2005), while minimizing fish activity along the vertical axis.

Each experimental individual was tested twice per each ontogenetic stage (i.e., juvenile, subadult, and adult stage), with an interval of 7 days between the first and the second trials for a total of six trials across the experimental campaign (Fig. 2). Before the beginning of the measurements at the subadult stage, eight experimental fish died and, thus, a total of 32 fish were tested at the subadult and adult ontogenetic stages. Experiments were performed between 11 a.m. and 6 p.m. from Monday to Friday, with fish tested in a randomized order to avoid confounding effects on their behavior resulting from measurements performed in a predetermined time window (e.g., hunger effects on risk-taking (Krause et al. 1998)). A bird's eye camera with high resolution (Bosch Dinion HD 1080p, Bosch, Grasbrunn, Germany) was placed approximately 1 m above the experimental arena and recorded fish movement on the *x-y* plane, while two lateral lights provided homogeneous illumination. All tests were performed in a second climate chamber, fully comparable with the one described above, to minimize external disturbances during experiments, while maintaining the water temperature within the experimental arena constantly at 23 °C.

For each trial, a single individual was captured from its cylinder and acclimatized in the closed refuge for 5 min. Then, the refuge was opened and the time spent before the individual left the refuge was measured with a stopwatch ("emergence latency," in s) for a maximum of 5 min, during which all fish had left the refuge spontaneously. Then, the movable door was closed and the behavior of fish was recorded for 10 min. At the conclusion of each trial, each individual was removed from the experimental arena and transferred back to its cylinder within the experimental housing tank.

For each individual, we recorded the total time spent not actively swimming ("freezing time," in s), "distance moved" (in cm), and the time spent within the shelter area ("hiding time," in s), using a dedicated video-tracking software (EthoVision XT Version 9.0; Noldus Information Technologies, Inc.). We additionally measured body mass (to the nearest 0.01 g), standard body length (to the nearest 0.1 mm), and Fulton's condition factor *K* ($(g/(mm)^3 \times 100{,}000$) (Froese 2006)) for each fish at each ontogenetic stage soon after the conclusion of the second behavioral trial. Fulton's *K* represents an index of fatness and nutritional state for a given individual in a given ontogenetic stage relative to its body size (Froese 2006).

Statistical analysis

We initially performed Pearson product moment correlations to explore the relationships among pairs of continuous explanatory variables (i.e., standard body length, body mass, and Fulton's condition factor *K*). Pearson correlations revealed that standard body length and body mass were highly correlated (Table S1 in the Electronic Supplementary Material). Thus, we included only standard body length and Fulton's *K* in all subsequent analyses. Since we did not find any average differences in behavior, standard body length, and Fulton's *K* between mosquitofish born from different mothers, we did not account for maternal identity in the statistical models.

To test whether average behaviors differed among ontogenetic stages, we fitted a linear mixed-effects model (LMM (Bates et al. 2014)) to each of the four behavioral traits ("emergence latency", "hiding time", "distance moved", and "freezing time"), using individual as a random effect and ontogenetic stage as a fixed factor to adjust for mean differences among ontogenetic stages. Notably, "emergence latency" was natural log transformed, while "freezing time" and "hiding time" were square root transformed for a better model fit. We used the Satterthwaite method to approximate degrees of freedom. Once a significant result was found, we performed post hoc comparisons between ontogenetic stages using the conservative Bonferroni method.

Since we were interested in testing whether personality was manifested in genetically similar mosquitofish, we used the resulting among-individual and within-individual variance estimates from the LMMs described above to estimate the overall repeatability of each behavioral trait over fish ontogeny (Nakagawa and Schielzeth 2010; Dingemanse and Dochtermann 2013). Repeatability represents the proportion of behavioral variation attributable to consistent differences among individuals (Dingemanse and Dochtermann 2013). The significance of individual differences was tested using both likelihood ratio tests (LRTs) and Akaike information criterions (AICs) between a full model that included individual as a random effect and the null model in which the random effect was excluded.

Lastly, we tested for the presence of personality differences on each given behavioral trait at each ontogenetic stage separately, as suggested by Brommer and Class (2015). Here, each full model included standard body length, Fulton's *K*, sex, and trial as fixed factors, while individual identities were included as a random effect. As above, the full model was compared through an LRT and AIC against a null model that did not include the random effect.

To test whether the within- and the among-individual variance for a given behavioral trait increased or decreased over the ontogeny, we estimated both within- and among-individual variances for each behavior at each stage by using a MCMC sampling method under a Bayesian framework to obtain the posterior distribution. Then, a linear regression was fitted to the variance scores randomly sampled from the posterior distribution at each stage using ontogenetic time (in weeks) as a predictor variable, since we assumed a linear change in behavior over ontogeny. However, a categorical variable has also been tested as a predictor to check for the consistency of results from the linear regressions. We repeated this procedure 10,000 times. Statistical significance was inferred from the empirical distribution of the 10,000 slopes. Repeatability and within- and among-individual variances were estimated using a non-informative prior, with 1,500,000 resamplings, 500,000 burn-ins, and 100 thinnings (R package "MCMCglmm" (Hadfield 2010)).

Data analysis was performed in R-3.1.1 version (R Core Team 2013), by using packages "lme4," "nlme," and "lmerTest" (Bates et al. 2014; Pinheiro et al. 2014; Kuznetsova et al. 2016), respectively. The significance level was set at $\alpha = 0.05$.

Results

On average, behavior of mosquitofish varied significantly in "distance moved" (LMM, $P < 0.01$; $F_{2,172} = 7.66$), "freezing time" ($P < 0.01$; $F_{2,166} = 98.80$), and "hiding time" ($P < 0.01$; $F_{2,174} = 5.60$), but not in "emergence latency" ($P = 0.08$; $F_{2,173} = 2.57$; Fig. 3). In particular, fish moved, on average,

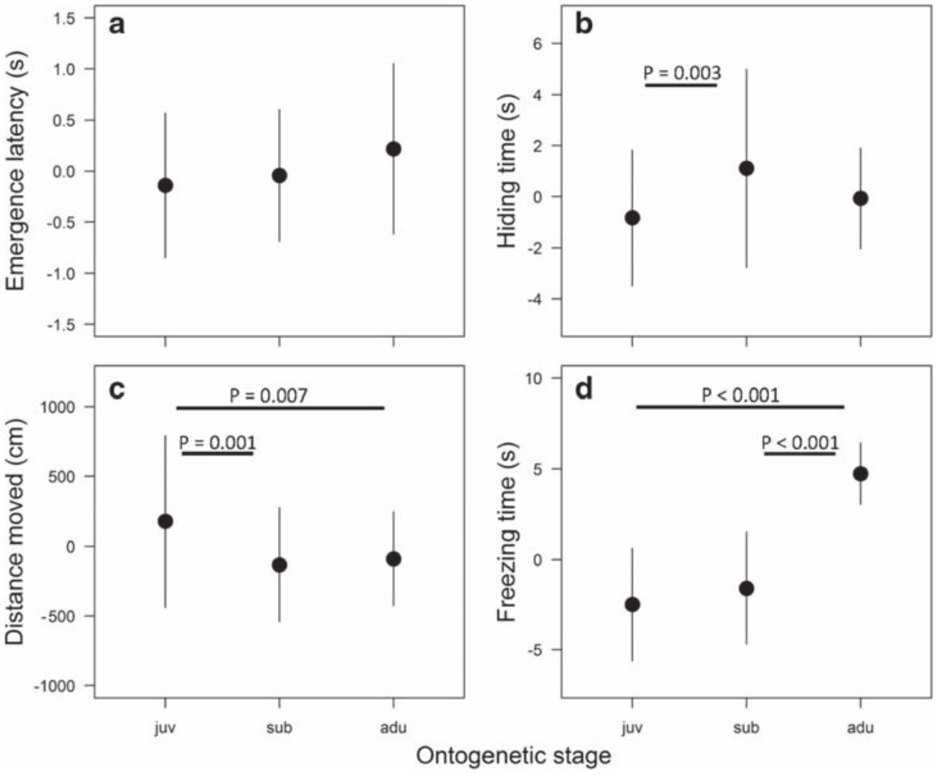


Fig. 3 Ontogenetic development of risk-taking and activity-related behaviors in mosquitofish. For each behavioral trait, *y*-axis values refer to the mean time interval ("emergence latency," "hiding time," and "freezing time" measured in s) and distance traveled ("distance moved" measured in cm) observed across 10-min trials in juvenile (juv), subadult (sub), and adult (adu) mosquitofish. Notably, "emergence latency" (**a**) is log transformed, while "hiding time" (**b**) and "freezing time" (**d**) are square root transformed. All variables are also centered around their grand mean. Post hoc comparisons were corrected with the conservative Bonferroni method, and corrected *P* values are shown. *Vertical bars* refer to standard deviations

less and for shorter distances with increasing age, while hiding time increased only until sexual maturity.

Across the entire experimental campaign, we detected personality differences among fish for each of the four behavioral traits measured, suggesting that individual differences in behavior were overall repeatable over the ontogeny of mosquitofish (Table 1). Nevertheless, the overall repeatability of fish behavior across the ontogeny was low (Table 1).

Thus, we tested whether personality differences were consistently low across the ontogeny of fish or, instead, whether personality strengthened over ontogeny. We did not find support for the presence of personality in fish at the juvenile stage (Table 2, Fig. S2 in the Electronic Supplementary Material), irrespective of the behavioral trait considered. However, personality emerged at the subadult stage with respect to "hiding time" and was detected at the adult stage for "hiding time", "distance moved", and "freezing time" (Table 2, Fig. S2 in the Electronic Supplementary Material). Furthermore, we observed a significant decline in the within-individual behavioral variance over ontogeny that was consistent to all behavioral traits for which personality was detected (Table 3; Fig. 4), whereas the among-individual variance did not vary with fish age for any trait (Table 3; Fig. 4).

Overall, the fixed factors standard body length, Fulton's *K*, and sex included in the models did not explain differences in behavior among fish for any of the ontogenetic stages analyzed separately. However, adult females (larger than males at equal age) emerged faster from the refuge and swam longer distances compared to males (Table S2 in the Electronic Supplementary Material).

Discussion

This study shows that the behavior of mosquitofish substantially changed over ontogeny, most obviously expressed by lower swimming activity in adult than in juvenile or subadult fish. Changes in average behaviors over ontogeny were accompanied by the emergence of personality differences among mosquitofish, which were absent in the juvenile stage, but became detectable in adult fish in three of the four behavioral traits measured. Interestingly, these increases in behavioral repeatability resulted from decreases in within-individual behavioral variance (i.e., behavioral plasticity) across ontogeny, while the among-individual variance in behavior did not vary with age. These results suggest that personality in animals could emerge even in the absence of environmental challenges (i.e., predation risk or intraspecific competition), but the primary mechanism to explain the strengthening of personality over ontogeny is a decline in individual behavioral plasticity.

Our results could be explained as the product of adaptive developmental plasticity (Nettle and Bateson 2015). Mosquitofish may have adaptively delayed their behavioral adjustments until the environmental information collected was sufficient to minimize costs of plasticity during adulthood, once benefits of plasticity become time limited (Stearns 1989). In this vein, recent studies have modeled the age dependency of behavioral plasticity in animals based on changes in costs and benefits of phenotypic adjustments (Fischer et al. 2014) and environmental uncertainty (Fawcett and Frankenhuis 2015) throughout ontogeny. Results from their analysis support our findings, predicting behavioral plasticity to decline from early life stages to adulthood as a function of the reduction in the environmental uncertainty facilitated by the accumulation of information on the environmental state (Fischer et al. 2014; Fawcett and Frankenhuis 2015). In our case, consistent decreases in behavioral plasticity over ontogeny have caused the emergence of personality in adult mosquitofish ultimately, in agreement with evidence on the age-related loss of plasticity well documented in human-behavior literature (reviewed by Baltes and Baltes 1993; Baltes 1997).

A second mechanism might have also contributed to the development of personality differences observed in this study. Despite experimental precautions, it is reasonable to assume that minor and elusive differences among individuals did

Table 1 Results from the linear mixed models with individual as a random effect and ontogenetic stage as a fixed factor

Variance components	V_{within}	V_{among}	Repeatability	ΔAIC	χ^2_1	*P* value
Emergence latency	0.84	0.10	0.11	2.64	4.64	0.03
Hiding time	10.47	1.83	0.15	5.6	7.62	<0.01
Distance moved	295,040	96,807	0.25	12.5	14.53	<0.01
Freezing time	10.22	2.45	0.20	7.7	9.64	<0.01

"Emergence latency," "hiding time," "distance moved," and "freezing time" represent the dependent variables, respectively. Within-individual variance (V_{within}), among-individual variance (V_{among}), and repeatability are shown with respect to each behavioral trait. Test statistics (χ^2_1) and significance levels of the random effect (i.e., intercepts) were estimated using LRTs (*P* values) and Akaike information criterions (AICs) between the full and the null models. Notably, ΔAIC corresponds to the difference in AIC between the null models minus the AIC from the full model. The significance level was set at $\alpha < 0.05$

Table 2 Results from the linear mixed models performed on each ontogenetic stage separately, with individual as a random effect and standard body length (mm), body condition (Fulton's *K*, g/(mm)^3 × 100,000), sex, and trial included as fixed factors

Behavioral trait	O. stage	V_{within} (95 % CI)	V_{among} (95 % CI)	R (95 % CI)	ΔAIC	χ^2_1	P value
Emergence latency	Juvenile	506.67 (320.67, 739.87)	31.88 (<0.01, 190.48)	0.06 (<0.01, 0.30)	−2.00	<0.01	0.99
	Subadult	105.42 (67.35, 156.77)	8.86 (<0.01, 46.19)	0.08 (<0.01, 0.33)	−1.97	0.03	0.87
	Adult	1564.36 (1007.35, 2292.50)	71.83 (<0.01, 482.51)	0.04 (<0.01, 0.26)	−2.00	<0.01	0.99
Hiding time	Juvenile	1009.62 (632.37, 1505.78)	96.13 (<0.01, 473.75)	0.08 (<0.01, 0.35)	−1.78	0.22	0.64
	Subadult	1202.40 (671.64, 2022.51)	1906.60 (698.28, 3210.22)	0.62 (0.36, 0.82)	12.61	14.61	<0.01
	Adult	332.00 (178.95, 551.60)	253.38 (<0.01, 535.15)	0.44 (0.09, 0.72)	4.60	6.60	0.01
Distance moved	Juvenile	152.54 (98.63, 226.70)	10.38 (<0.01, 60.02)	0.06 (<0.01, 0.31)	−1.97	0.02	0.87
	Subadult	27.54 (15.88, 43.03)	7.12 (<0.01, 22.62)	0.21 (<0.01, 0.50)	−0.23	1.77	0.18
	Adult	10.39 (5.68, 17.45)	9.50 (<0.01, 19.46)	0.48 (0.13, 0.74)	4.23	6.23	0.01
Freezing time	Juvenile	8221.27 (5394.92, 11855.54)	182.33 (<0.01, 1758.39)	0.02 (<0.01, 0.17)	−2.00	<0.01	0.99
	Subadult	2436.60 (1378.27, 3799.27)	600.04 (<0.01, 1891.70)	0.20 (<0.01, 0.50)	0.50	2.50	0.11
	Adult	1366.76 (767.54, 2335.77)	1671.21 (379.73, 3387.49)	0.55 (0.24, 0.78)	9.52	11.52	<0.01

"Emergence latency," "hiding time," "distance moved," and "freezing time" represent the dependent variables, respectively. Medians of the within-individual variances (V_{within}), among-individual variances (V_{among}), repeatability estimates (R), and their 95 % credible intervals (95 % CI) are shown with respect to each behavioral trait at each ontogenetic stage (O. stage). Test statistics (χ^2_1) and significance levels of the random effect (i.e., intercepts) were estimated using LRTs (*P* values) and Akaike information criterions (AICs) between the full and the null models for each behavioral trait at each ontogenetic stage. Notably, ΔAIC corresponds to the difference in AIC between the null models minus the AIC from the full model. The significance level was set at $\alpha < 0.05$

occur in their early development, for example with respect to food and/or hormonal contents among eggs (Brooks et al. 1997) and neighbors' phenotype at specific and sensitive time windows (Arnold and Taborsky 2010). As observed by Freund et al. (2013) in clonal mice, early phenotypic discrepancies, albeit small, can activate physiological and cognitive processes that, in turn, increasingly limit the plasticity of individual behavior and favor their personality differentiation (Wolf and Weissing 2010).

Since personality differences increased with age, it is possible that our limited sample size may have impeded the detection of personality in juvenile fish or may have blurred ontogenetic trends in the emergence of personality. Independent of the fact whether personality differences were marginal or absent in young individuals, the development of personality was consistent over ontogeny in three of the four behavioral traits measured, indicating that personality of mosquitofish indeed develops over lifetime. Increases in the repeatability of mosquitofish behavior with increasing age are supported by theoretical predictions (Fischer et al. 2014) and by empirical evidence from ontogenetic studies performed on field crickets (*Gryllus campestris* (Fisher et al. 2015)), mangrove killifish (*Kryptolebias marmoratus* (Edenbrow and Croft 2013)), red junglefowls (*Gallus gallus* (Favati et al. 2015)), and laboratory mice (*Mus musculus* (Freund et al. 2013)).

Table 3 Results from the linear regressions on the change in within- and among-individual behavioral variances over ontogeny

Variance components	P_{within}	P_{among}
Emergence latency	<0.001	0.69
Hiding time	<0.001	0.29
Distance moved	<0.001	0.94
Freezing time	<0.001	0.12

"Emergence latency," "hiding time," "distance moved," and "freezing time" represent the dependent variables, respectively. The *P* values (*P*) of the linear regressions are shown with respect to within- and among-individual variances over ontogeny

The study's key finding on the development of personality over lifetime is contingent with untraceable personality differences in these fish early in life (i.e., at the juvenile stage). Behavioral strategies of young animals are known to vary in response to parental inheritance (i.e., genetics, hormones, and parental behavior), which is capable of preparing offspring to face environmental challenges (Drent et al. 2003; Carere et al. 2005; Reddon 2011; Ayroles et al. 2015; Dochtermann et al. 2015; Han and Brooks 2015; Bell et al. 2016). Our results may suggest that minimizing genetic and parental sources of behavioral differentiation can effectively suppress the expression of personality differences of social animals early in life, as also observed by Edenbrow and Croft (2013). Additionally, competition for resources was prevented among juvenile individuals, thus minimizing differences in their energy reserves (i.e., body length, mass, and condition) that can, in turn, contribute to behavioral differentiation in young animals (Biro and Stamps 2008). In support of this, personality was successfully detected in juvenile mosquitofish from the very same population used in this study once environmental

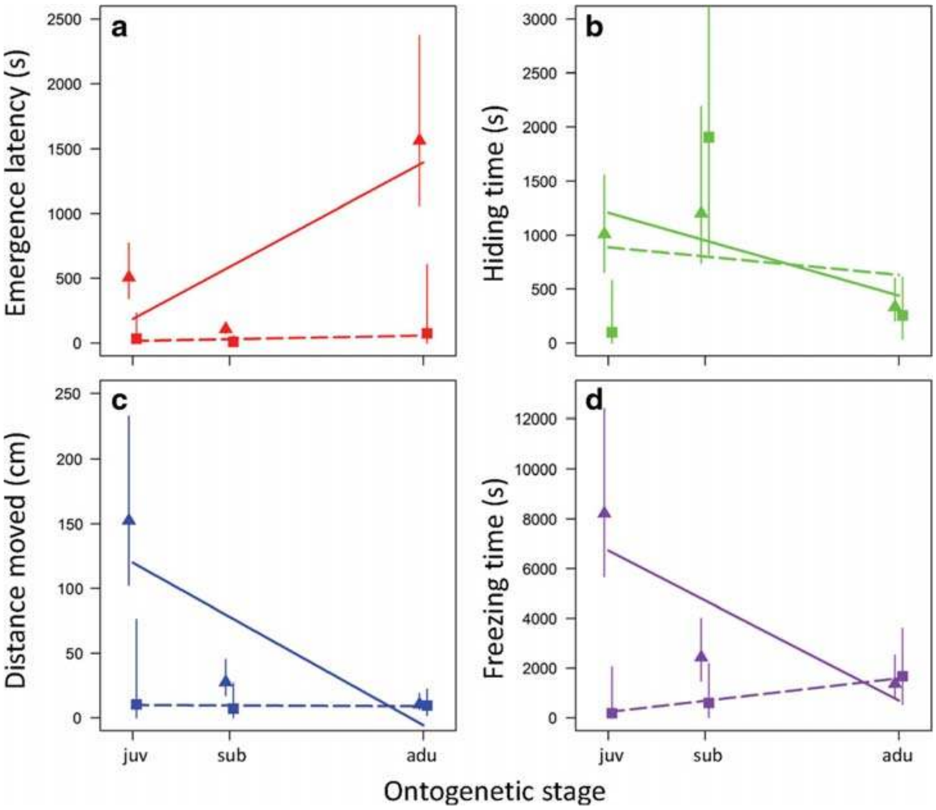


Fig. 4 Change in the variance components of behavioral traits within and among individuals across ontogeny, as estimated by linear regressions. Within-individual variance (solid regression lines, medians as triangles) and among-individual variance (dashed regression lines, medians as squares) are shown with respect to "emergence latency" (**a**), "hiding time" (**b**), "distance moved" (**c**), and "freezing time" (**d**). Medians are represented with respect to juvenile (~5 weeks old), subadult (~10 weeks old), and adult fish (~20 weeks old). *Vertical lines* represent 95 % credible intervals

challenges associated with their social lives were allowed (i.e., competition for space and food resources (Polverino et al. 2016)). In contrast, we observed here that personality was not manifested in juvenile mosquitofish and was never explained by differences in their energy reserves (i.e., body length and condition factor) at any of the ontogenetic stages considered, except at the adult stage, in which the sexual dimorphism of the species imposes mature females to be larger than males at equal age (Pyke 2005).

To the best of our knowledge, this study provides the first empirical evidence that personality may emerge during the lifetime of animals when genetic differences are low and environmental sources of behavioral differentiation after birth are rigorously controlled for. These results might be well explained through two non-mutually exclusive explanations, that is, the adaptive developmental plasticity theory (or age-dependent plasticity) and the accumulation of stochastic events over the lifetime. Our results suggest that the presence of personality during the lifetime of animals should not be simply assumed but have to be tested for each ontogenetic stage separately. Furthermore, the variable expression of personality over lifetime requires the explicit consideration of the animal's age in models which consider individual behavioral variation. In particular, we suggest that considering effects of age on personality expression may add insights into the understanding of dynamics within populations and among ecological levels.

Acknowledgments The authors would like to acknowledge K. Kuntze, T. Ruberto, and N. Gamrath for the help provided with the laboratory procedures and F. Del Sette for the support to realize Fig. 1a. We also thank all members of the B-Types group for providing important feedbacks during all phases of the study and the research group Evolutionary Ecology of Variation for the statistical support. A further acknowledgement goes to all participants to the workshop "New perspectives in behavioral development: adaptive shaping of behavior over a lifetime?" at the Center for Interdisciplinary Research (ZiF), Bielefeld University, Bielefeld (Germany), for the fundamental insights that contributed to the realization of this study.

Compliance with ethical standards

Funding This work was part of the B-Types project (http://www.b-types.igb-berlin.de/) funded through the Wissenschaftsgemeinschaft Leibniz (WGL, SAW-2013-IGB-2).

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

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The experimental procedure was approved through an animal care permit (G 0074/15) granted by the Landesamt für Gesundheit und Soziales Berlin (LAGeSo). Both housing and experimental procedures were designed to minimize stress in the tested animals.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

Ariani AP, Camassa MM, Wittmann KJ (2000) The dolinas of Torre Castiglione (Gulf of Tarent, Italy): environmental and faunistic aspects of a semi-hypogean water system. Mem Biospeol 27:1–14

Arnold C, Taborsky B (2010) Social experience in early ontogeny has lasting effects on social skills in cooperatively breeding cichlids. Anim Behav 79:621–630

Ayroles JF, Buchanan SM, O'Leary C, Skutt-Kakaria K, Grenier JK, Clark AG, Hartl DL, de Bivort BL (2015) Behavioral idiosyncrasy reveals genetic control of phenotypic variability. P Natl Acad Sci USA 112:6706–6711

Baltes PB (1997) On the incomplete architecture of human ontogeny: selection, optimization, and compensation as foundation of developmental theory. Am Psychol 52:366–380

Baltes PB, Baltes MM (1993) Successful aging: perspectives from the behavioral sciences, vol 4. Cambridge University Press, Cambridge

Bates D, Maechler M, Bolker B, Walker S (2014) lme4: linear mixed-effects models using Eigen and S4. R package version 1.1–5, https://cran.r-project.org/web/packages/lme4/index.html

Bell AM, Hankison SJ, Laskowski KL (2009) The repeatability of behaviour: a meta-analysis. Anim Behav 77:771–783

Bell AM, McGhee KE, Stein LR (2016) Effects of mothers' and fathers' experience with predation risk on the behavioral development of their offspring in threespined sticklebacks. Curr Opin Behav Sci 7: 28–32

Bestion E, Teyssier A, Aubret F, Clobert J, Cote J (2014) Maternal exposure to predator scents: offspring phenotypic adjustment and dispersal. Proc R Soc B 281:–20140701

Biro PA, Stamps JA (2008) Are animal personality traits linked to life-history productivity? Trends Ecol Evol 23:361–368

Biro PA, Stamps JA (2010) Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? Trends Ecol Evol 25:653–659

Biro PA, Stamps JA (2015) Using repeatability to study physiological and behavioural traits: ignore time-related change at your peril. Anim Behav 105:223–230

Bisazza A (1993) Male competition, female mate choice and sexual size dimorphism in poeciliid fishes. Mar Freshw Behav Phy 23:257–286

Brommer JE, Class B (2015) The importance of genotype-by-age interactions for the development of repeatable behavior and correlated behaviors over lifetime. Front Zool 12:S2

Brooks S, Tyler CR, Sumpter JP (1997) Egg quality in fish: what makes a good egg? Rev Fish Biol Fish 7:387–416

Carere C, Drent PJ, Koolhaas JM, Groothuis TG (2005) Epigenetic effects on personality traits: early food provisioning and sibling competition. Behaviour 142:1329–1355

Dadda M, Pilastro A, Bisazza A (2005) Male sexual harassment and female schooling behavior in the eastern mosquitofish. Anim Behav 70:463–471

Dingemanse NJ, Dochtermann NA (2013) Quantifying individual variation in behaviour: mixed-effect modelling approaches. J Anim Ecol 82:39–54

DiRienzo N, Pruitt JN, Hedrick AV (2012) Juvenile exposure to acoustic sexual signals from conspecifics alters growth trajectory and an adult personality trait. Anim Behav 84:861–868

Dochtermann NA, Schwab T, Sih A (2015) The contribution of additive genetic variation to personality variation: heritability of personality. Proc R Soc B 282:20142201

Drent PJ, van Oers K, van Noordwijk AJ (2003) Realized heritability of personalities in the great tit (*Parus major*). Proc R Soc Lond B 270: 45–51

Earley RL, Edwards JT, Aseem O, Felton K, Blumer LS, Karom M, Grober MS (2006) Social interactions tune aggression and stress responsiveness in a territorial cichlid fish (*Archocentrus nigrofasciatus)*. Physiol Behav 88:353–363

Edenbrow M, Croft DP (2013) Environmental and genetic effects shape the development of personality traits in the mangrove killifish *Kryptolebias marmoratus*. Oikos 122:667–681

Favati A, Zidar J, Thorpe H, Jensen P, Løvlie H (2015) The ontogeny of personality traits in the red junglefowl, *Gallus gallus*. Behav Ecol 27:484–493

Fawcett TW, Frankenhuis WE (2015) Adaptive explanations for sensitive windows in development. Front Zool 12:1–14

Fernández-Delgado C, Rossomanno S (1997) Reproductive biology of the mosquitofish in a permanent natural lagoon in south-west Spain: two tactics for one species. J Fish Biol 51:80–92

Fischer B, van Doorn GS, Dieckmann U, Taborsky B (2014) The evolution of age-dependent plasticity. Am Nat 183:108–125

Fisher DN, David M, Tregenza T, Rodríguez-Muñoz R (2015) Dynamics of among-individual behavioral variation over adult lifespan in a wild insect. Behav Ecol 26:975–985

Frederick JL (1997) Evaluation of fluorescent elastomer injection as a method for marking small fish. Bull Mar Sci 61:399–408

Freund J, Brandmaier AM, Lewejohann L, Kirste I, Kritzler M, Krüger A, Sachser N, Lindenberger U, Kempermann G (2013) Emergence of individuality in genetically identical mice. Science 340:756–775

Froese R (2006) Cube law, condition factor and weight–length relationships: history, meta-analysis and recommendations. J Appl Ichthyol 22:241–253

Gorski JN, Dunn-Meynell AA, Hartman TG, Levin BE (2006) Postnatal environment overrides genetic and prenatal factors influencing offspring obesity and insulin resistance. Am J Physiol-Reg I 291: R768–R778

Gosling SD (2001) From mice to men: what can we learn about personality from animal research? Psychol Bull 127:45

Haake PW, Dean JM (1983) Age and growth of four Everglades fishes using otolith techniques. Technical Report SFRC83/03 South Florida Research Center, Everglades National Park Homestead, FL

Hadfield JD (2010) MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. J Stat Softw 33:1–22

Halperin JRP, Dunham DW, Ye S (1992) Social isolation increases social display after priming in *Betta splendens* but decreases aggressive readiness. Behav Process 28:13–31

Han CS, Brooks RC (2015) The interaction between genotype and juvenile and adult density environment in shaping multidimensional reaction norms of behaviour. Funct Ecol 29:78–87

Hannes RP, Franck D (1983) The effect of social isolation on androgen and corticosteroid levels in a cichlid fish (*Haplochromis burtoni*) and in swordtails (*Xiphophorus helleri)*. Horm Behav 17:292–301

Herborn KA, Macleod R, Miles WT, Schofield AN, Alexander L, Arnold KE (2010) Personality in captivity reflects personality in the wild. Anim Behav 79:835–843

Kain JS, Stokes C, de Bivort BL (2012) Phototactic personality in fruit flies and its suppression by serotonin and white. P Natl Acad Sci USA 109:19834–19839

Killen SS, Marras S, Metcalfe NB, McKenzie DJ, Domenici P (2013) Environmental stressors alter relationships between physiology and behaviour. Trends Ecol Evol 28:651–658

Krause J, Loader SP, McDermott J, Ruxton GD (1998) Refuge use by fish as a function of body length–related metabolic expenditure and predation risks. Proc R Soc Lond B 265:2373–2379

Kuznetsova A, Brockhoff PB, Christensen RHB (2016) lmerTest: tests in linear mixed effect models. R package version 2–6, https://cran.r-project.org/web/packages/lmerTest/index.html

Laskowski KL, Pruitt JN (2014) Evidence of social niche construction: persistent and repeated social interactions generate stronger personalities in a social spider. Proc R Soc B 281:20133166

Laskowski KL, Montiglio PO, Pruitt JN (2016) Individual and group performance suffers from social niche disruption. Am Nat 187: 776–785

Matthews SA, Wong MY (2015) Temperature-dependent resolution of conflict over rank within a size-based dominance hierarchy. Behav Ecol 26:947–958

Nakagawa S, Schielzeth H (2010) Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biol Rev 85:935–956

Nettle D, Bateson M (2015) Adaptive developmental plasticity: what is it, how can we recognize it and when can it evolve? Proc R Soc B 282: 20151005

Nilsson PA, Brönmark C (2000) Prey vulnerability to a gape-size limited predator: behavioural and morphological impacts on northern pike piscivory. Oikos 88:539–546

Oliveira RF, Almada VC, Canario AV (1996) Social modulation of sex steroid concentrations in the urine of male cichlid fish *Oreochromis mossambicus*. Horm Behav 30:2–12

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2014) nlme: linear and nonlinear mixed effects models. R package version 3.1–118, http://CRAN.R-project.org/package=nlme

Polverino G, Liao JC, Porfiri M (2013) Mosquitofish (*Gambusia affinis*) preference and behavioral response to animated images of conspecifics altered in their color, aspect ratio, and swimming depth. PLoS One 8:e54315

Polverino G, Ruberto T, Staaks G, Mehner T (2016) Tank size alters mean behaviours and individual rank orders in personality traits of fish depending on their life stage. Anim Behav 115:127–135

Pyke GH (2005) A review of the biology of *Gambusia affinis* and *G. holbrooki*. Rev Fish Biol Fish 15:339–365

R Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, https://www.r-project.org/

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ (2007) Integrating animal temperament within ecology and evolution. Biol Rev 82:291–318

Reddon AR (2011) Parental effects on animal personality. Behav Ecol 23: 242–245

Rittschof CC, Bukhari SA, Sloofman LG, et al. (2014) Neuromolecular responses to social challenge: common mechanisms across mouse, stickleback fish, and honey bee. P Natl Acad Sci USA 111:17929–17934

Seeman TE, McEwen BS (1996) Impact of social environment characteristics on neuroendocrine regulation. Psychosom Med 58:459–471

Senner NR, Conklin JR, Piersma T (2015) An ontogenetic perspective on individual differences. Proc R Soc B 282:20151050

Sih A, Mathot KJ, Moiron M, Montiglio PO, Wolf M, Dingemanse NJ (2015) Animal personality and state–behaviour feedbacks: a review and guide for empiricists. Trends Ecol Evol 30:50–60

Sogard SM (1997) Size-selective mortality in the juvenile stage of teleost fishes: a review. Bull Mar Sci 60:1129–1157

Stamps JA, Krishnan VV (2014) Combining information from ancestors and personal experiences to predict individual differences in developmental trajectories. Am Nat 184:647–657

Stearns SC (1989) The evolutionary significance of phenotypic plasticity. Bioscience 39:436–445

Trillmich F, Hudson R (2011) The emergence of personality in animals: the need for a developmental approach. Dev Psychobiol 53:505–509

Trillmich F, Günther A, Müller C, Reinhold K, Sachser N (2015) New perspectives in behavioural development: adaptive shaping of behaviour over a lifetime? Front Zool 12:S1

Turner CL (1941) Morphogenesis of the gonopodium in *Gambusia affinis affinis*. J Morphol 69:161–185

Urszán TJ, Garamszegi LZ, Nagy G, Hettyey A, Török J, Herczeg G (2015) No personality without experience? A test on *Rana dalmatina* tadpoles. Ecol Evol 5:5847–5856

van Oers K, de Jong G, van Noordwijk AJ, Kempenaers B, Drent PJ (2005) Contribution of genetics to the study of animal personalities: a review of case studies. Behaviour 142:1185–1206

van Overveld T, Matthysen E (2010) Personality predicts spatial responses to food manipulations in free-ranging great tits (*Parus major*). Biol Lett 6:187–190

Ward AJW, Mehner T (2010) Multimodal mixed messages: the use of multiple cues allows greater accuracy in social recognition and predator detection decisions in the mosquitofish, *Gambusia holbrooki*. Behav Ecol 21:1315–1320

West-Eberhard MJ (1989) Phenotypic plasticity and the origins of diversity. Annu Rev Ecol Syst 20:249–278

Wolf M, McNamara JM (2012) On the evolution of personalities via frequency-dependent selection. Am Nat 179:679–692

Wolf M, Weissing FJ (2010) An explanatory framework for adaptive personality differences. Philos T Roy Soc B 365:3959–3968

Wolf M, van Doorn GS, Leimar O, Weissing FJ (2007) Life-history trade-offs favour the evolution of animal personalities. Nature 447:581–584

Wong M, Balshine S (2011) Fight for your breeding right: hierarchy re-establishment predicts aggression in a social queue. Biol Lett 7:190–193

Wright CM, Holbrook CT, Pruitt JN (2014) Animal personality aligns task specialization and task proficiency in a spider society. P Natl Acad Sci USA 111:9533–9537

Electronic Supplementary Material

MATERIALS AND METHODS

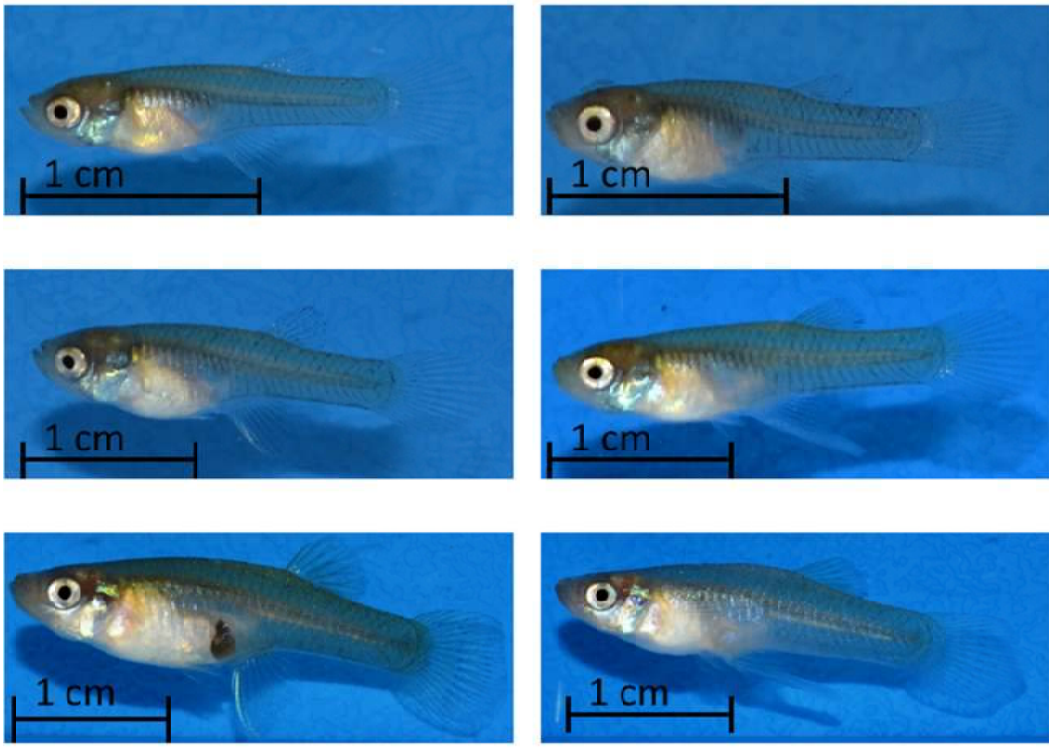


Fig. S1 Representation of the morphogenetic development of mosquitofish during ontogeny. Fish in the juvenile, subadult, and adult ontogenetic stages are represented from top to bottom, respectively. From the left to the right side, females and males are shown, respectively. Notably, the characteristic sexual dimorphism of the species is fully evident in the adult stage only (last row), in which female show the characteristic periproctal spot on the ventral area while males display the fully mature gonopodium (Pyke, 2005) used by mature males for the intromission of sperm during copulation (Turner, 1941). The morphogenesis of the anal fin is partially visible in subadult males (middle row), while no morphological differences are visible between sexes at the juvenile stage (first row)

RESULTS

Table S1 Pearson correlation coefficients between pairs of log-transformed continuous explanatory variables measured in mosquitofish. Values above the diagonal represent the correlation coefficients, while values below the diagonal represent their *P*-values. Significant results ($P < 0.05$) are represented in bold. Note that the significant correlation between standard body length (mm) and body mass (g) did not allow to include both variables into the models. Thus body mass was not included and, instead, body condition of the fish was estimated with Fulton's *K* (g/(mm)^3 x 100000), independent of body length, and included for the statistical analysis

	Body Length	Body mass	Body condition
Body length		**0.994**	0.136
Body mass	**<0.001**		**0.237**
Body condition	0.054	**<0.001**	

Table S2 Fixed factor's results from the linear mixed models on each ontogenetic stage separately. "Emergence latency", "hiding time", "distance moved", and "freezing time" represent the dependent variables, respectively. Standard body length (mm), body condition (Fulton's *K*, g/(mm)^3 × 100000), sex, and trial are included as fixed factors, while random intercepts represented the random effect. Estimate, standard error (*SE*), t-value (*T*), degrees of freedom (*df*), and *P*-value (*P*) are reported for each fixed factor

Ontogenetic stage	Juvenile					Subadult					Adult				
Fixed Factors	Estimate	*SE*	*T*	*df*	*P*	Estimate	*SE*	*T*	*df*	*P*	Estimate	*SE*	*T*	*df*	*P*
Emergence latency															
Body length	-1.65	3.44	-0.48	55.00	0.63	0.83	1.33	0.62	26.00	0.54	-12.19	3.82	-3.19	54.99	<0.01
K	-0.20	24.21	-0.01	55.00	0.99	15.97	12.61	1.27	26.00	0.22	-60.97	46.09	-1.32	54.99	0.19
Sex	3.44	6.39	0.54	55.00	0.59	-0.58	2.98	-0.19	26.00	0.85	-46.64	19.20	-2.43	54.99	0.02
Trial	-10.37	5.95	-1.74	55.00	0.09	4.43	2.70	1.64	26.00	0.11	-3.80	10.39	-0.37	54.99	0.72
Hiding time															
Body length	-1.01	5.16	-0.20	26.00	0.85	-11.36	8.90	-1.28	26.00	0.21	-2.78	2.75	-1.01	26.00	0.32
K	-27.25	36.37	-0.75	26.00	0.46	-104.79	84.11	-1.25	26.00	0.22	21.02	33.22	0.63	26.00	0.53
Sex	-14.29	9.59	-1.49	26.00	0.15	7.21	19.85	0.36	26.00	0.72	-6.21	13.84	-0.45	26.00	0.66
Trial	30.06	8.19	3.67	26.00	<0.01	19.65	8.75	2.25	26.00	0.03	-2.65	4.56	-0.58	26.00	0.56
Distance moved															
Body length	135.73	111.64	1.21	26.00	0.23	-39.99	55.00	-0.73	26.00	0.48	-97.41	35.44	-2.75	26.00	0.01
K	-416.97	786.34	-0.53	26.00	0.60	140.46	519.69	0.27	26.00	0.79	-482.81	427.85	-1.13	26.00	0.27
Sex	155.57	207.47	0.75	26.00	0.46	86.98	122.65	0.71	26.00	0.48	-495.60	178.20	-2.78	26.00	<0.01
Trial	20.93	187.69	0.11	26.00	0.91	-523.08	89.03	-5.87	26.00	<0.01	-110.77	59.60	-1.86	26.00	0.07
Freezing time															
Body length	-9.82	13.59	-0.72	55.01	0.47	-7.91	7.81	-1.01	26.00	0.32	6.71	6.61	1.01	26.00	0.32
K	91.90	95.74	0.96	55.01	0.34	-76.08	73.75	-1.03	26.00	0.31	50.25	79.82	0.63	26.00	0.53
Sex	-3.04	25.26	-0.12	55.10	0.90	8.05	17.41	0.46	26.00	0.65	54.46	33.25	1.64	26.00	0.11
Trial	-29.20	23.55	-1.24	55.10	0.22	27.39	12.03	2.28	26.00	0.03	16.86	9.27	1.82	26.00	0.08

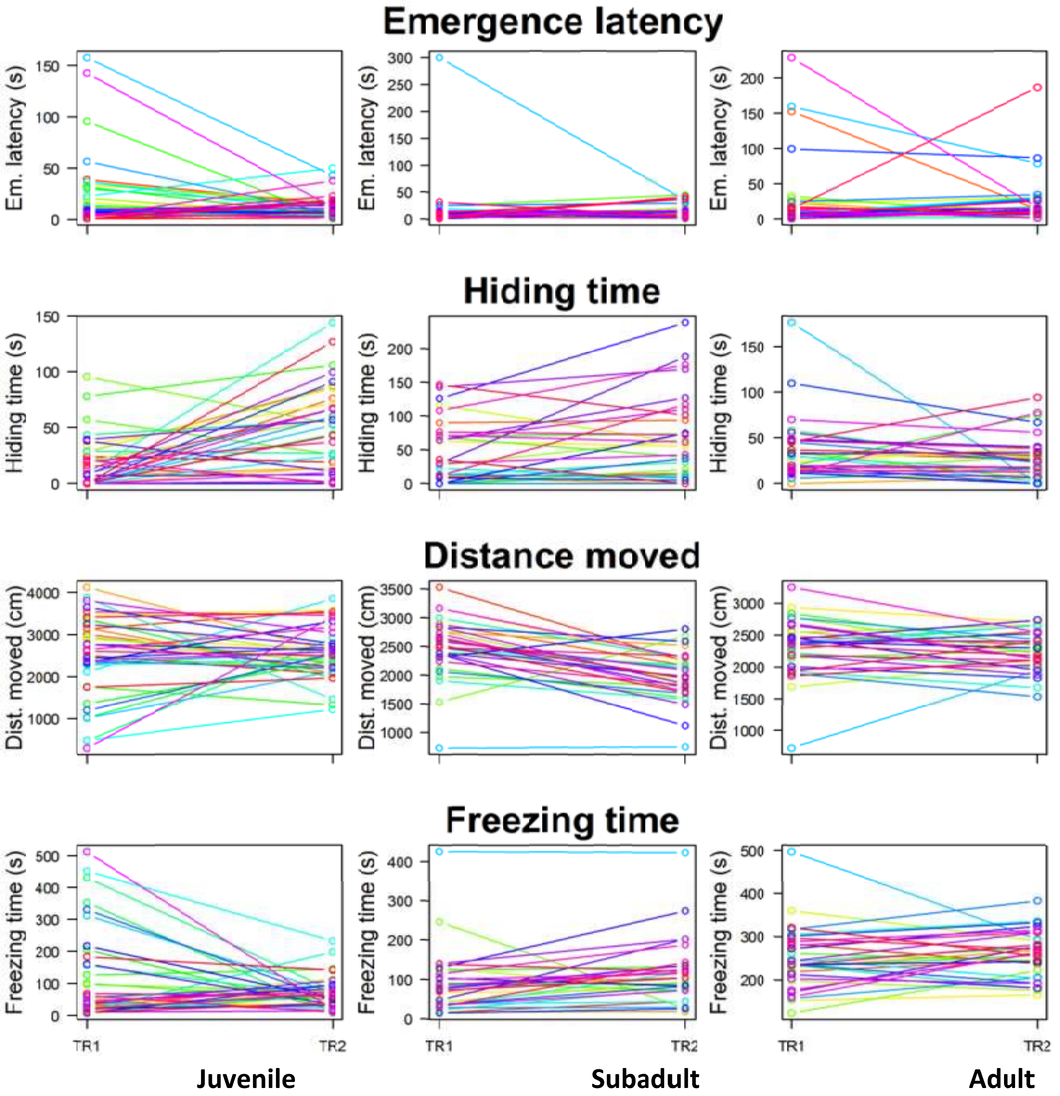


Fig. S2 Behavioral repeatability across ontogenetic stages. For each behavioral trait (rows), behavioral scores are represented for each individual with respect to the first (TR1) and the second (TR2) experimental trial across the three ontogenetic stages (columns)

REFERENCES

Pyke, G. H. (2005). A review of the biology of *Gambusia affinis* and *G. holbrooki*. Rev Fish Biol Fisher, 15(4), 339-365.

Turner, C. L. (1941). Morphogenesis of the gonopodium in *Gambusia affinis affinis*. J Morphol, 69(1), 161-185.

Chapter 3

Tank size alters mean behaviours and individual rank orders in personality traits of fish depending on their life stage

This chapter consists of the following publication:

G Polverino, T Ruberto, G Staaks, T Mehner, 2016. Tank size alters mean behaviours and individual rank orders in personality traits of fish depending on their life stage. *Animal Behaviour* 115:127-135.

Authors' contributions (*i.e., entire, large, predominant, partial):*

GP (**large**), GS, and TM developed the concept; **GP** (**entire**) did the literature research; **GP** (**entire**) developed the methods; **GP** (**predominant**) and TR collected the data; **GP** (**large**) and TR analyzed the data; **GP** (**large**), TR, and TM discussed the results; **GP** (**entire**) wrote the manuscript; **GP** (**predominant**) and TM revised the manuscript.

Animal Behaviour 115 (2016) 127–135

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Tank size alters mean behaviours and individual rank orders in personality traits of fish depending on their life stage

Giovanni Polverino [a], [*], Tommaso Ruberto [b], Georg Staaks [a], Thomas Mehner [a]

[a] *Department of Biology and Ecology of Fishes, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany*
[b] *Department of Ecophysiology and Aquaculture, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany*

ARTICLE INFO

Article history:
Received 14 September 2015
Initial acceptance 2 December 2015
Final acceptance 19 February 2016

MS. number: 15-00793R

Keywords:
behavioural plasticity
context-dependent behaviour
Gambusia
ontogeny
personality
repeatability
size scaling
temperament

Animals adjust their behaviour over time and contexts to cope with ecological and environmental variation. However, the presence of consistent between-individual differences in behaviour (i.e. personality) suggests that individual behaviour may be less flexible than previously thought. Here, we tested whether the size of the experimental tank and the ontogenetic stage of individuals affect estimates of average and consistent individual variation in activity and risk-taking behaviours in the eastern mosquitofish, *Gambusia holbrooki*, a fish model widely utilized for behavioural research. We measured risk taking and activity in juvenile, subadult and adult mosquitofish in a standard open-field test, in which the size of test tank varied linearly. We found strong evidence that spatial constraints alter mosquitofish behaviour. In particular, we observed that average activity increased with tank size, while the willingness of fish to take risks was independent of tank size. Moreover, juvenile fish exhibited, on average, lower risk-taking behaviours than older individuals. We highlight that the use of differently sized tanks may result in unequal variation in the average behaviour between juvenile and older fish, with escaping abilities of juvenile fish being underestimated in small environments. Most interestingly, we observed that variation in tank size triggered changes in the individual rank order for both risk taking and activity in juvenile fish, thus altering their personality estimates. In contrast, adult fish maintained consistent individual rank orders across all tank sizes. This study supports the hypothesis that behavioural repeatability increases with age, suggesting that personality estimates on adult animals may be less vulnerable to variation in laboratory contexts than those on juvenile ones.

Behavioural adjustments represent the initial response that allows animals to cope rapidly with changes in the surrounding environment (Dingemanse, Both, Drent, & Tinbergen, 2004; Sol, Lapiedra, & González-Lagos, 2013) and individuals are evolutionarily predisposed to behavioural flexibility. To study the mechanisms that contribute to the behavioural flexibility of animals in nature, experimental enclosures exposing animals to controlled and comparable conditions are commonly utilized. However, controlled conditions are rarely standardized across laboratories and, hence, may constitute a hidden contribution to the treatment results. For example, it has been documented that the behaviour of animals can be altered by minor variation in the spatial dimension of the experimental enclosures (Mason, 1991). Enclosures that are too small trigger repetitive and invariant behaviours in a variety of laboratory animals, such as primates (Paulk, Dienske, & Ribbens, 1977), rodents (Manosevitz & Pryor, 1975) and birds (Polverino, Manciocco, Vitale, & Alleva, 2015), whereas enclosures that are too large, with extended and potentially unsafe areas, typically cause the emergence of fearful and anxiety states that can severely disrupt the behavioural response even in humans (Madge, 1997). As a result, the size of the experimental enclosure affects remarkably the behaviour of animals under captive conditions. Yet, despite the increasing utilization of fishes as animal models for behavioural studies and applications to technological (Polverino, Liao, & Porfiri, 2013; Polverino & Porfiri, 2013), biomedical (Gerlai, 2010), ecological (Lienart, Mitchell, Ferrari, & McCormick, 2014) and evolutionary (Uusi-Heikkilä et al., 2015) research questions, few studies have explored whether fish behaviour varies on average as a consequence of changing the size of the experimental enclosure (Brown, Davidson, & Laland, 2003; Stewart, Gaikwad, Kyzar, & Kalueff, 2012; Tang & Boisclair, 1993). Indeed, in agreement with findings on nonhuman primates (Bassett, Buchanan-Smith, McKinley, & Smith, 2003), ungulates (Hogan, Houpt, & Sweeney,

---

[*] Correspondence: G. Polverino, Department of Biology and Ecology of Fishes, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, Berlin 12587, Germany.
E-mail address: polverino@igb-berlin.de (G. Polverino).

http://dx.doi.org/10.1016/j.anbehav.2016.03.013

1988), rodents (Manosevitz & Pryor, 1975), birds (Polverino et al., 2015) and reptiles (Warwick, 1990) in which activity varied with the size of the experimental enclosure, it is reasonable to expect that fish behaviour can also be altered by the size of the test tank. Ignoring the effects caused by variation in the size of the test tank on fish behaviour may contribute substantially to biased and conflicting outcomes in the literature (Carter, Feeney, Marshall, Cowlishaw, & Heinsohn, 2013), in which behavioural studies on fishes reflect a variety of experimental conditions with respect to the ratio between fish length and the size of the test tank.

The vast majority of animals undergo morphological changes and substantial increments in size during their lives, accompanied by shifts in ecological niches and phenotypic traits (Clark, 1994; Dial, Greene, & Irschick, 2008; Guenther & Trillmich, 2015; Healy, McNally, Ruxton, Cooper, & Jackson, 2013; Oikawa, Itazawa, & Gotoh, 1991; Werner & Gilliam, 1984; Wilbur, 1980). Thus, behavioural strategies and tactics of animals can naturally change over their ontogeny (Werner & Gilliam, 1984) in response to changes in hidden states of individuals (Sih et al., 2015), such as energy requirements for their basic metabolism (Careau, Thomas, Humphries, & Réale, 2008), stress levels (Polverino et al., 2015) and energy reserves (Krause, Loader, McDermott, & Ruxton, 1998). Significant shifts in average behaviour can, thus, occur during the ontogenetic development of animals as a consequence of different optimal strategies with respect to foraging, risk taking and social interactions (Biro, Post, & Abrahams, 2005; Clark, 1994; Matthews & Wong, 2015). In this vein, age and size of fishes are expected to have major consequences for their behavioural responses (Dial et al., 2008; Nilsson & Brönmark, 2000). For example, risk-taking behaviour can vary extensively across the ontogeny of fishes because predation is a strong selective force in most aquatic ecosystems, with fish size being a key predictor to discriminate who eats whom (Lundvall, Svanbäck, Persson, & Byström, 1999). Indeed, predator–prey theories suggest that adult and large fish should be more inclined to take risks than juveniles because they are less vulnerable to predation by gape-limited predators (Nilsson & Brönmark, 2000; Sogard, 1997).

Despite the advantage of adjusting behaviour to changing environmental contexts (Dingemanse et al., 2004; Skelhorn, Rowland, Delf, Speed, & Ruxton, 2011; van Oers, Klunder, & Drent, 2005), behavioural plasticity of individuals is limited (Buskirk, 2012; Dingemanse et al., 2004). Indeed, consistent between-individual differences in behaviour (i.e. personality or behavioural types) have been observed in numerous animal taxa (Bell, Hankison, & Laskowski, 2009; Gosling, 2001). A hallmark of personality is that individuals differ consistently in their behaviour over time and across contexts (Réale, Reader, Sol, McDougall, & Dingemanse, 2007). However, behavioural plasticity is expected to decline with age because benefits no longer outweigh the costs of behavioural adjustments in older animals (Fischer, van Doorn, Dieckmann, & Taborsky, 2014). In support of this, longitudinal studies have reported increases in the repeatability of individual behaviour during the lifetime of animals (Edenbrow & Croft, 2013; Favati, Zidar, Thorpe, Jensen, & Løvlie, 2016; Fisher, David, Tregenza, & Rodríguez-Muñoz, 2015; Freund et al., 2013). From this perspective, it can be assumed that personality estimates on young individuals may be more vulnerable to variation in contexts than those on adult animals.

In this study, we measured the behavioural responses of juvenile, subadult and adult eastern mosquitofish, *Gambusia holbrooki*, in a standard open field (Walsh & Cummins, 1976) that varied in size. Our aim was to find out whether average behavioural traits and estimates of personality in mosquitofish were sensitive to this variation in spatial context and whether the effect of spatial context was similarly expressed across ontogenetic stages. We hypothesized that (1) risk-taking behaviours and activity of fish vary, on average, as a function of the size of the test tank, (2) average risk-taking behaviours and activity increase from juvenile to adult individuals and (3) the individual rank order in personality traits (i.e. risk taking and activity) across the range of tank sizes becomes more predictable from juvenile to adult animals.

METHODS

Ethical Note

The experimental procedure was approved through an animal care permit (G 0074/15) granted by the Landesamt für Gesundheit und Soziales Berlin (LAGeSo). Both the housing and the experimental procedure were designed to minimize stress in the tested animals. All experiments were performed at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) in Berlin, Germany.

Animal Care and Maintenance

Fish ($N = 45$) were first-generation progeny of wild-caught individuals from Torre Castiglione (Italy, $+40°17'20.44''$, $+17°49'24.30''$; Ariani, Camassa, & Wittmann, 2000). Wild-caught fish were housed in 50-litre aquaria with a maximum density of 0.4 fish/litre. Each aquarium was equipped with an external filtration system (EHEIM Professional 2222, EHEIM GmbH & Co KG, Deizisau, Germany), a heater to maintain the water temperature constantly at 23 ± 0.5 °C, plastic tubes, rocks and filter material to provide refuges for fish.

After a minimum of 5 months acclimatization after capture, pregnant females were removed from their housing tanks, individually transferred into 10-litre aquaria and checked twice a day until they gave birth. Each 10-litre aquarium was filled with filtered water and equipped with an air filter (Quick-Draw Corner Aquarium Filter, Penn Plax, NY), plastic plants and filter material to provide refuge to juveniles after birth. The 10-litre aquaria were housed in a climate chamber (Climate test chamber, Feutron Klimasimulation GmbH, Greiz, Germany) to minimize external disturbances, while maintaining the water temperature constantly at 23 °C. Illumination was provided via fluorescent light bulbs on a 12:12 h cycle (0800–2000 hours) in accordance with the circadian rhythm of the species (Pyke, 2005). Soon after giving birth, each female was returned to its original housing tank and its clutch of juveniles (on average 20 individuals each) was retained in the 10-litre aquarium for the first 15 days after birth. Subsequently, juveniles born within the same 5-day period were grouped and transferred into 50-litre tanks equipped as described above for the wild-caught fish, with a maximum density of 1 fish/litre. Clutches continued to be obtained until fish were available from all ontogenetic stages. At this point, 15 focal fish from each ontogenetic stage were haphazardly selected from the tanks as follows: juvenile (1.6 cm mean total length at approximately 1 month old), subadult (2.7 cm mean total length at approximately 2 months old) and adult fish (4.2 cm mean total length at approximately 10 months old). The subadult stage was visually determined based on the formation of the gonopodium (modified anal fin used for internal fertilization; Pyke, 2005) observed in male mosquitofish. Since male and female mosquitofish do not differ in time to reach sexual maturity (Pyke, 2005), females were considered as subadult when males from the same clutch were determined to be subadult according to the formation of the gonopodium. All subadults identified as females during the experiment were confirmed to be females once sexually mature. All fish were fed twice a day, alternately with flake food (Tetramin Tropical Flakes, Tetra, Melle, Germany) and live *Artemia* nauplii.

Open-field Test

The test tank used for the experiment was a rectangular green tank (60 × 42 cm and 30 cm high) with a capacity of 75 litres. Opaque Plexiglas panels, comparable in coloration to the test tank, were used to divide the test tank (Large tank) into smaller tanks of a half (Medium tank) and a quarter (Small tank) of the total volume (Fig. 1).

A white Plexiglas panel was positioned on the bottom of the test tank to maximize the colour contrast and facilitate fish identification. A white plastic cylinder (5 cm in diameter and 10 cm high) was glued to a transparent Plexiglas base (10 × 10 cm and 2 cm high) and fixed in a corner of the test tank to be used as the experimental refuge (Fig. 1). A square entrance (2 × 2 cm) was created at the base of the cylinder to allow the fish to leave the refuge. A larger concentric cylinder (6 cm in diameter and 10 cm high) was used as a movable 'door' to ensure that fish could not escape from the refuge before the conclusion of the acclimatization period or re-enter the refuge once out. Fish were videorecorded by a top-view high-resolution camera (Bosch Dinion HD 1080p, Bosch, Grasbrunn, Germany) placed approximately 1 m above the test tank. The experimental tank was filled with 7 cm of conditioned water to approximate fish motion on the x–y plane only, while guaranteeing natural shallow-water conditions to the fish (Pyke, 2005). Tests were performed in a second climate chamber, fully comparable to the one described above, to minimize external disturbances during experiments, while maintaining the water temperature constantly at 23 °C.

Behavioural Assay

Experiments were performed over 4 consecutive weeks between 1100 and 1700 hours, from Monday to Friday. To begin an assay, a fish was captured from the housing tank and placed into the closed refuge of the test tank for acclimatization. After 5 min of acclimatization, the door of the refuge was opened and the time for the fish to leave the refuge ('emergence latency', in s) was recorded manually with a stopwatch. We interpreted 'emergence latency' as the willingness of an individual to approach a novel and potentially dangerous environment (Krause et al., 1998). Once the fish left the refuge, the door was closed and the video recording started. If the fish did not leave the refuge within the 5 min, it was gently induced to leave the refuge and the second part of the test was initiated. Once the fish left the refuge, fish behaviour was recorded for 10 min. After the 10 min trial, the fish was removed from the test tank and transferred into a 3-litre isolation tank located in the experimental climate chamber and equipped as described above for the 10-litre tanks utilized for collecting juveniles. On the following 2 days, each fish was tested again twice, once per day, as described above but in test tanks that differed in size to that used on the first day. In other words, each fish was tested on 3 consecutive days, experiencing the Large, Medium and Small tank in a randomized order. The order of the individuals was also randomized every day during the measurements so that the same fish was not measured consistently in the first or last daily trial.

Fish behaviour was quantified using automated video analysis software (Ethovision X10, Noldus IT, Wageningen, Germany) with a maximum sample rate of 30 frames/s. Fish activity and risk taking were calculated for each trial by dividing the surface of the test tank into two zones: 'sheltered area' and 'open water'. The 'sheltered area' was the square zone delimited by the Plexiglas base of the refuge and 'open water' was the remaining part of the tank.

The time spent within the 'sheltered area' ('shelter', in s), the total time spent swimming ('swimming', in s), 'distance moved' (in cm), 'mean velocity' (in cm/s) and 'velocity variance' (in cm^2/s^2) were calculated from each video. We interpreted short time periods spent within the 'sheltered area' as the fish's willingness to take risks in exploring open spaces that were unfamiliar and potentially dangerous. 'Swimming', 'distance moved' and 'mean velocity' characterized common measurements of activity and were interpreted as measures of exploration. 'Velocity variance' denoted the activity pattern of fish and high values were related to an increase in the potential escaping abilities of the individuals. The threshold between swimming and nonswimming behaviour was calibrated separately for each ontogenetic stage at half of the mean body length of fish (juveniles: 1 cm/s; subadult: 1.5 cm/s; adult: 2 cm/s). Data acquisition started 10 s after the beginning of each 10 min trial to minimize the presence of shadows and was interrupted 5 s before the conclusion of the video. In other words, fish behaviour was measured for 585 s for each trial.

Statistical Analysis

Prior to all analyses, 'emergence latency', 'shelter' and 'velocity variance' were log-transformed to normalize error distribution. To reduce the dimensionality of the data set, we first explored correlations between the behavioural traits measured. To do this, we estimated the phenotypic correlation (i.e. the overall correlation jointly contributed by between- and within-individual correlations; Dingemanse & Dochtermann, 2013) between each pair of the six behavioural traits by using bivariate linear mixed-effects models (LMMs), as suggested by Dingemanse and Dochtermann

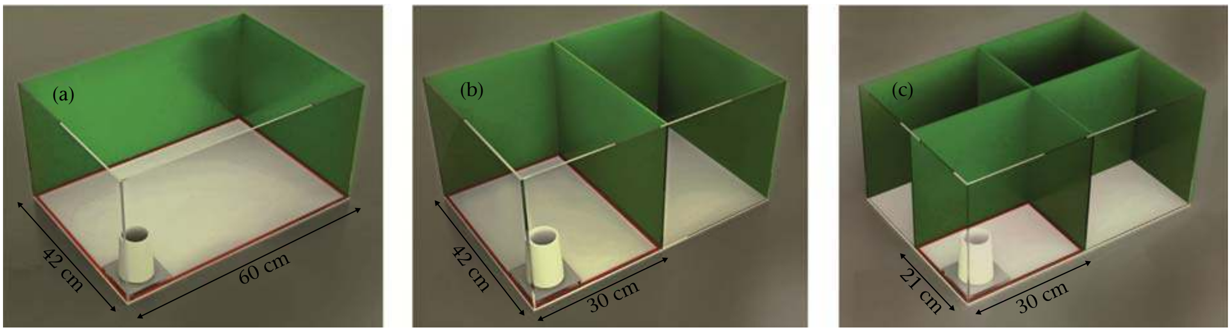


Figure 1. Schematics of the test tanks used in the experiment: (a) Large, (b) Medium and (c) Small tank, respectively. The white plastic cylinder refers to the refuge used to acclimatize each fish before the beginning of the test. The two frontal sides of each tank are here represented as transparent to facilitate the identification of the total area and the refuge. The perimeter in red outlines the surface available for the fish in each tank size.

(2013). We specified the individual as a random effect (i.e. random intercepts) to account for repeated measures of the same individuals across different tank sizes. The six behavioural traits recorded in the open-field test were overall highly correlated (Appendix Table A1). Thus, we used 'mean velocity' in subsequent analyses because of computational correspondence to 'velocity variance', but excluded 'distance moved' and 'swimming' because of strong correlations with 'mean velocity' (Appendix Table A1). Nevertheless, 'emergence latency' and 'shelter' did not correlate with each other, suggesting that latency to emerge from a refuge and time spent in a 'sheltered area' did not represent the same risk-taking axis (Appendix Table A1).

We tested whether average risk-taking behaviours and activity varied as a function of the size of the test tank and the ontogenetic stage of the fish. We ran LMMs separately for each behavioural trait (Dingemanse & Dochtermann, 2013) with the individual as a random effect (i.e. random intercepts) and tank size, ontogenetic stage and their interactions as fixed factors. When the interaction between the two fixed factors was not significant for a given behavioural trait, we removed the interaction term from the model. When significant main effects were found, differences of least squares means from pairwise comparisons were corrected with the conservative Bonferroni method and used for post hoc comparisons.

Then, we tested whether the individual rank order for each behavioural trait was maintained across the range of tank sizes in juvenile, subadult and adult fish. Thus, as suggested by Biro, Beckmann, and Stamps (2010), we performed an LMM for each behavioural trait on each ontogenetic stage separately, specifying the individual as a random effect (i.e. random intercepts) and tank size as a fixed factor, and compared this model with a null model without the individual as a random effect using a likelihood ratio test (LRT). By this approach, a significant random intercept (i.e. individual) indicates that the individual rank order in fish from the same ontogenetic stage was maintained across tank sizes (for further details see Biro et al., 2010). Finally, for each behavioural trait we checked whether estimates of behavioural repeatability (measured separately for juvenile, subadult and adult fish) varied consistently between ontogenetic stages. Thus, the resulting between-individual (intercept) and within-individual (residual) variance estimates from the LMMs described above were used to estimate the repeatability of each behavioural trait and its corresponding 95% credible interval (CI) within a given ontogenetic stage (Hadfield, 2010; Nakagawa & Schielzeth, 2010). Repeatability is defined as the proportion of the behavioural variation attributable to differences between individuals (Dingemanse & Dochtermann, 2013).

Data analysis was performed in R 3.0.2 version (R Core Team, 2013). The bivariate LMMs and repeatability estimates were performed using MCMC sampling methods under a Bayesian framework (R package 'MCMCglmm'; Hadfield, 2010). The parameters were estimated using a noninformative prior (bivariate LMMs) and a semi-informative prior (repeatability estimates), with 1 500 000 resamplings, 500 000 burn-ins and 100 thinnings. LMMs were performed with 'lme4', 'lmerTest' and 'nlme' R packages (Bates, Maechler, Bolker, & Walker, 2014; Kuznetsova, Brockhoff, & Christensen, 2014; Pinheiro, Bates, DebRoy, Sarkar, & CoreTeam, 2014), respectively. The Satterthwaite method was used to approximate degrees of freedom. The significance level was set at $\alpha < 0.05$.

RESULTS

Average Activity and Risk Taking Across Tank Sizes and Ontogenetic Stages

Fish behaviour varied significantly between ontogenetic stages in response to variation in tank size, as reflected by the four behavioural traits (Table 1, Fig. 2). Yet, the LRT between the full model and the model without interaction between fixed factors was not significant for 'mean velocity' and 'velocity variance' ($P = 0.790$; $P = 0.850$; respectively). Accordingly, the interaction between fixed factors was not included in the final models for these two behavioural traits.

When looking at the variation in average behaviours between ontogenetic stages in response to variation in tank size, we observed that 'mean velocity' increased significantly with tank size, independently of the ontogenetic stage (Table 1, Fig. 2). In contrast, 'velocity variance' was independent of tank size, while a significant effect of the ontogenetic stage was observed, with juvenile fish exhibiting the highest 'velocity variance' and adult fish the lowest (Table 1, Fig. 2). We also found that tank size, ontogenetic stage and their interaction all had a significant effect on 'shelter'. In particular, fish spent more time within the 'sheltered area' when tested in the Small tank, with juvenile fish hiding consistently longer than adult fish. Interestingly, average differences in 'shelter' increased consistently between ontogenetic stages with the reduction in tank size (Fig. 2). Similarly, our results indicate a significant interaction between tank size and ontogenetic stage in 'emergence latency', for which the effects of tank size on fish behaviour varied significantly between ontogenetic stages (Table 1, Fig. 2).

Stability of the Rank Order of Individual Behaviour Across Tank Sizes

For each behavioural trait, we tested whether individuals from a given ontogenetic stage maintained their rank order across test tanks that varied in size. We observed an inconsistent individual rank order (i.e. nonsignificant effects of the random intercepts) for all four behavioural traits for at least one ontogenetic stage. This indicated that the rank order of individuals within a given ontogenetic stage was not maintained across observations performed in differently sized tanks (Table 2). In particular, we observed inconsistent rank orders primarily in juvenile fish, in which individual rank orders were never maintained across tank sizes in any of the four behavioural traits measured. In contrast, rank orders were consistently maintained in adult fish across variation in tank size (Table 2, Fig. 3). For subadults, intermediate results were found, with an inconsistent individual rank order observed with respect to 'mean velocity' and 'velocity variance'.

We also verified that behavioural repeatability varied between juvenile, subadult and adult individuals. In agreement with the results given above on individual rank orders, we observed higher behavioural repeatabilities in adult fish than in juveniles for all behavioural traits (Table 2, Fig. 3). Subadult fish showed

Table 1
Results from the LMMs with ontogenetic stage (O.stage) and tank size (T.size) included as fixed factors

Behavioural trait	Fixed factor	*F*	*df*	*P*
Emergence latency	O.stage	0.52	2,45	0.59
	T.size	1.45	2,90	0.24
	O.stage∗T.size	3.77	4,90	<0.01
Shelter	O.stage	9.77	2,45	<0.01
	T.size	39.51	2,90	<0.01
	O.stage∗T.size	2.64	4,90	0.04
Mean velocity	O.stage	0.42	2,45	0.66
	T.size	50.90	2,90	<0.01
Velocity variance	O.stage	19.70	2,45	<0.01
	T.size	0.76	2,90	0.47

The interaction between fixed factors is considered only when significant. 'Emergence latency', 'shelter', 'mean velocity' and 'velocity variance' represents the dependent variables, respectively. Random intercepts are also included for each model to account for repeated measures. The significance level was set at $\alpha < 0.05$.

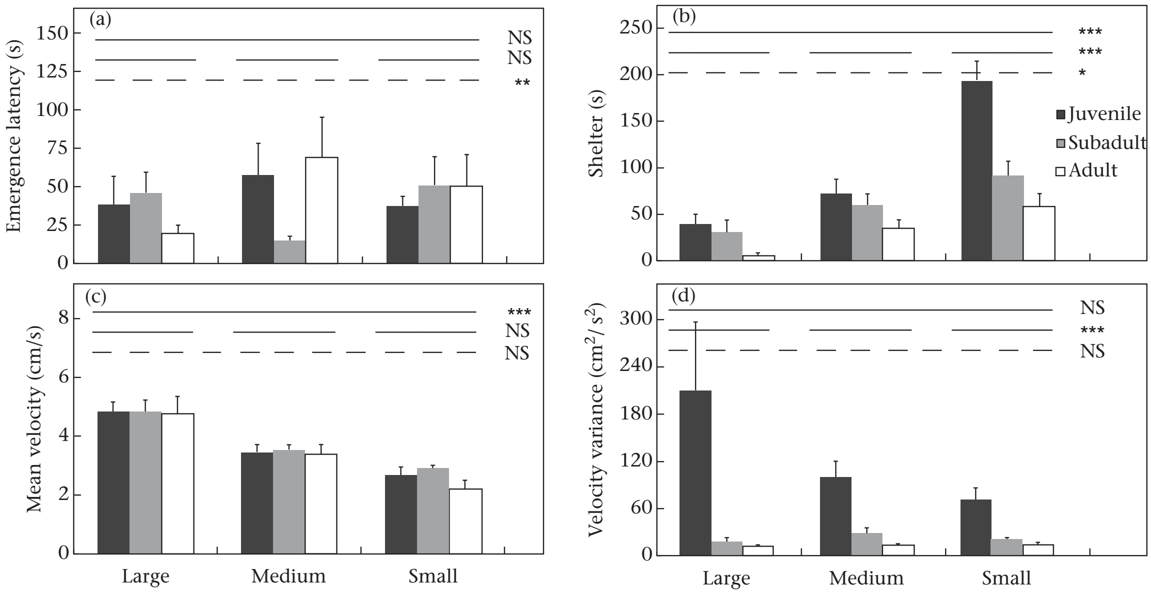


Figure 2. Effects of tank size and ontogenetic stage on average fish behaviour. For each behavioural trait, y-axis values refer to the mean time interval ((a) 'emergence latency' and (b) 'shelter' measured in s) and velocity ((c) 'mean velocity' and (d) 'velocity variance' measured in cm/s and cm^2/s^2, respectively) observed across 10 min trials. Note that 'emergence latency', 'shelter' and 'velocity variance' are not log transformed. Error bars refer to the SEM. For each behavioural trait, continuous lines above histograms refer to the effects of tank size, dashed lines at three segments refer to the effect of the ontogenetic stage, while dashed lines at multiple segments refer to the effect of the interaction between tank size and ontogenetic stage (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$).

Table 2

Stability of the rank order of individual behaviour within ontogenetic stages in response to variation in tank size

Behavioural trait	O.stage	AIC Full model Null model	*P*	V_{within}±SEM	V_{among}±SEM	*R* (95% CI)
Emergence latency	Juvenile	136.24 137.72	0.06	0.74±0.13	0.32±0.08	0.28 (0.04, 0.55)
	Subadult	133.05 145.36	<0.01	0.51±0.11	0.75±0.13	0.58 (0.31, 0.82)
	Adult	154.48 158.66	0.01	1.04±0.15	0.70±0.12	0.38 (0.10, 0.66)
Shelter	Juvenile	160.22 159.03	0.37	1.51±0.18	0.25±0.07	0.13 (0.01, 0.38)
	Subadult	148.20 153.77	<0.01	0.86±0.14	0.69±0.12	0.43 (0.14, 0.69)
	Adult	167.85 174.51	<0.01	1.34±0.17	1.21±0.16	0.46 (0.16, 0.71)
Mean velocity	Juvenile	149.49 147.49	1.0	1.34±0.17	<0.01±<0.01.	<0.01 (<0.01, <0.01)
	Subadult	136.85 134.85	1.0	0.99±0.15	<0.01±<0.01	<0.01 (<0.01, 0.06)
	Adult	165.80 176.44	<0.01	1.16±0.16	1.50±0.18	0.55 (0.26, 0.79)
Velocity variance	Juvenile	159.72 161.52	0.05	1.27±0.17	0.59±0.11	0.29 (0.06, 0.57)
	Subadult	99.07 98.98	0.17	0.33±0.08	0.09±0.05	0.20 (0.02, 0.47)
	Adult	108.07 116.24	<0.01	0.31±0.08	0.32±0.08	0.50 (0.21, 0.76)

Results from the LMMs with 'emergence latency', 'shelter', 'mean velocity' and 'velocity variance' as dependent variables are shown with respect to the Akaike information criterion (AIC) from each LMM. Significance of the random effect (intercepts) was estimated using LRTs (*P*). Significant values indicate that individual rank orders were maintained across observations performed in different tank sizes. The significance level was set at $\alpha < 0.05$. Random intercepts also allowed variance decomposition. Thus, residuals (V_{within}), intercepts (V_{among}) and repeatabilities (*R*) with their 95% CI are shown with respect to each behavioural trait for a given ontogenetic stage (O.stage).

intermediate values of repeatability, except for 'emergence latency', in which subadult fish showed the highest repeatability score, and 'velocity variance', in which they showed the lowest repeatability score (Table 2, Fig. 3).

DISCUSSION

In this study, we have explored the consequences of variation in the size of the test tank on risk-taking behaviours and activity in

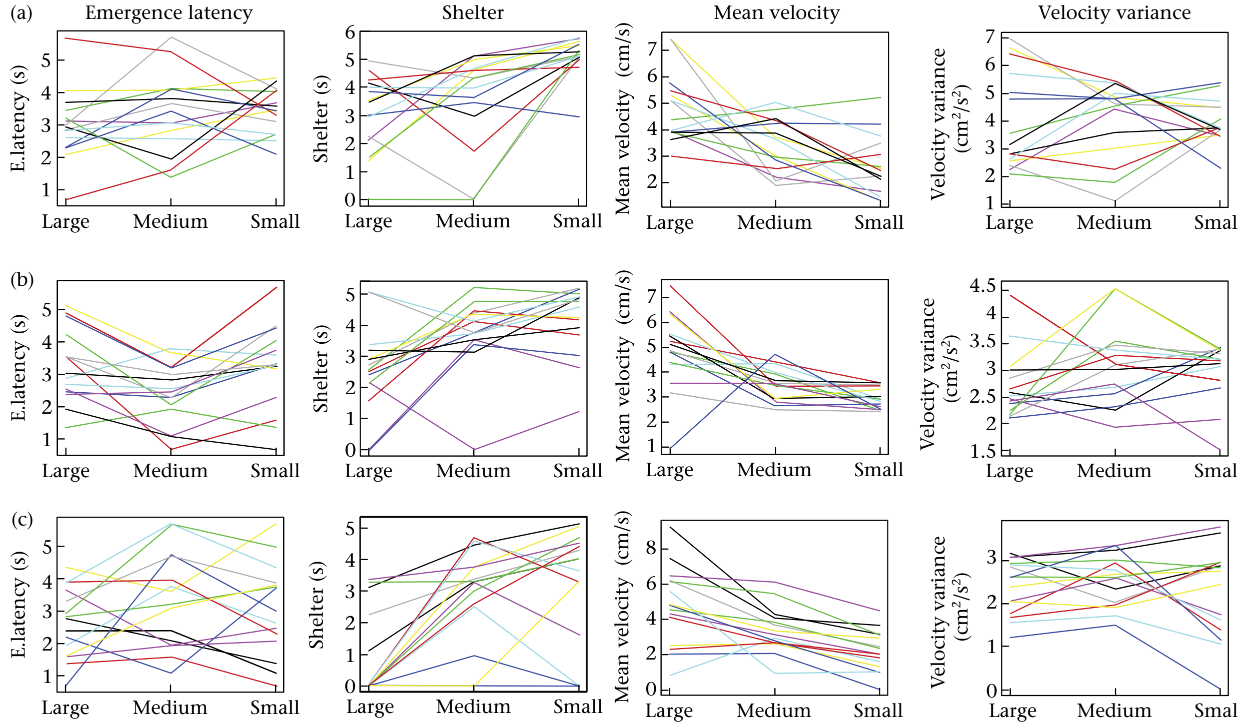


Figure 3. Repeatability of behavioural traits within each ontogenetic stage in response to variation in tank size. Behavioural scores of fish across tank sizes are expressed for each ontogenetic stage: (a) juvenile, (b) subadult and (c) adult. Each coloured line represents a single individual measured across the three tank sizes. Note that coordinates of data points in ‘emergence latency’, ‘shelter’ and ‘velocity variance’ are log transformed.

mosquitofish from different ontogenetic stages. We observed that both tank size and ontogenetic stage of fish had, on average, a strong effect on the four behavioural traits measured. Furthermore, we observed that the individual rank order of juvenile and in part subadult fish was not maintained consistently across different tank sizes, with behavioural repeatability being consistently higher in adult individuals than juveniles. Thus, our results suggest that the size of the experimental enclosures may affect the outcome of personality tests in immature animals under laboratory contexts, whereas the spatial context is less important when studying the behaviour of adult animals.

One goal of this study was to test whether average fish behaviour varied as a function of the size of the test tank. Indeed, meta-analyses indicate that behaviour in ectotherms (such as fishes) is more sensitive to environmental variation than in endotherms (Bell et al., 2009). Therefore, fish behaviour may be more likely to be affected by variation in laboratory contexts than in traditional endothermal animal models, such as primates (Paulk et al., 1977), rodents (Manosevitz & Pryor, 1975) and birds (van Oers et al., 2005). Despite the large number of laboratory studies on fish behaviour, the contribution of the size of the test tank to the behavioural response of fishes remains controversial (Maszczyk & Gliwicz, 2014; Näslund, Bererhi, & Johnsson, 2015; Stewart et al., 2012; Tang & Boisclair, 1993). In one study, the distance travelled by zebrafish, *Danio rerio*, in an open-field test was found to scale with the size of the test tank, while other activity-related measurements (i.e. velocity and freezing time) and temporal patterns of activity were independent of the tank size (Stewart et al., 2012). In another study, the swimming pattern of brook trout, *Salvelinus fontinalis*, varied with the size of the submerged enclosures in a field experiment, suggesting that measuring fish behaviour in small tanks may result in biased conclusions on the complexity of their swimming features (Tang & Boisclair, 1993). Moreover, roach, *Rutilus rutilus*, were observed to adapt their foraging behaviour as a function of the tank size (Maszczyk & Gliwicz, 2014). Our results confirm that tank size modifies behaviour also in mosquitofish, an animal model widely utilized in this research field (Cote, Fogarty, Weinersmith, Brodin, & Sih, 2010; Matthews & Wong, 2015; Polverino et al., 2013; Polverino & Porfiri, 2013; Ward & Mehner, 2010). Nevertheless, in contrast to the above studies, we observed that average measurements of activity increased with tank size. In agreement with evidence on mammals (Eilam, Dank, & Maurer, 2003), our results may suggest that larger enclosures can offer more opportunities for explorative behaviour also in fishes, as reflected by their higher activity levels. We also observed that the time spent in the ‘sheltered area’ decreased with increasing tank size, with an increment in space that proportionally reduced the probability of a fish passing through the ‘sheltered area’, whose dimension was maintained constant across different tank sizes. Thus, our results suggest that, contrary to observations in humans (Madge, 1997), fishes may not perceive larger open fields as more dangerous.

As a second goal, we investigated whether the average behaviour of mosquitofish differs between ontogenetic stages. As expected, juvenile fish exhibited a lower propensity to take risks when in an unfamiliar context (i.e. high time spent hiding in the ‘sheltered area’) compared to older fish. Juvenile animals are particularly susceptible to predation risk (Childs, 1986; Sousa, 1993) and freshwater ecosystems are not an exception (Lundvall et al., 1999; Sogard, 1997). Then, size-dependent predation should be especially favoured in cannibalistic species such as mosquitofish, in which larger conspecifics represent a threat for young individuals

(Pyke, 2005). Thus, we argue that these fish may have been affected by strong selection pressures to survive their most critical life stage, favouring the evolution of cautious behaviours (Magurran & Seghers, 1990) among juveniles as a consequence of increased vulnerability to gape-size limited predators (Nilsson & Brönmark, 2000). Furthermore, our results support the ‘escaping hypothesis’ (Tang & Boisclair, 1993), which suggests that escaping abilities of juvenile and small animals may be underestimated if the experimental space is limited. Indeed, the rapid accelerations (i.e. high ‘velocity variance’) that characterize the activity pattern of juvenile and small animals (reviewed by Dial et al., 2008) increased substantially from the Small to the Large tank only in juvenile mosquitofish, whereas prolonged swimming at constant speeds was observed primarily in adult individuals. In contrast, we did not find any significant variation between ontogenetic stages in the other risk-taking behaviour (i.e. ‘emergence latency’). As mosquitofish feed at the air–water interface and live prevalently in shallow waters (Pyke, 2005), our results may suggest that the latency to leave the submerged refuge may not be relevant to mosquitofish's ecology (Koski, 2014) and, hence, it does not vary across their ontogeny. However, it is also possible that the latency to emerge from a refuge actually represents a critical trait for mosquitofish survival that is consistent during their ontogeny and, therefore, it does not vary with age. This alternative perspective would also explain the relatively high repeatability observed for ‘emergence latency’ across juvenile (i.e. 0.28), subadult (i.e. 0.58) and adult fish (i.e. 0.38) compared to other behavioural traits. A similar ontogenetic coherence in the tendency to emerge from a safe refuge was also observed during the lifetime of female crickets, *Gryllus integer* (Hedrick & Kortet, 2012), and European green lizards, *Lacerta viridis* (Bajer et al., 2015), for which the variation in antipredator behaviour was not, as in this study, dictated by size-dependent metabolic constraints, such as thermal costs (Martín & López, 2003) or risk of starvation (Krause et al., 1998).

Finally, we tested whether variation in the size of the test tank altered the individual rank order for a given personality trait (Biro et al., 2010). We observed that some individuals in the sample had much stronger behavioural responses to context variation than others. Both theoretical and empirical studies indicate higher repeatabilities of animal behaviour in natural contexts than in laboratory studies (Bell et al., 2009; Carere & Maestripieri, 2013; Niemelä & Dingemanse, 2014), suggesting that personality measurements, by definition consistent over time and contexts (Réale et al., 2007), may be biased by certain laboratory contexts. For example, between-individual differences in risk taking of brown trout, *Salmo trutta*, fry were found to vary with the characteristics of the test tank, such as the gate size of the refuge from which fish initiated the experiment (Näslund et al., 2015). Moreover, juvenile damselfish, *Pomacentrus moluccensis*, were found to vary their individual response to minor temperature variation, with the rank order of individual behaviour changing across the temperature gradient (Biro et al., 2010). Surprisingly, there is no evidence in the literature for a similar context dependency of personality estimates in adult animals. Our results indicated that adult and in part subadult fish exhibited consistent individual rank orders across all behavioural traits independently of the size of the test tank, suggesting that there was no or only a weak context dependency of behaviour in these ontogenetic stages. In contrast, the individual rank order was never maintained across tank sizes in juvenile fish. This result suggests that low behavioural repeatabilities can affect the experimental determination of personality primarily in immature animals, whereby changes in space availability (i.e. laboratory contexts) might be sufficient to compromise the stability of their individual rank orders. In support of this hypothesis, we observed that the repeatability of individual behaviour was consistently lower in juvenile than in adult fish, while subadult individuals mostly showed intermediate repeatability scores for each behavioural trait. Longitudinal studies have reported a similar increase in behavioural repeatability during ontogeny of several animal taxa (Edenbrow & Croft, 2013; Favati et al., 2016; Fisher et al., 2015; Freund et al., 2013). Thus, we conclude that the increased behavioural repeatability over the ontogeny of animals, coupled with the simultaneous decrease in their behavioural plasticity over lifetime (i.e. ‘age-dependent plasticity’; Fischer et al., 2014; Freund et al., 2013), may cause personality tests on young individuals to be more vulnerable to environmental variation under laboratory contexts than those on adults. In particular, behavioural plasticity in risk-taking behaviours and activity is expected to be higher in juvenile animals as a coping mechanism to respond to environmental uncertainties in their most critical life stage (Fischer et al., 2014). Then, behavioural plasticity is expected to decrease with age because costs of plasticity may be higher than benefits in older and larger animals due to lowered vulnerability to predation (Fischer et al., 2014).

Animals are capable of adjusting their behaviour across contexts to cope with variation in their environment. Here, we have demonstrated that the variation in space availability (i.e. tank size) triggers context dependency of several behavioural traits in mosquitofish. In addition to space availability, variation in other abiotic environmental variables was found to determine consequent variation in the behaviour of animals. Indeed, variation in temperature can also alter mean behaviours (Angilletta, Wilson, Navas, & James, 2003) and individual responses of animals, for example with respect to personality estimates (Biro et al., 2010). Similarly, behavioural traits of individuals have been found to rely on the environmental context with respect to photoperiod (Finkemeier, Trillmich, & Guenther, 2016; Reparaz et al., 2014), abundance of nesting sites (Jacot, Valcu, van Oers, & Kempenaers, 2009), food abundance (Dingemanse et al., 2004), current velocity within a water body (Sinclair, Souza, Ward, & Seebacher, 2014) and presence of environmental enrichments (Näslund et al., 2015). Thus, we acknowledge that variation in other context variables, aside from the planned variation of the treatment variable, might also alter both mean behaviours and the individual rank order in personality traits of animals. Furthermore, the context dependency of behaviour may vary with the age of the experimental animals, as shown here for mosquitofish. Therefore, it is recommended that these context variables are standardized across experimental protocols.

In summary, our study provides strong evidence on the effects of spatial constraints on fish behaviour under common laboratory conditions. At the best of our knowledge, this study represents the first complete evidence for the confounding consequences that the lack of experimental standardization may have in terms of average behavioural outcomes and personality estimates on animals (Carter et al., 2013; Niemelä & Dingemanse, 2014). In particular, we highlight how the size of the test tank can influence both average behavioural responses and the individual rank order in the behaviour of fishes, with unbalanced variation in behaviour depending on the ontogenetic stage. Thus, we encourage detailed and standardized descriptions of the test tanks adopted to favour comparisons among studies on fish behaviour.

Acknowledgments

We gratefully acknowledge C. Baal, K. Kuntze and H. Zwadlo for their valuable support on the realization of the experimental setup. We also thank S. Nakayama for providing important feedback on the statistics and D. Bierbach and A. Schroeder for comments which helped revising of the manuscript. We also thank the two anonymous referees, K. van Oers, K. Laskowski and M. Wolf for their useful suggestions that have helped to improve the presentation. This work was part of the B-Types project (http://www.b-types.igb-berlin.de/) funded through the Leibniz Competition (SAW-2013-IGB-2).

References

Angilletta, M. J., Wilson, R. S., Navas, C. A., & James, R. S. (2003). Tradeoffs and the evolution of thermal reaction norms. *Trends in Ecology & Evolution, 18*(5), 234–240.

Ariani, A. P., Camassa, M. M., & Wittmann, K. J. (2000). The dolinas of Torre Castiglione (Gulf of Tarent, Italy): environmental and faunistic aspects of a semi-hypogean water system. *Mémoires de Biospéologie, 27*(XXVII), 1–14.

Bajer, K., Horváth, G., Molnár, O., Török, J., Garamszegi, L. Z., & Herczeg, G. (2015). European green lizard (*Lacerta viridis*) personalities: linking behavioural types to ecologically relevant traits at different ontogenetic stages. *Behavioural Processes, 111*, 67–74.

Bassett, L., Buchanan-Smith, H. M., McKinley, J., & Smith, T. E. (2003). Effects of training on stress-related behavior of the common marmoset (*Callithrix jacchus*) in relation to coping with routine husbandry procedures. *Journal of Applied Animal Welfare Science, 6*(3), 221–233.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2014). *lme4: Linear mixed-effects models using Eigen and S4 (version R package version 1.1-5)*. Retrieved from https://cran.r-project.org/web/packages/lme4.

Bell, A. M., Hankison, S. J., & Laskowski, K. L. (2009). The repeatability of behaviour: a meta-analysis. *Animal Behaviour, 77*(4), 771–783.

Biro, P. A., Beckmann, C., & Stamps, J. A. (2010). Small within-day increases in temperature affects boldness and alters personality in coral reef fish. *Proceedings of the Royal Society B: Biological Sciences, 277*(1678), 71–77.

Biro, P. A., Post, J. R., & Abrahams, M. V. (2005). Ontogeny of energy allocation reveals selective pressure promoting risk-taking behaviour in young fish cohorts. *Proceedings of the Royal Society B: Biological Sciences, 272*(1571), 1443–1448.

Brown, C., Davidson, T., & Laland, K. (2003). Environmental enrichment and prior experience of live prey improve foraging behaviour in hatchery-reared Atlantic salmon. *Journal of Fish Biology, 63*(s1), 187–196.

Buskirk, J. V. (2012). Behavioural plasticity and environmental change. In U. Candolin, & B. B. Wong (Eds.), *Behavioural responses to a changing world: Mechanisms and consequences* (pp. 145–158). Oxford, U.K.: Oxford University Press.

Careau, V., Thomas, D., Humphries, M. M., & Réale, D. (2008). Energy metabolism and animal personality. *Oikos, 117*(5), 641–653.

Carere, C., & Maestripieri, D. (2013). *Animal personalities: Behavior, physiology, and evolution*. Chicago, IL: University of Chicago Press.

Carter, A. J., Feeney, W. E., Marshall, H. H., Cowlishaw, G., & Heinsohn, R. (2013). Animal personality: what are behavioural ecologists measuring? *Biological Reviews, 88*(2), 465–475.

Childs, J. E. (1986). Size-dependent predation on rats (*Rattus norvegicus*) by house cats (*Felis catus*) in an urban setting. *Journal of Mammalogy, 67*(1), 196–199.

Clark, C. W. (1994). Antipredator behavior and the asset-protection principle. *Behavioral Ecology, 5*(2), 159–170.

Cote, J., Fogarty, S., Weinersmith, K., Brodin, T., & Sih, A. (2010). Personality traits and dispersal tendency in the invasive mosquitofish (*Gambusia affinis*). *Proceedings of the Royal Society B: Biological Sciences, 277*(1687), 1571–1579.

Dial, K. P., Greene, E., & Irschick, D. J. (2008). Allometry of behavior. *Trends in Ecology & Evolution, 23*(7), 394–401.

Dingemanse, N. J., Both, C., Drent, P. J., & Tinbergen, J. M. (2004). Fitness consequences of avian personalities in a fluctuating environment. *Proceedings of the Royal Society B: Biological Sciences, 271*(1541), 847–852.

Dingemanse, N. J., & Dochtermann, N. A. (2013). Quantifying individual variation in behaviour: mixed-effect modelling approaches. *Journal of Animal Ecology, 82*(1), 39–54.

Edenbrow, M., & Croft, D. P. (2013). Environmental and genetic effects shape the development of personality traits in the mangrove killifish *Kryptolebias marmoratus*. *Oikos, 122*(5), 667–681.

Eilam, D., Dank, M., & Maurer, R. (2003). Voles scale locomotion to the size of the open-field by adjusting the distance between stops: a possible link to path integration. *Behavioural Brain Research, 141*(1), 73–81.

Favati, A., Zidar, J., Thorpe, H., Jensen, P., & Løvlie, H. (2016). The ontogeny of personality traits in the red junglefowl, *Gallus gallus*. *Behavioral Ecology, 27*(2), 484–493.

Finkemeier, M. A., Trillmich, F., & Guenther, A. (2016). Match–mismatch experiments using photoperiod expose developmental plasticity of personality traits. *Ethology, 122*(1), 80–93.

Fischer, B., van Doorn, G. S., Dieckmann, U., & Taborsky, B. (2014). The evolution of age-dependent plasticity. *American Naturalist, 183*(1), 108–125.

Fisher, D. N., David, M., Tregenza, T., & Rodríguez-Muñoz, R. (2015). Dynamics of among-individual behavioral variation over adult lifespan in a wild insect. *Behavioral Ecology, 26*(4), 975–985.

Freund, J., Brandmaier, A. M., Lewejohann, L., Kirste, I., Kritzler, M., Krüger, A., et al. (2013). Emergence of individuality in genetically identical mice. *Science, 340*(6133), 756–775.

Gerlai, R. (2010). High-throughput behavioral screens: the first step towards finding genes involved in vertebrate brain function using zebrafish. *Molecules, 15*(4), 2609–2622.

Gosling, S. D. (2001). From mice to men: what can we learn about personality from animal research? *Psychological Bulletin, 127*(1), 45.

Guenther, A., & Trillmich, F. (2015). Within-litter differences in personality and physiology relate to size differences among siblings in cavies. *Physiology & Behavior, 145*, 22–28.

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *Journal of Statistical Software, 33*(2), 1–22.

Healy, K., McNally, L., Ruxton, G. D., Cooper, N., & Jackson, A. L. (2013). Metabolic rate and body size are linked with perception of temporal information. *Animal Behaviour, 86*(4), 685–696.

Hedrick, A. V., & Kortet, R. (2012). Sex differences in the repeatability of boldness over metamorphosis. *Behavioral Ecology and Sociobiology, 66*(3), 407–412.

Hogan, E. S., Houpt, K. A., & Sweeney, K. (1988). The effect of enclosure size on social interactions and daily activity patterns of the captive Asiatic wild horse (*Equus przewalskii*). *Applied Animal Behaviour Science, 21*(1), 147–168.

Jacot, A., Valcu, M., van Oers, K., & Kempenaers, B. (2009). Experimental nest site limitation affects reproductive strategies and parental investment in a hole-nesting passerine. *Animal Behaviour, 77*(5), 1075–1083.

Koski, S. E. (2014). Broader horizons for animal personality research. *Frontiers in Ecology and Evolution, 2*, 70.

Krause, J., Loader, S. P., McDermott, J., & Ruxton, G. D. (1998). Refuge use by fish as a function of body length–related metabolic expenditure and predation risks. *Proceedings of the Royal Society B: Biological Sciences, 265*(1413), 2373–2379.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2014). *lmerTest: Tests in linear mixed effects models*. Retrieved from http://CRAN.R-project.org/package=lmerTest.

Lienart, G. D. H., Mitchell, M. D., Ferrari, M. C. O., & McCormick, M. I. (2014). Temperature and food availability affect risk assessment in an ectotherm. *Animal Behaviour, 89*, 199–204.

Lundvall, D., Svanbäck, R., Persson, L., & Byström, P. (1999). Size-dependent predation in piscivores: interactions between predator foraging and prey avoidance abilities. *Canadian Journal of Fisheries and Aquatic Sciences, 56*(7), 1285–1292.

Madge, C. (1997). Public parks and the geography of fear. *Tijdschrift Voor Economische en Sociale Geografie, 88*(3), 237–250.

Magurran, A. E., & Seghers, B. H. (1990). Population differences in predator recognition and attack cone avoidance in the guppy *Poecilia reticulata*. *Animal Behaviour, 40*(3), 443–452.

Manosevitz, M., & Pryor, J. B. (1975). Cage size as a factor in environmental enrichment. *Journal of Comparative and Physiological Psychology, 89*(6), 648.

Martín, J., & López, P. (2003). Ontogenetic variation in antipredator behavior of Iberian rock lizards (*Lacerta monticola*): effects of body-size-dependent thermal-exchange rates and costs of refuge use. *Canadian Journal of Zoology, 81*(7), 1131–1137.

Mason, G. J. (1991). Stereotypies: a critical review. *Animal Behaviour, 41*(6), 1015–1037.

Maszczyk, P., & Gliwicz, Z. M. (2014). Selectivity by planktivorous fish at different prey densities, heterogeneities, and spatial scales. *Limnology and Oceanography, 59*(1), 68–78.

Matthews, S. A., & Wong, M. Y. (2015). Temperature-dependent resolution of conflict over rank within a size-based dominance hierarchy. *Behavioral Ecology, 26*(3), 947–958.

Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biological Reviews, 85*(4), 935–956.

Näslund, J., Bererhi, B., & Johnsson, J. I. (2015). Design of emergence test arenas can affect the results of boldness assays. *Ethology, 121*(6), 556–565.

Niemelä, P. T., & Dingemanse, N. J. (2014). Artificial environments and the study of 'adaptive' personalities. *Trends in Ecology & Evolution, 29*(5), 245–247.

Nilsson, P. A., & Brönmark, C. (2000). Prey vulnerability to a gape-size limited predator: behavioural and morphological impacts on northern pike piscivory. *Oikos, 88*(3), 539–546.

van Oers, K., Klunder, M., & Drent, P. J. (2005). Context dependence of personalities: risk-taking behavior in a social and a nonsocial situation. *Behavioral Ecology, 16*(4), 716–723.

Oikawa, S., Itazawa, Y., & Gotoh, M. (1991). Ontogenetic change in the relationship between metabolic rate and body mass in a sea bream *Pagrus major* (Temminck & Schlegel). *Journal of Fish Biology, 38*(4), 483–496.

Paulk, H. H., Dienske, H., & Ribbens, L. G. (1977). Abnormal behavior in relation to cage size in rhesus monkeys. *Journal of Abnormal Psychology, 86*(1), 87.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & CoreTeam, R. (2014). *nlme: Linear and nonlinear mixed effects models (R package version 3.1-118)*. Retrieved from http://CRAN.R-project.org/package=nlme.

Polverino, G., Liao, J. C., & Porfiri, M. (2013). Mosquitofish (*Gambusia affinis*) preference and behavioral response to animated images of conspecifics altered in their color, aspect ratio, and swimming depth. *PLoS One, 8*(1), e54315.

Polverino, G., Manciocco, A., Vitale, A., & Alleva, E. (2015). Stereotypic behaviours in *Melopsittacus undulatus*: behavioural consequences of social and spatial limitations. *Applied Animal Behaviour Science, 165*, 143–155.

Polverino, G., & Porfiri, M. (2013). Zebrafish (*Danio rerio*) behavioural response to bioinspired robotic fish and mosquitofish (*Gambusia affinis*). *Bioinspiration & Biomimetics, 8*(4), 044001.

Pyke, G. H. (2005). A review of the biology of *Gambusia affinis* and *G. holbrooki*. *Reviews in Fish Biology and Fisheries, 15*(4), 339–365.

R Core Team. (2013). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews, 82*(2), 291–318.

Reparaz, L. B., van Oers, K., Naguib, M., Doutrelant, C., Visser, M. E., & Caro, S. P. (2014). Mate preference of female blue tits varies with experimental photoperiod. *PLoS One, 9*(3), e92527.

Sih, A., Mathot, K. J., Moiron, M., Montiglio, P. O., Wolf, M., & Dingemanse, N. J. (2015). Animal personality and state–behaviour feedbacks: a review and guide for empiricists. *Trends in Ecology & Evolution, 30*(1), 50–60.

Sinclair, E. L. E., Souza, C. R. N., Ward, A. J. W., & Seebacher, F. (2014). Exercise changes behaviour. *Functional Ecology, 28*(3), 652–659.

Skelhorn, J., Rowland, H. M., Delf, J., Speed, M. P., & Ruxton, G. D. (2011). Density-dependent predation influences the evolution and behavior of masquerading prey. *Proceedings of the National Academy of Sciences of the United States of America, 108*(16), 6532–6536.

Sogard, S. M. (1997). Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bulletin of Marine Science, 60*(3), 1129–1157.

Sol, D., Lapiedra, O., & González-Lagos, C. (2013). Behavioural adjustments for a life in the city. *Animal Behaviour, 85*(5), 1101–1112.

Sousa, W. P. (1993). Size-dependent predation on the salt-marsh snail *Cerithidea californica Haldeman*. *Journal of Experimental Marine Biology and Ecology, 166*(1), 19–37.

Stewart, A. M., Gaikwad, S., Kyzar, E., & Kalueff, A. V. (2012). Understanding spatio-temporal strategies of adult zebrafish exploration in the open field test. *Brain Research, 1451*, 44–52.

Tang, M., & Boisclair, D. (1993). Influence of the size of enclosures on the swimming characteristics of juvenile brook trout (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences, 50*(8), 1786–1793.

Uusi-Heikkilä, S., Whiteley, A. R., Kuparinen, A., Matsumura, S., Venturelli, P. A., Wolter, C., et al. (2015). The evolutionary legacy of size-selective harvesting extends from genes to populations. *Evolutionary Applications, 8*(6), 597–620.

Walsh, R. N., & Cummins, R. A. (1976). The open-field test: a critical review. *Psychological Bulletin, 83*(3), 482.

Ward, A. J. W., & Mehner, T. (2010). Multimodal mixed messages: the use of multiple cues allows greater accuracy in social recognition and predator detection decisions in the mosquitofish, *Gambusia holbrooki*. *Behavioral Ecology, 21*(6), 1315–1320.

Warwick, C. (1990). Reptilian ethology in captivity: observations of some problems and an evaluation of their aetiology. *Applied Animal Behaviour Science, 26*(1), 1–13.

Werner, E. E., & Gilliam, J. F. (1984). The ontogenetic niche and species interactions in size-structured populations. *Annual Review of Ecology and Systematics, 15*, 393–425.

Wilbur, H. M. (1980). Complex life cycles. *Annual review of Ecology and Systematics, 11*, 67–93.

APPENDIX

Table A1
Phenotypic-correlation estimates between pairs of behavioural traits

	Emergence latency	Shelter	Swimming	Distance moved	Mean velocity	Velocity variance
Emergence latency		0.02	−0.16	−0.22	−0.23	−0.14
Shelter	−0.16 0.20		0.07	0.02	0.03	0.57
Swimming	−0.33 0.03	−0.11 0.25		0.76	0.72	0.24
Distance moved	−0.39 −0.04	−0.16 0.22	0.68 0.83		0.97	0.33
Mean velocity	−0.40 −0.05	−0.15 0.22	0.63 0.80	0.95 0.98		0.37
Velocity variance	−0.33 0.07	0.44 0.69	0.07 0.42	0.15 0.49	0.21 0.546	

The best estimate of correlation coefficients (i.e. values above the diagonal) and their 95% confidence intervals (i.e. values below the diagonal) are represented for each pair of behavioural traits. We used bivariate linear mixed-effects models using Markov Chain Monte Carlo techniques, while including individuals as a random effect (i.e. random intercepts). Significant results correspond to correlation coefficients whose confidence intervals do not overlap with zero.

Chapter 4

Pace-of-life syndrome in fishes under laboratory and natural settings

This chapter consists of the following publications:

G Polverino, D Bierbach, SS Killen, S Uusi-Heikkilä, R Arlinghaus, 2016. Body length rather than routine metabolic rate and body condition correlates with activity and risk-taking in juvenile zebrafish *Danio rerio*. *Journal of Fish Biology* 0:1-17.

> Authors' contributions (*i.e., entire, large, predominant, partial):*
>
> SSK and RA developed the concept; **GP** (**large**), DB, and RA did the literature research; SSK, SUH, and RA developed the methods; SSK collected the data; **GP** (**large**), DB, and SSK analyzed the data; **GP** (**predominant**), DB, SSK, SUH, and RA discussed the results; **GP** (**entire**) wrote the manuscript; **GP** (**predominant**), SSK, and RA revised the manuscript.

KL Laskowski, CT Monk, **G Polverino**, J Alós, S Nakayama, G Staaks, T Mehner, R Arlinghaus, 2016. Behaviour in a standardized assay, but not metabolic or growth rate, predicts behavioural variation in an adult aquatic top predator *Esox lucius* in the wild. *Journal of Fish Biology* 88:1544-1563.

> Authors' contributions (*i.e., entire, large, predominant, partial):*
>
> KLL, CTM, **GP** (**partial**), GS, TM, and RA developed the concept; KLL and RA did the literature research; KLL, CTM, **GP** (**partial**), GS, TM, and RA developed the methods; KLL, CTM, and **GP** (**partial**) collected the data; KLL, CTM, **GP** (**partial**), JA, and SN analyzed the data; KLL, CTM, **GP** (**partial**), TM, and RA discussed the results; KLL wrote the manuscript; KLL, CTM, **GP** (**partial**), JA, SN, TM, and RA revised the manuscript.

Journal of FISH BIOLOGY
fsbi An International Society for Fish Biology

Journal of Fish Biology (2016)
doi:10.1111/jfb.13100, available online at wileyonlinelibrary.com

Body length rather than routine metabolic rate and body condition correlates with activity and risk-taking in juvenile zebrafish *Danio rerio*

G. POLVERINO*†, D. BIERBACH*, S. S. KILLEN‡, S. UUSI-HEIKKILÄ*§ AND R. ARLINGHAUS*||

**Department of Biology and Ecology of Fishes, Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Muggelseedamm 310, 12587 Berlin, Germany, ‡College of Medical, Veterinary and Life Sciences, Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow G12 8QQ, U.K., §Division of Genetics and Physiology, Department of Biology, University of Turku Itäinen Pitkäkatu 4, Turku, 20014, Finland and ||Division of Integrative Fisheries Management, Faculty of Life Sciences, Humboldt-Universität zu Berlin, Unter den Linden 6, 10099 Berlin, Germany*

(Received 9 February 2016, Accepted 28 June 2016)

In this study, the following hypotheses were explored using zebrafish *Danio rerio*: (1) individuals from the same cohort differ consistently in activity and risk-taking and (2) variation in activity and risk-taking is linked to individual differences in metabolic rate, body length and body condition. To examine these hypotheses, juvenile *D. rerio* were tested for routine metabolic rate and subsequently exposed to an open field test. Strong evidence was found for consistent among-individual differences in activity and risk-taking, which were overall negatively correlated with body length, *i.e.* larger *D. rerio* were found to be less active in a potentially dangerous open field and a similar trend was found with respect to a more direct measure of their risk-taking tendency. In contrast, routine metabolic rate and body condition were uncorrelated with both activity and risk-taking of juvenile *D. rerio*. These findings suggest that body length is associated with risk-related behaviours in juvenile *D. rerio* for which larger, rather than smaller, individuals may have a higher risk of predation, while the role for routine metabolic rate is relatively limited or non-existent, at least under the conditions of the present study.

Key words: allometry; behavioural type; body size; boldness; metabolism; personality.

INTRODUCTION

Individual animals within a given species commonly exhibit consistent differences in behavioural traits such as activity, risk-taking, exploration, aggressiveness and sociability, referred to as animal personalities or behavioural types (Réale *et al.*, 2007). Theoretical and empirical studies suggest that animal personality is ultimately caused by ecological and evolutionary adaptation to ecological gradients and corresponding

---

†Author to whom correspondence should be addressed. Tel.: +49 (30)64181609; email: polverino@igb-berlin.de

selection pressures, such as risk of predation (Wolf *et al.*, 2007), resource availability (Biro & Stamps, 2008) or social environment (Pruitt & Riechert, 2011). Individuals do not only differ in their experienced extrinsic conditions but also in intrinsic factors such as body size and metabolic (*i.e.* energetic) demands, both of which can also promote consistent differences in behaviour among animals and are commonly referred to as 'state variables' (Biro & Stamps, 2010; Wolf & Weissing, 2010; Sih *et al.*, 2015). A state variable represents any measurable trait that varies dynamically over time, often influencing the costs and benefits associated with a particular behaviour [*e.g.* age, size, body condition or physiological condition (Wolf & Weissing, 2010)]. The optimal behavioural response of an individual then depends on the interplay between both (often stochastically varying) extrinsic and intrinsic conditions in light of trade-offs (Carere & Maestripieri, 2013), which in turn may lead to consistent among-individual differences in expressed behavioural phenotypes and maintain behavioural variation in natural populations (Réale *et al.*, 2007; Killen *et al.*, 2013; Sih *et al.*, 2015).

Behavioural plasticity allows animals to rapidly adapt to changing ecological and state conditions on very short time scales to safeguard access to resources and mates and avoid predation (Ahrens *et al.*, 2012; Buskirk, 2012). For example, as physiological and morphological states change (*e.g.* hunger levels, body condition or size) it is likely that the risk-taking propensity of individuals also changes (Krause *et al.*, 1998; Brown & Braithwaite, 2004; Brown *et al.*, 2007). In this context, hungry fishes, fishes with small body size and low energy reserves should be more inclined to explore potentially dangerous areas and take risks in the attempt to search for food than well-fed, large individuals and those with a good nutritional state (Krause *et al.*, 1998; Brown & Braithwaite, 2004; Killen *et al.*, 2011). Energy reserves are generally known to affect animal behaviour (Uchmanski, 1985; Filby *et al.*, 2010), resulting in consistent foraging, social and risk-taking responses of individuals characterized by varying energetic states (Clark, 1994; Brown *et al.*, 2007; Dial *et al.*, 2008; Filby *et al.*, 2010). In contrast, the effects of body size on activity and risk-taking behaviour have not been consistent in the literature. For example, large individuals of some fish species have been reported to be more active (Kobler *et al.*, 2008) and bolder (Grant, 1990; Polverino *et al.*, 2016) than smaller individuals, and these larger individuals thus tend to more readily expose themselves to potential predators (Johnsson, 1993), probably because the risk of predation typically declines with increasing body length and height (Lorenzen, 2000; Nilsson & Brönmark, 2000). In other fish species, smaller size classes were reported to be generally bolder (Krause *et al.*, 1998; Brown & Braithwaite, 2004) and to show higher activity in the wild (Landsman *et al.*, 2015). One explanation for these inconsistent findings may rest in the size-dependency of predation risk. In small-bodied species it is possible that larger, rather than smaller, individuals may be under greater risk of predation because these larger individuals are preferentially consumed due to the larger absolute amount of energy and nutrition they offer for predators compared to smaller conspecifics (Persson *et al.*, 2003; Johansson *et al.*, 2004). From an ecological and evolutionary perspective, it would then be beneficial to decrease exploration rates in unsafe environments and risk-taking as body size increases, as found for various poeciliids (Laland & Reader, 1999; Brown & Braithwaite, 2004).

In addition to state-effects on behaviour, animal personality has also been related to consistent individual variation in maintenance metabolism, with spill-over effects on the expression of life-history traits, commonly expressed as the pace-of-life-syndrome hypothesis (Réale *et al.*, 2007, 2010; Wolf & Weissing, 2010; Dwyer *et al.*, 2014).

In fishes, higher metabolic demands are expected to promote foraging (Krause *et al.*, 1998), which in turn usually involve exposing oneself to predators and overall risky situations (Ahrens *et al.*, 2012), providing a link between routine metabolic rate (RMR), activity and risk-taking (Metcalfe *et al.*, 2016). A commonly expressed hypothesis is that individuals with a high RMR should also be consistently bold (Biro & Stamps, 2010; Killen *et al.*, 2013), manifesting in a high propensity to take risks as a function of the elevated energy demand to satisfy. An alternative perspective is that bold fishes may also be highly active and therefore develop a high RMR to be able to sustain the high muscular activity and the cellular machinery (*e.g.* mitochondria) needed to maintain a high scope for activity (White & Kearney, 2013). Independent of the exact mechanism, any correlation between metabolic rate and boldness is expected to in turn affect individual life-history productivity by carrying over effects on growth, maturation and reproduction (Biro & Stamps, 2010; Guenther & Trillmich, 2015).

Several recent papers, however, have questioned the generality of the correlation between routine metabolism and behaviour (Killen *et al.*, 2013; Mathot & Dingemanse, 2015). In fact, it has been suggested that the covariance between RMR and activity or risk-taking is only revealed in environments that physiologically and ecologically challenge individuals to express ecologically relevant behaviours (Killen *et al.*, 2012). A key consideration is that many physiological rates, including metabolic rates, scale disproportionately with size (Uchmanski, 1985). Hence, variance in body size can significantly affect a cascade of physiological (Clarke & Johnston, 1999; White & Kearney, 2014) and behavioural traits (Clark, 1994; Dial *et al.*, 2008; Filby *et al.*, 2010; Healy *et al.*, 2013). As a result, differences in body size can strongly affect phenotypic differences among individuals and potentially override differences in behaviour stemming from variation in basic metabolic demand. Generally, studying cause–effect relationships among behaviour, metabolic rate and body size remain controversial (Oikawa *et al.*, 1991; Metcalfe & Monaghan, 2001; Nussey *et al.*, 2007), which motivated the present study.

This study contributes to the literature on metabolism and behaviour (Metcalfe *et al.*, 2016) by reporting correlations between routine metabolism and activity and risk-taking in juvenile zebrafish *Danio rerio* (Hamilton 1822), while controlling for body size and body condition in individuals of the same cohort. The rate of routine oxygen consumption (Schurmann & Steffensen, 1997) and boldness-related behaviours (Réale *et al.*, 2007) were characterized using intermittent-flow respirometry and open field tests, respectively. Based on the body of literature reviewed above, the following predictions were explored: (1) individuals differ consistently in activity and risk-taking behaviours and (2) these differences are explained by differences in state variables like body condition, body length and routine metabolism.

MATERIALS AND METHODS

STUDY ORGANISM AND MAINTENANCE

Experimental animals ($n = 68$) were laboratory-raised *D. rerio* [mean ± s.d.; total length (L_T) and mass = 22·4 ± 2·9 mm and 0·105 ± 0·04 g] of the same cohort. All *D. rerio* were F10 generation descendants of wild-caught individuals originally sampled in a river system west of Coochibar [West Bengal, India (Whiteley *et al.*, 2011)]. Before experimentation, *D. rerio* were housed in opaque tanks (320 l each) connected to a single recirculation system at 27° C with 14L:10D, which reflected the circadian rhythm of the species (Cahill, 2002). *Danio rerio* density never exceeded 1·1 individual l^{-1}. *Danio rerio* were fed five times per day with a combination of *Artemia* sp. nauplii and commercial flake food (TetraMin; www.tetra-fish.com; 47% protein, 10% fat). Water quality was monitored regularly for temperature (mean ± s.d.; 27·01 ± 1·23° C), pH (mean ± s.d.; 8·41 ± 0·11) and daily for oxygen content (mean ± s.d.; 8·00 ± 0·21 mg l^{-1}). A week before the experiment began, *D. rerio* were transferred to 30 l tanks located in a temperature-controlled room. Here, external disturbances were minimized, while water temperature was maintained at a constant 25° C. *Danio rerio* density and feeding were maintained as described before.

METABOLIC RATE ASSAYS

Danio rerio were fasted for 24 h prior to being transferred individually into 5 ml glass respirometers [Loligo Systems; www.loligosystems.com (White & Kearney, 2014)]. The RMR was then estimated as the minimum rate of oxygen consumption. Pilot trials, in which *D. rerio* were left overnight in respirometers for 18 h, determined that the metabolic rate of juvenile *D. rerio* stabilised after 2 h in the chambers. Thus, the time that *D. rerio* spent individually in the respirometers was minimised because extended isolation periods are known to negatively affect both health of social animals and hence data reliability (Seeman & McEwen, 1996). During the experimental trials, *D. rerio* were given a 2 h acclimation period, after which RMR, *i.e.* the minimum cost of maintenance measured at a particular temperature and post-absorptive state while also allowing spontaneous activity, of each individual was taken as the mean level of metabolism over the subsequent 3 h period (Steffensen, 1989). Notably, the metabolic measures performed here did not allow disentangling the extra oxygen consumption beyond standard metabolic rate caused by spontaneous motion activity. Therefore, such measures are defined as RMR to account for the presence of intrinsic factors that may have altered the individual cost of self-maintenance and activity (Schurmann & Steffensen, 1997).

An array of eight cylindrical glass chambers was utilized to perform measurements on eight *D. rerio* simultaneously. The chambers were submerged in an aerated water bath maintained at a constant temperature of 25·0° C, range ±0·1° C using a thermostat. Water oxygen content within the chambers was measured once every 2 s using optodes (Firesting 4-Channel oxygen meters, Pyroscience; www.pyro-science.com). After placing the chambers into the water bath, the system was covered with opaque polystyrene to minimize disturbance during measurements. The entire apparatus was located within a second temperature-controlled room comparable with the one mentioned above for the housing of the holding tanks. This experimental controlled room remained closed during measurements.

The oxygen-saturated water from the water bath was periodically flushed into each chamber of the respirometer through an external pump that was set to turn on and off for alternating 15 min periods. The decrease in oxygen content in the closed chambers was measured at intervals of 2 s for each 15 min period (*i.e.* closed phase). Subsequently, each chamber was automatically flushed with aerated water for 15 min before the start of the next measurement. For each closed phase, specific oxygen consumption of *D. rerio* was calculated by using linear least squares regression (decrease in oxygen concentration over time expressed as mass-specific RMR in the unit mg O_2 kg^{-1} h^{-1}). The first and final 2 min were excluded from each measure such that only the linear component of O_2 degradation was captured (Dupont-Prinet *et al.*, 2010). For each individual, six slopes of oxygen decrease were recorded, and the mass-specific RMR was calculated as the mean of the final three measurements. During each experimental session, one of the chambers measured the oxygen decline in blank conditions without a *D. rerio* in the chamber. As a result, a simultaneous and consistent measurement of microbial respiration was obtained and subtracted from the estimation of *D. rerio* oxygen consumption provided by the system. The oxygen consumption measured in this study was similar to standard metabolic rates observed previously in 2 month old juvenile *D. rerio* of similar L_T (Lucas *et al.*, 2014). Because (mass-specific) RMR was correlated with L_T (Table I), the residuals of RMR from a regression of $\log_{10}$-transformed (mass-specific) RMR *v.* $\log_{10}$-transformed L_T were used to derive a size-controlled index of RMR.

TABLE I. Pearson correlation coefficients of fixed factors in juvenile *Danio rerio*. Values above the diagonal represent the correlation coefficients between pairs of fixed factors, while values below the diagonal represent their *P*-values. Note that residuals from the $\log_{10}$-transformed mass-specific routine metabolic rate (RMR, mg O_2 kg^{-1} h^{-1}) and $\log_{10}$-transformed Fulton's *K* (g mm^{-3} × 100 000) were both independent of total length (L_T; mm) and used for the statistical analysis

	L_T	Fulton's *K*	RMR
L_T		−0·142	−0·442
Fulton's *K*	>0·05		−0·117
RMR	<0·001	0·174	

BEHAVIOURAL ASSAY TO ASSESS ACTIVITY AND RISK-TAKING

After the completion of the metabolic measurements, *D. rerio* were housed individually in 5 l transparent tanks (25 cm × 15 cm × 15 cm) for 24 h before conducting the behavioural experiments. Because of the importance of visual cues in *D. rerio* behavioural responses (Polverino *et al.*, 2012; Kopman *et al.*, 2013), visual interaction between individuals during the 24 h of isolation was allowed to minimize stress on the focal individuals induced by isolation. Activity and risk-taking of each *D. rerio* were tested individually in a standard open field test (Ariyomo & Watt, 2012) during two distinct measuring events (referred to as the first and second trial) to assess the repeatability of these two behavioural traits. The open field test is widely utilized to characterize activity rates and the individual position in the shy-bold continuum for teleosts and it is based on the assumption that a novel, open and structureless field is considered dangerous. Under this perspective, highly exploratory individuals or those that move especially in the central danger zone might be more willing to take risks (Burns, 2008; Ariyomo & Watt, 2012). The circular experimental arena (48·5 cm in diameter) used in the present study was covered with white contact paper on its bottom and lateral surfaces to guarantee a high colour contrast and to facilitate *D. rerio* identification. Notably, the colour of the contact paper used for the experimental arena matched the internal colouration of the housing tanks where *D. rerio* were housed since birth. In this way, stressful responses of individuals to the high contrast background of the experimental arena were prevented. Two neon tubes on the room ceiling assured standardized light conditions between housing and experimental tanks and provided diffuse illumination. The water level in the experimental arena was kept constant at 7 cm depth to minimize *D. rerio* motion across the water column and approximate movements along the horizontal plane.

At the beginning of each trial, a single *D. rerio* was acclimated for 5 min in a transparent plastic cylinder placed in the centre of the arena (acclimation period). A bird's eye webcam (C920 HD Pro, Logitech; www.logitech.com) recorded *D. rerio* movement for a total of 5 min after the cylinder had been carefully removed (first behavioural trial). Subsequently, each individual was transferred back into its individual tank for 30 min before repeating the measurement (second behavioural trial), as described above. After the finalization of the second behavioural trial, both L_T (to the nearest 0·1 mm) and body mass (to the nearest 0·01 g) of each individual were measured.

Videos were analysed using the video tracking software EthoVision XT Version 9.0 (Noldus Information Technologies Inc.; www.noldus.com), with position scoring starting 10 s after the cylinder had been removed. A total of 250 s were analysed for each individual per trial, with smoothing positions based on 10 samples. The space use of each *D. rerio* was calculated by virtually dividing the circular arena into three concentric zones: inner region (approximately six body-lengths in diameter), middle region (approximately three body-lengths in diameter wider than the inner region) and external region (approximately three body-lengths in diameter wider than the middle region). Preliminary analyses, however, showed comparable scores for the time spent by *D. rerio* between the inner and middle region, while individuals were observed to spend most of the time within the three body-lengths region away from the tank wall (*i.e.* external region). Thus, inner and middle regions were combined and two main zones of the arena were considered only: open-water area (approximately nine body lengths from the centre of the arena) and proximity to the wall [approximately three body lengths from the wall of the arena, a measure consistent with Wright & Krause (2006) and Ladu *et al.* (2015)]. Distance moved (a proxy for activity and exploration and defined as total swimming distance per 250 s, measured in cm), time spent freezing (a proxy for inactivity and defined as time an individual was moving at speeds lower than 20 mm s^{-1} within any given 250 s, measured in s) and swimming in open water (a measure of risk-taking and defined as time an individual was moving in the open-water area at speeds higher than 20 mm s^{-1} within any given 250 s, measured in s) were extracted from the *xy*-co-ordinates obtained for each frame. Mean velocity was then calculated as distance moved per (250 s – time spent freezing). Notably, since freezing behaviour can be also associated with anxiogenic states in *D. rerio* and to antipredatory responses (Gerlai, 2010), the risk-taking propensity of juvenile *D. rerio* was estimated here with respect to their time spent actively swimming in the open-water area of arena, while the lack of mobility (*i.e.* freezing) was not included in the analysis.

STATISTICAL ANALYSIS

Prior to all analyses, distance moved was square-root transformed and swimming in open water was log$_{10}$-transformed to achieve normal error distributions. Subsequently, dependent and continuous explanatory variables were centred on their grand mean following Dingemanse & Dochtermann (2013).

As distance moved, mean velocity, freezing and swimming in open water were measured synchronously, these behavioural traits may all represent components of the same personality axis [*i.e.* trait (Réale *et al.*, 2007; Carter *et al.*, 2013)]. Therefore, in a first step bi-variate correlations between all behavioural traits were estimated through phenotypic correlations [*i.e.* the overall correlation jointly contributed by between- and within-individual correlations (Dingemanse & Dochtermann, 2013)] by using bivariate linear mixed-effects models (LMMs), as suggested by Dingemanse & Dochtermann (2013). The individual was specified as a random effect (*i.e.* random intercepts) to account for repeated measures of the same individual across trials.

To represent *D. rerio* condition, the Fulton's condition factor *K* (Pope & Kruse, 2007) was calculated for each individual. Although the correlation between Fulton's *K* (g mm^{-3} × 100 000) and L_T was not significant, the *P*-value was low (Table I). To be conservative, a size-controlled index of Fulton's *K* was derived by using the residuals of a regression of log$_{10}$-transformed *K* on log$_{10}$-transformed L_T. Thus, residuals from Fulton's *K* were used for subsequent analysis serving as a condition index that was independent of length; it indicated the mass or nutritional state for a given individual relative to the standard size.

The presence of significant among-individual differences in each behavioural trait was tested separately using LMMs (Dingemanse & Dochtermann, 2013) with individual random intercepts. Gape-limited predators will be more constrained by prey length, as opposed to prey mass (Lorenzen, 2000). Thus, L_T (mm), residuals of (mass-specific) RMR (controlled for L_T), residuals of Fulton's *K* (controlled for L_T) and trial were included as fixed factors, and the resulting among-individual (intercept) and within-individual (residual) variance estimates were used to calculate repeatability for each behavioural trait (Nakagawa & Schielzeth, 2010; Dingemanse & Dochtermann, 2013). Repeatability was defined as the proportion of the behavioural variation attributable to differences among individuals (Dingemanse & Dochtermann, 2013). As models included fixed factors, estimates of adjusted repeatability (Nakagawa & Schielzeth, 2010) are presented, *i.e.* the proportion of phenotypic variance not explained by fixed factors, which is attributable to consistent among-individual differences in behaviour. Subsequently, the contribution of each fixed factor to the overall among-individual variance in behaviour was investigated by including L_T, residuals of RMR and residuals of Fulton's *K* independently in each LMM. Note that the fixed factor trial was consistently included in the ANOVA components as it does not represent a state variable (Sih *et al.*, 2015). Significance levels of fixed (L_T, residuals RMR, residuals *K* and trial) and random factors were calculated using likelihood ratio tests.

The base model described above was inspired by the idea that L_T would exert a fundamental effect on activity and risk-taking but that individuals that are in a poorer nutritional state or that carry higher-than-average mass-specific metabolic demands would be more explorative and bolder. Models were therefore built with residuals of mass-specific RMR controlling for L_{T}. Alternative models are also possible, notably substituting absolute RMR for mass-specific RMR and body mass as an index for L_{T}. These alternative model variants were also tested, with residuals of log-transformed absolute RMR (mg O_2 h^{-1}) controlling for L_{T} and using L_{T} as the fixed factor. Models were also built substituting body mass for L_{T} (because length and mass are highly correlated) taking residuals from both the log-transformed absolute RMR and log-transformed Fulton's K when regressed on log body mass. Results of these alternative models, that used measure of absolute rather than mass-specific RMR and body mass rather than L_{T} as a morphological measure, entirely agreed with the model introduced in the previous paragraph. Alternative model variants are presented in Tables SI and SII, Supporting information.

The data analysis was performed in R 3.0.2 version (www.r-project.org). The bivariate LMMs were performed using MCMC sampling methods under a Bayesian framework [R package 'MCMCglmm'; (Hadfield, 2010)]. The parameters were estimated using a non-informative prior, with 1 500 000 resamplings, 500 000 burn-ins and 100 thinnings. Other LMMs were performed with lme4 and nlme R packages (Pinheiro *et al.*, 2007; Bates *et al.*, 2014), respectively. The significance level was set at $P \leq 0{\cdot}05$. The S.E. of the mean is provided for each variance estimate in the results.

RESULTS

All four behavioural traits recorded in the open field were strongly correlated (Table II). LMMs detected significant effects, however, of the fixed factor L_{T} in relation to distance moved and freezing only. Accordingly, larger individuals swam on average shorter distances and spent more time freezing than smaller individuals of the same cohort (Table III and Fig. 1). Such effects of L_{T} on behaviour were not observed with respect to swimming in open water and mean velocity (Table III and Fig. 1). Nevertheless, the ANOVA yielded the highest F-value, and the corresponding lowest P-value, for the fixed factor L_{T} also in relation to swimming in open water, suggesting L_{T} to influence risk-taking behaviour in *D. rerio*, albeit marginally, more than the other state variables measured in this study. There were no significant effects

TABLE II. Phenotypic-correlation estimates between pairs of behavioural traits. The best estimate of correlation coefficients (*i.e.* values above the diagonal) and their 95% C.I. (*i.e.* values below the diagonal) are represented for each pair of behavioural traits. Bivariate linear mixed-effects models were used with Markov Chain Monte Carlo techniques, while including individuals as a random effect (*i.e.* random intercepts) to account for repeated measures. Significant results correspond to correlation coefficients whose 95% C.I. do not overlap with zero

	Distance moved	Mean velocity	Freezing	Swimming in open water
Distance moved		0·416	−0·944	0·533
Mean velocity	0·269 0·551		−0·245	−0·025
Freezing	−0·963 −0·923	−0·402 −0·081		−0·568
Swimming in open water	0·393 0·655	−0·055 0·272	−0·681 −0·437	

TABLE III. Results from the LMMs with distance moved, mean velocity, freezing and swimming in open water as dependent variables. Trial, total length (L_T; mm) and L_T-corrected residuals from the $\log_{10}$-transformed mass-specific routine metabolic rate (RMR, mg O_2 kg^{-1} h^{-1}) and $\log_{10}$-transformed Fulton's K (g mm^{-3} × 100 000) are included as fixed factors. Random intercepts are also included for each individual, which allowed variance decomposition. Intercepts (V_{among}), residuals (V_{within}) and adjusted repeatabilities are also shown with respect to each behavioural trait. Note that the fixed factor trial was consistently included for the ANOVA components: LMMs with one fixed factor per time (first three rows of the variance components) and LMM that includes all fixed factors (last row of the variance components)

Distance moved				
Fixed factors	Estimate	F	d.f.$_2$, d.f.$_1$	P
L_T	−2·93	4·41	4, 5	<0·05
RMR	2·47	0·17	4, 5	>0·05
K	−17·43	0·22	4, 5	>0·05
Trial	−0·78	0·03	4, 5	>0·05
Random factor		F	d.f.$_2$, d.f.$_1$	P
Individual		–	6, 7	<0·001
Variance components		V_{within} ± S.E.	V_{among} ± S.E.	Repeatability
L_T		710·9 ± 2·3	720·6 ± 2·3	0·50
RMR		710·9 ± 2·3	787·0 ± 2·4	0·53
K		710·9 ± 2·3	785·2 ± 2·4	0·53
L_T, RMR, K		710·9 ± 2·3	714·3 ± 2·3	**0·50**
Mean velocity				
Fixed factors	Estimate	F	d.f.$_2$, d.f.$_1$	P
L_T	−0·78	0·76	4, 5	>0·05
RMR	6·01	0·92	4, 5	>0·05
K	20·88	0·87	4, 5	>0·05
Trial	−5·17	1·58	4, 5	>0·05
Random factor		F	d.f.$_2$, d.f.$_1$	P
Individual		–	6, 7	0·05
Variance components		V_{within} ± S.E.	V_{among} ± S.E.	Repeatability
L_T		575·6 ± 2·1	160·7 ± 1·1	0·22
RMR		575·6 ± 2·1	159·8 ± 1·1	0·22
K		575·6 ± 2·1	162·4 ± 1·1	0·22
L_T, RMR, K		575·6 ± 2·1	149·4 ± 1·1	**0·21**
Freezing				
Fixed factors	Estimate	F	d.f.$_2$, d.f.$_1$	P
L_T	7·85	6·45	4, 5	<0·05
RMR	−5·59	0·24	4, 5	>0·05
K	64·34	0·62	4, 5	>0·05
Trial	−2·67	0·08	4, 5	>0·05
Random factor		F	d.f.$_2$, d.f.$_1$	P
Individual		–	6, 7	<0·001
Variance components		V_{within} ± S.E.	V_{among} ± S.E.	Repeatability
L_T		3112 ± 5	3770 ± 5	0·55
RMR		3112 ± 5	4248 ± 6	0·58
K		3112 ± 5	4209 ± 6	0·57
L_T, RMR, K		3112 ± 5	3701 ± 5	**0·54**

TABLE III. Continued

Swimming in open water				
Fixed factors	Estimate	*F*	d.f.$_2$, d.f.$_1$	*P*
L_T	−0·07	2·59	4, 5	>0·05
RMR	−0·09	<0·01	4, 5	>0·05
K	−1·57	1·91	4, 5	>0·05
Trial	<0·01	<0·01	4, 5	>0·05
Random factor		*F*	d.f.$_2$, d.f.$_1$	*P*
Individual		–	6, 7	<0·05
Variance components		V_{within} ± S.E.	V_{among} ± S.E.	Repeatability
L_T		1·32 ± 0·10	0·44 ± 0·06	0·25
RMR		1·32 ± 0·10	0·48 ± 0·06	0·27
K		1·32 ± 0·10	0·45 ± 0·06	0·25
L_T, RMR, *K*		1·32 ± 0·10	0·41 ± 0·05	**0·24**

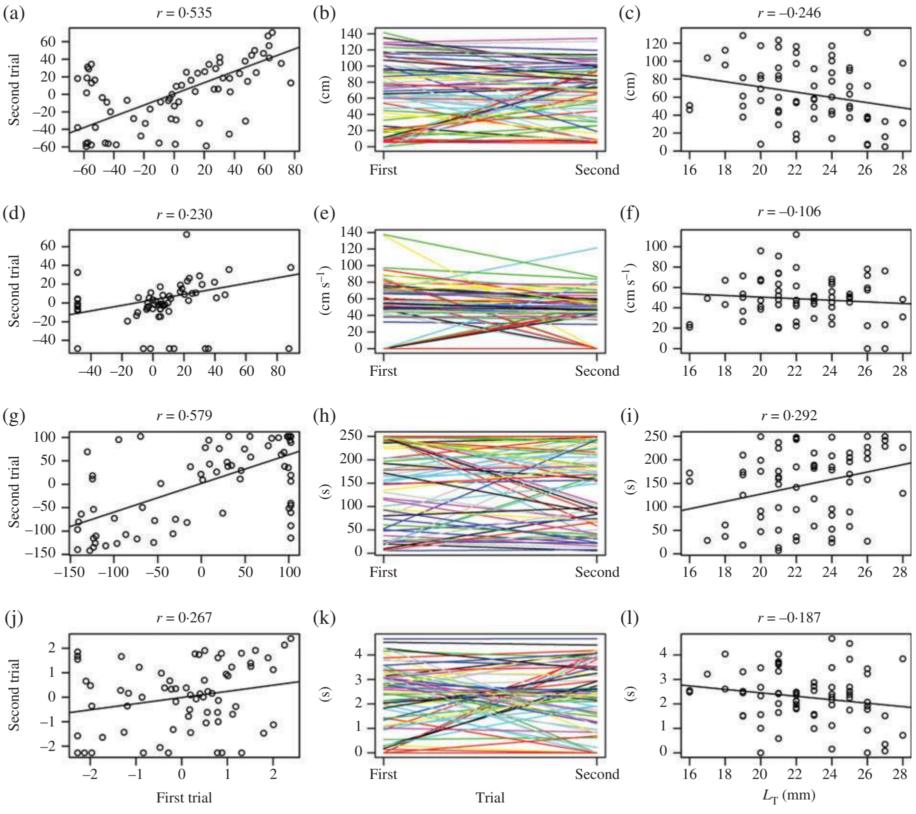


FIG. 1. (a), (b), (d), (e), (g), (h), (j), (k) Behavioural repeatability and (c), (f), (i), (l) correlation of *Danio rerio* behaviour with total length (L_T) are expressed with respect to (a)–(c) distance moved, (d)–(f) mean velocity, (g)–(i) freezing and (j)–(l) swimming in open water. Correlation coefficients (*r*) are given for each behavioural trait. Note co-ordinates of each data point in (c), (f), (i), (l) represent the mean value calculated from the two behavioural measurements performed on each individual.

of mass-specific RMR (residuals), Fulton's K (residuals) and trial on any of the four behavioural traits measured in the present study (Table III).

After accounting for L_T and other fixed factors, significant among-individual variation in each behavioural trait remained, yielding repeatability estimates which comprised between 0·21 and 0·54 (Table III and Fig. 1). In other words, even after controlling for L_T effects, consistent variation in *D. rerio* behaviour in the open field was present among similar sized individuals. Further analyses using LMMs including all fixed factors revealed that among-individual differences for each behavioural trait were significantly affected by the random effect (individual ID; Table III), confirming the presence of personality (or behavioural types) in juvenile *D. rerio*.

DISCUSSION

In the present study, smaller juvenile *D. rerio* exhibited consistently higher activity (*i.e.* higher distance moved and lower freezing) in a novel and potentially dangerous open field compared to larger conspecifics of the same cohort, while swimming in open water (*i.e.* a direct measure of risk-taking) was only marginally affected by individual size. On the contrary, no effects of either RMR or nutritional state were found on the propensity to explore and take risks in an open field. The present study thus supports the hypothesis that body length (*i.e.* L_T) *per se* has a stronger effect than metabolism on activity and risk-taking behaviours and in fact suggests that no relationship among RMR and these behaviours may exist in juvenile *D. rerio*, at least under the conditions examined here.

The study's key finding of a lack of relationship of size-independent RMR and tendency to explore and take risks in *D. rerio* is contingent on the presence of personality in this species. The experimental design, however, has the limitation of a short time interval (30 min) between the two behavioural trials. It is unknown whether the fairly robust consistency in activity and risk-taking reported here would persist over longer time periods (*e.g.* weeks or months). There is a large body of literature on *D. rerio* personality, however, all finding evidence of consistent individual variation in a range of personality traits, such as activity, risk-taking, aggression and sociability (Moretz *et al.*, 2007; Dahlbom *et al.*, 2011; Ariyomo & Watt, 2012; Tran & Gerlai, 2013). It is thus believed that personality is present in *D. rerio*, which adds weight to the conclusion that routine metabolism may play only a limited role, or no role at all, in determining consistent variation in activity and risk-taking in this species when body length (*i.e.* L_T) is accounted for.

In the present study, the L_T of juvenile *D. rerio* was found to be a strong predictor of activity, which is strongly associated with boldness in *D. rerio* (Moretz *et al.*, 2007), but only marginally linked to a more direct measure of risk-taking behaviour in individuals from the same cohort. In particular, smaller individuals were found to be more active and risk prone in a novel, shelterless and potentially dangerous environment, which is in agreement with earlier studies on other fish species (Krause *et al.*, 1998; Brown & Braithwaite, 2004; Biro *et al.*, 2005). Size-dependent phenomena are well known in ecology and size scaling relationships have been well described in fishes (Schmidt-Nielsen, 1984; Clarke & Johnston, 1999; Killen *et al.*, 2010; White & Kearney, 2014). Indeed, small fishes of a given cohort typically possess lower energy reserves and higher mass-specific metabolic demands, which is believed to generally favour elevated propensities to explore new environments and take risks because smaller individuals should have more immediate needs for finding food (Wootton, 1994; Krause *et al.*, 1998). Following this argument, Krause *et al.* (1998) observed that small three-spined sticklebacks *Gasterosteus aculeatus* L. 1758 were consistently faster in emerging from a refuge and spent more time exploring a novel environment compared to larger conspecifics. These smaller *G. aculeatus* also showed larger mass loss when deprived of food, motivating the explanation that the increased activity and risk-taking of small fishes may be associated with metabolic demands (Krause *et al.*, 1998). Here, a metabolism effect in *D. rerio* that differed in size was controlled for, in turn rendering the metabolic explanation for the size-effect on activity and boldness unlikely. Instead, the present work may suggest that smaller *D. rerio* of a given cohort were outcompeted for food in their holding tanks, in turn motivating elevated exploratory and risk-taking tendencies in these starved individuals when observed in isolation. Effects of nutritional states on activity and marginally on risk-taking, however, were only present in relation to using L_T as fixed factor, with no further role related to body condition. L_T variation in juvenile *D. rerio* might more readily indicate variation of nutritional states than variation in Fulton' *K*. Indeed, findings from the present study do not support an effect of Fulton's *K* in shaping different risk strategies in *D. rerio* and other works have also raised cautionary notes about the index values of Fulton's *K* to properly capture nutritional variation in fishes (Morton & Routledge, 2006; Klefoth *et al.*, 2013). This explanation might help understanding the lack of predictive power of body condition in the present work.

A second evolutionary mechanism may have contributed to the present study's findings in relation to the correlation between L_T and activity and boldness. In contrast to most species (Lorenzen, 2000), larger size classes may be under greater risk of predation in small-bodied fish species because they offer greater energy packs for predators compared to smaller conspecifics (Persson *et al.*, 2003; Johansson *et al.*, 2004). Hence, larger fish of small-bodied species, in which large size does not necessarily confer a mortality advantage, may reduce their propensity to take risks as an evolutionary adaptation to predation risk in their natural environment. Such adaptation would in turn show up as greater activity and boldness of smaller fishes in laboratory tests. Fishes have indeed developed a wide array of strategies in the juvenile stage aimed at trading-off key fitness components, particularly growth and mortality (Werner & Hall, 1988; Werner & Anholt, 1993; Clark, 1994; Sogard, 1997; Metcalfe & Monaghan, 2001; Biro *et al.*, 2005; Dial *et al.*, 2008). One of these strategies is feeding in the presence of risk rather than waiting for safer feeding opportunities, which can lead to starvation and possibly mortality in the long term (Werner & Hall, 1988; Werner & Anholt, 1993; Clark, 1994; Biro *et al.*, 2005). In this context, the risk-taking behaviour of juvenile rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) was found to decrease with time in favour of exploration as a function of size, *i.e.* only small individuals were highly motivated to explore the environment and feed under risk of predation to increase growth and minimize the time spent in vulnerable sizes (Biro *et al.*, 2005). Moreover, small guppies *Poecilia reticulata* Peters 1859 have been found to be more exploratory and successful in discovering novel food sources in a novel and potentially dangerous environment compared to larger conspecifics (Laland & Reader, 1999). Similar results were found in other small-bodied poeciliids (Brown & Braithwaite, 2004), where smaller individuals emerged consistently sooner from a sheltered area compared to larger ones, probably in an attempt to balance predation risk with foraging rate and energy gain. An evolutionary adaptation to reduce predation risk of large-bodied animals, however, cannot be ruled out. Similarly, it is possible that the individuals used in the present study were evolutionarily adapted to the predators in their home habitat, *e.g.* the Indian leaf fish *Nandus nandus* (Hamilton 1822) (Bass & Gerlai, 2008). Therefore, the presented finding that smaller juvenile *D. rerio* were more active and, thus, more risk-prone than larger ones aligns with two non-mutually exclusive explanations: either these behaviours are shown to permit the smallest size classes of a given cohort to safeguard access to food in order to grow and reach sizes that substantially reduce the probability of predatory attacks, or the pattern can be explained by evolutionary adaptation to combat greater predation risk in large size classes by becoming inactive and shyer as the individual's length increases. Only ontogenetic studies where individuals are repeatedly assayed in high and low food situations can disentangle these competing hypotheses.

The present results did not support the expected covariance between RMR and personality in *D. rerio*, which agrees with previous works reporting inconsistent associations between metabolic rates and behavioural types in a range of animals (Seppänen *et al.*, 2009; Vaz-Serrano *et al.*, 2011; Bouwhuis *et al.*, 2014; Guenther *et al.*, 2014; Killen *et al.*, 2014). In European sea bass *Dicentrarchus labrax* (L. 1758) the correlation between metabolic rate and spontaneous swimming activity, as an index of risk-taking behaviour, was found to vary across environmental contexts (Killen *et al.*, 2012). After a predator attack, individuals with higher RMR tended to emerge sooner from a sheltered area and were more active than their conspecifics with lower metabolic demands only under hypoxia, while no relationship between activity or risk-taking and metabolic rate was observed when oxygen availability was high (Killen *et al.*, 2012). Killen *et al.* (2013) have recently suggested that the presence or intensity of environmental stressors can modulate covariation between metabolic traits and animal personality. In particular, moderate levels of food deprivation or risk of predation may persuade individuals with high energetic demands to become more active or prone to take risks as they attempt to find food, thus pronouncing the latent links between metabolic traits, activity and risk-taking (Killen *et al.*, 2013). A corollary of the argument is that relationships between metabolism and behavioural phenotypes might not be manifested under relatively benign conditions (Killen *et al.*, 2013). Although the open field test is assumed to be perceived as a threat by experimental animals, this stress alone may not have been sufficient to reveal ecologically relevant variation in behaviours among *D. rerio* stemming from variation in RMR. In fact, as is common in laboratory studies, water quality variables adopted here were optimal for *D. rerio* (Avdesh *et al.*, 2011). Focal individuals were also free from starvation and actual risk of predation since no real predators were involved in the experimental design, while age at the time of testing was selected to prevent the manifestation of aggressive and territorial tendencies among *D. rerio* that are typical at reproductive maturity (Spence *et al.*, 2008). Therefore, in the absence of strong environmental stressors that may challenge individuals and require them to adjust behaviour to fit their intrinsic variation in RMR, fluctuations in behavioural and metabolic traits of juvenile *D. rerio* may have acted independently and hence the two phenotypes were uncorrelated. Nevertheless, the data suggest that even if there is a relationship of metabolism and behaviour in different environmental contexts, the relationship is subtle and may be overridden by state effects related to body size. Note that the present work used *D. rerio* of the same cohort and hence variation in L_T was controlled to a small length range, such that effects of L_T on activity and risk-taking may be more pronounced if a greater length range is examined.

In conclusion, activity and in part risk-taking in juvenile *D. rerio* were found to vary as a function of individual L_T, while there was little evidence for correlations between these behaviours and length-independent RMR or body condition. Future studies shall explore behavioural and metabolic responses along ontogenetic transitions of *D. rerio* (or other species) and in different environments to uncover the relative role of state and physiological underpinning in shaping behavioural variation and personality.

The authors would like to gratefully acknowledge K. Laskowski and F. Pilotto for their valuable help on the statistical analysis and T. Mehner and the participants of the workshop 'Scientific Writing' at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) for helpful discussions on an early stage of the manuscript. The authors would also like to thank the two anonymous reviewers for the useful suggestions that have helped to improve the presentation of this manuscript. S.S.K. was supported by a NERC Advanced Fellowship NE/J019100/1. This work was part of the B-Types project (http://www.b-types.igb-berlin.de/) funded through the Leibniz Competition (SAW-2013-IGB-2) and was also funded by the Adaptfish grant funded through the Leibniz-Competition to R.A. The experiments described here were approved under HO Project License 60/4461.

Supporting Information

Supporting Information may be found in the online version of this paper:

Table SI. Results from the LMMs with distance moved, mean velocity, freezing and swimming in open water as dependent variables. Trial, L_T (mm) and L_T-corrected residuals from the log-transformed absolute RMR (mg O_2 h^{-1}) and log-transformed Fulton's K (g $mm^{-3} \times 100\,000$) are included as fixed factors. Random intercepts are also included for each individual, which allowed variance decomposition. Intercepts (V_{among}), residuals (V_{within}) and adjusted repeatabilities are also shown with respect to each behavioural trait. Note that the fixed factor trial was consistently included for the analysis of the variance components: LMMs with one fixed factor per time (first three rows of the variance components) and LMM that includes all fixed factors (last row of the variance components)

Table SII. Results from the LMMs with distance moved, mean velocity, freezing and swimming in open water as dependent variables. Trial, body mass (g) and mass-corrected residuals from the log-transformed absolute RMR (mg O_2 h^{-1}) and log-transformed Fulton's K (g $mm^{-3} \times 100\,000$) are included as fixed factors. Random intercepts are also included for each individual, which allowed variance decomposition. Intercepts (V_{among}), residuals (V_{within}) and adjusted repeatabilities are also shown with respect to each behavioural trait. Note that the fixed factor trial was consistently included for the analysis of the variance components: LMMs with one fixed factor per time (first three rows of the variance components) and LMM that includes all fixed factors (last row of the variance components)

References

Ahrens, R. N., Walters, C. J. & Christensen, V. (2012). Foraging arena theory. *Fish and Fisheries* **13,** 41–59.

Ariyomo, T. O. & Watt, P. J. (2012). The effect of variation in boldness and aggressiveness on the reproductive success of zebrafish. *Animal Behaviour* **83,** 41–46.

Avdesh, A., Chen, M., Martin-Iverson, M. T., Mondal, A., Ong, D., Rainey-Smith, S., Taddei, K., Lardelli, M., Groth, D. M., Verdile, G. & Martins, R. N. (2011). Regular care and maintenance of a zebrafish (*Danio rerio*) laboratory: an introduction. *Journal of Visualized Experiments* **69**, e4196.

Bass, S. L. & Gerlai, R. (2008). Zebrafish (*Danio rerio*) responds differentially to stimulus fish: the effects of sympatric and allopatric predators and harmless fish. *Behavioural Brain Research* **186,** 107–117.

Biro, P. A. & Stamps, J. A. (2008). Are animal personality traits linked to life-history productivity? *Trends in Ecology & Evolution* **23,** 361–368.

Biro, P. A. & Stamps, J. A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends in Ecology & Evolution* **25,** 653–659.

Biro, P. A., Post, J. R. & Abrahams, M. V. (2005). Ontogeny of energy allocation reveals selective pressure promoting risk-taking behaviour in young fish cohorts. *Proceedings of the Royal Society B* **272,** 1443–1448.

Bouwhuis, S., Quinn, J. L., Sheldon, B. C. & Verhulst, S. (2014). Personality and basal metabolic rate in a wild bird population. *Oikos* **123,** 56–62.

Brown, C. & Braithwaite, V. A. (2004). Size matters: a test of boldness in eight populations of the poeciliid *Brachyraphis episcopi*. *Animal Behaviour* **68,** 1325–1329.

Brown, C., Jones, F. & Braithwaite, V. A. (2007). Correlation between boldness and body mass in natural populations of the poeciliid *Brachyrhaphis episcopi*. *Journal of Fish Biology* **71,** 1590–1601.

Burns, J. G. (2008). The validity of three tests of temperament in guppies (*Poecilia reticulata*). *Journal of Comparative Psychology* **122,** 344.

Buskirk, J. V. (2012). Behavioural plasticity and environmental change. In *Behavioural Responses to a Changing World: Mechanisms and Consequences* (Candolin, U. & Wong, B. B. M., eds), pp. 145–158. Oxford: Oxford University Press.

Cahill, G. M. (2002). Clock mechanisms in zebrafish. *Cell and Tissue Research* **309,** 27–34.

Carere, C. & Maestripieri, D. (2013). *Animal Personalities: Behavior, Physiology, and Evolution*. Chicago, IL: University of Chicago Press.

Carter, A. J., Feeney, W. E., Marshall, H. H., Cowlishaw, G. & Heinsohn, R. (2013). Animal personality: what are behavioural ecologists measuring? *Biological Reviews* **88,** 465–475.

Clark, C. W. (1994). Antipredator behavior and the asset-protection principle. *Behavioral Ecology* **5,** 159–170.

Clarke, A. & Johnston, N. M. (1999). Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology* **68,** 893–905.

Dahlbom, S. J., Lagman, D., Lundstedt-Enkel, K., Sundström, L. F. & Winberg, S. (2011). Boldness predicts social status in zebrafish (*Danio rerio*). *PLoS One* **6,** e23565.

Dial, K. P., Greene, E. & Irschick, D. J. (2008). Allometry of behavior. *Trends in Ecology & Evolution* **23,** 394–401.

Dingemanse, N. J. & Dochtermann, N. A. (2013). Quantifying individual variation in behaviour: mixed-effect modelling approaches. *Journal of Animal Ecology* **82,** 39–54.

Dupont-Prinet, A., Chatain, B., Grima, L., Vandeputte, M., Claireaux, G. & McKenzie, D. J. (2010). Physiological mechanisms underlying a trade-off between growth rate and tolerance of feed deprivation in the European sea bass (*Dicentrarchus labrax*). *The Journal of Experimental Biology* **213,** 1143–1152.

Dwyer, G. K., Stoffels, R. J. & Pridmore, P. A. (2014). Morphology, metabolism and behaviour: responses of three fishes with different lifestyles to acute hypoxia. *Freshwater Biology* **59,** 819–831.

Filby, A. L., Paull, G. C., Bartlett, E. J., Van Look, K. J. & Tyler, C. R. (2010). Physiological and health consequences of social status in zebrafish (*Danio rerio*). *Physiology & Behavior* **101,** 576–587.

Gerlai, R. (2010). Zebrafish antipredatory responses: a future for translational research? *Behavioural Brain Research* **207,** 223–231.

Grant, J. W. (1990). Aggressiveness and the foraging behaviour of young-of-the-year brook charr (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences* **47,** 915–920.

Guenther, A. & Trillmich, F. (2015). Within-litter differences in personality and physiology relate to size differences among siblings in cavies. *Physiology & Behavior* **145,** 22–28.

Guenther, A., Finkemeier, M. A. & Trillmich, F. (2014). The ontogeny of personality in the wild guinea pig. *Animal Behaviour* **90,** 131–139.

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *Journal of Statistical Software* **33,** 1–22.

Healy, K., McNally, L., Ruxton, G. D., Cooper, N. & Jackson, A. L. (2013). Metabolic rate and body size are linked with perception of temporal information. *Animal Behaviour* **86,** 685–696.

Johansson, J., Turesson, H. & Persson, A. (2004). Active selection for large guppies, *Poecilia reticulata*, by the pike cichlid, *Crenicichla saxatilis*. *Oikos* **105,** 595–605.

Johnsson, J. I. (1993). Big and brave: size selection affects foraging under risk of predation in juvenile rainbow trout, *Oncorhynchus mykiss*. *Animal Behaviour* **45,** 1219–1225.

Killen, S. S., Atkinson, D. & Glazier, D. S. (2010). The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. *Ecology Letters* **13,** 184–193.

Killen, S. S., Marras, S. & McKenzie, D. J. (2011). Fuel, fasting, fear: routine metabolic rate and food deprivation exert synergistic effects on risk-taking in individual juvenile European sea bass. *Journal of Animal Ecology* **80,** 1024–1033.

Killen, S. S., Marras, S., Ryan, M. R., Domenici, P. & McKenzie, D. J. (2012). A relationship between metabolic rate and risk-taking behaviour is revealed during hypoxia in juvenile European sea bass. *Functional Ecology* **26,** 134–143.

Killen, S. S., Marras, S., Metcalfe, N. B., McKenzie, D. J. & Domenici, P. (2013). Environmental stressors alter relationships between physiology and behaviour. *Trends in Ecology & Evolution* **28,** 651–658.

Killen, S. S., Mitchell, M. D., Rummer, J. L., Chivers, D. P., Ferrari, M. C., Meekan, M. G. & McCormick, M. I. (2014). Aerobic scope predicts dominance during early life in a tropical damselfish. *Functional Ecology* **28,** 1367–1376.

Klefoth, T., Skov, C., Aarestrup, K. & Arlinghaus, R. (2013). Reliability of non-lethal assessment methods of body composition and energetic status exemplified by applications to eel (*Anguilla anguilla*) and carp (*Cyprinus carpio*). *Fisheries Research* **146,** 18–26.

Kobler, A., Klefoth, T., Wolter, C., Fredrich, F. & Arlinghaus, R. (2008). Contrasting pike (*Esox lucius* L.) movement and habitat choice between summer and winter in a small lake. *Hydrobiologia* **601,** 17–27.

Kopman, V., Laut, J., Polverino, G. & Porfiri, M. (2013). Closed-loop control of zebrafish response using a bioinspired robotic-fish in a preference test. *Journal of the Royal Society Interface* **10,** 20120540.

Krause, J., Loader, S. P., McDermott, J. & Ruxton, G. D. (1998). Refuge use by fish as a function of body length-related metabolic expenditure and predation risks. *Proceedings of the Royal Society of London B* **265,** 2373–2379.

Ladu, F., Bartolini, T., Panitz, S. G., Chiarotti, F., Butail, S., Macrì, S. & Porfiri, M. (2015). Live predators, robots, and computer-animated images elicit differential avoidance responses in zebrafish. *Zebrafish* **12,** 205–214.

Laland, K. N. & Reader, S. M. (1999). Foraging innovation in the guppy. *Animal Behaviour* **57,** 331–340.

Landsman, S. J., Martins, E. G., Gutowsky, L. F., Suski, C. D., Arlinghaus, R. & Cooke, S. J. (2015). Locomotor activity patterns of muskellunge (*Esox masquinongy*) assessed using tri-axial acceleration sensing acoustic transmitters. *Environmental Biology of Fishes* **98,** 2109–2121.

Lorenzen, K. (2000). Allometry of natural mortality as a basis for assessing optimal release size in fish-stocking programmes. *Canadian Journal of Fisheries and Aquatic Sciences* **57,** 2374–2381.

Lucas, J., Schouman, A., Lyphout, L., Cousin, X. & Lefrancois, C. (2014). Allometric relationship between body mass and aerobic metabolism in zebrafish *Danio rerio*. *Journal of Fish Biology* **84,** 1171–1178.

Mathot, K. J. & Dingemanse, N. J. (2015). Energetics and behavior: unrequited needs and new directions. *Trends in Ecology & Evolution* **30,** 199–206.

Metcalfe, N. B. & Monaghan, P. (2001). Compensation for a bad start: grow now, pay later? *Trends in Ecology & Evolution* **16,** 254–260.

Metcalfe, N. B., Van Leeuwen, T. E. & Killen, S. S. (2016). Does individual variation in metabolic phenotype predict behaviour and performance in fish? *Journal of Fish Biology* **88,** 298–321.

Moretz, J. A., Martins, E. P. & Robison, B. D. (2007). Behavioral syndromes and the evolution of correlated behavior in zebrafish. *Behavioral Ecology* **18,** 556–562.

Morton, A. & Routledge, R. D. (2006). Fulton's condition factor: is it a valid measure of sea lice impact on juvenile salmon? *North American Journal of Fisheries Management* **26,** 56–62.

Nakagawa, S. & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biological Reviews* **85,** 935–956.

Nilsson, P. A. & Brönmark, C. (2000). Prey vulnerability to a gape-size limited predator: behavioural and morphological impacts on northern pike piscivory. *Oikos* **88,** 539–546.

Nussey, D. H., Wilson, A. J. & Brommer, J. E. (2007). The evolutionary ecology of individual phenotypic plasticity in wild populations. *Journal of Evolutionary Biology* **20,** 831–844.

Oikawa, S., Itazawa, Y. & Gotoh, M. (1991). Ontogenetic change in the relationship between metabolic rate and body mass in a sea bream *Pagrus major* (Temminck & Schlegel). *Journal of Fish Biology* **38,** 483–496.

Persson, L., De Roos, A. M., Claessen, D., Byström, P., Lövgren, J., Sjögren, S., Svanbäck, R., Wahlström, E. & Westman, E. (2003). Gigantic cannibals driving a whole-lake trophic cascade. *Proceedings of the National Academy of Sciences* **100,** 4035–4039.

Polverino, G., Abaid, N., Kopman, V., Macrì, S. & Porfiri, M. (2012). Zebrafish response to robotic fish: preference experiments on isolated individuals and small shoals. *Bioinspiration & Biomimetics* **7,** 036019.

Polverino, G., Ruberto, T., Staaks, G. & Mehner, T. (2016). Tank size alters mean behaviours and individual rank orders in personality traits of fish depending on their life stage. *Animal Behaviour* **115,** 127–135.

Pope, K. L. & Kruse, C. G. (2007). Condition. In *Analysis and Interpretation of Freshwater Fisheries Data* (Guy, C. S. & Brown, M. L., eds), pp. 423–471. Maryland, MD: American Fisheries Society.

Pruitt, J. N. & Riechert, S. E. (2011). How within-group behavioural variation and task efficiency enhance fitness in a social group. *Proceedings of the Royal Society B* **278,** 1209–1215.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T. & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews* **82,** 291–318.

Réale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V. & Montiglio, P. O. (2010). Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions of the Royal Society, B: Biological Sciences* **365,** 4051–4063.

Schmidt-Nielsen, K. (1984). *Scaling: Why Is Animal Size so Important?* Cambridge: Cambridge University Press.

Schurmann, H. & Steffensen, J. F. (1997). Effects of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. *Journal of Fish Biology* **50,** 1166–1180.

Seeman, T. E. & McEwen, B. S. (1996). Impact of social environment characteristics on neuroendocrine regulation. *Psychosomatic Medicine* **58,** 459–471.

Seppänen, E., Tiira, K., Huuskonen, H. & Piironen, J. (2009). Metabolic rate, growth and aggressiveness in three Atlantic salmon *Salmo salar* populations. *Journal of Fish Biology* **74,** 562–575.

Sih, A., Mathot, K. J., Moiron, M., Montiglio, P. O., Wolf, M. & Dingemanse, N. J. (2015). Animal personality and state-behaviour feedbacks: a review and guide for empiricists. *Trends in Ecology & Evolution* **30,** 50–60.

Sogard, S. M. (1997). Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bulletin of Marine Science* **60,** 1129–1157.

Spence, R., Gerlach, G., Lawrence, C. & Smith, C. (2008). The behaviour and ecology of the zebrafish, *Danio rerio*. *Biological Reviews* **83,** 13–34.

Steffensen, J. F. (1989). Some errors in respirometry of aquatic breathers: how to avoid and correct for them. *Fish Physiology and Biochemistry* **6,** 49–59.

Tran, S. & Gerlai, R. (2013). Individual differences in activity levels in zebrafish (*Danio rerio*). *Behavioural Brain Research* **257,** 224–229.

Uchmanski, J. (1985). Differentiation and frequency distributions of body weights in plants and animals. *Philosophical Transactions of the Royal Society B* **310,** 1–75.

Vaz-Serrano, J., Ruiz-Gomez, M. L., Gjøen, H. M., Skov, P. V., Huntingford, F. A., Øverli, Ø. & Höglund, E. (2011). Consistent boldness behaviour in early emerging fry of domesticated Atlantic salmon (*Salmo salar*): decoupling of behavioural and physiological traits of the proactive stress coping style. *Physiology & Behavior* **103,** 359–364.

Werner, E. E. & Anholt, B. R. (1993). Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *American Naturalist* **142,** 242–272.

Werner, E. E. & Hall, D. J. (1988). Ontogenetic habitat shifts in bluegill: the foraging rate-predation risk trade-off. *Ecology* **69,** 1352–1366.

White, C. R. & Kearney, M. R. (2013). Determinants of inter-specific variation in basal metabolic rate. *Journal of Comparative Physiology B* **183,** 1–26.

White, C. R. & Kearney, M. R. (2014). Metabolic scaling in animals: methods, empirical results, and theoretical explanations. *Comprehensive Physiology* **4,** 231–256.

Whiteley, A. R., Bhat, A., Martins, E. P., Mayden, R. L., Arunachalam, M., Uusi-Heikkilä, S., Ahmed, A. T. A., Shrestha, J., Clark, M., Stemple, D. & Bernatchez, L. (2011). Population genomics of wild and laboratory zebrafish (*Danio rerio*). *Molecular Ecology* **20,** 4259–4276.

Wolf, M. & Weissing, F. J. (2010). An explanatory framework for adaptive personality differences. *Philosophical Transactions of the Royal Society B* **365,** 3959–3968.

Wolf, M., van Doorn, G. S., Leimar, O. & Weissing, F. J. (2007). Life-history trade-offs favour the evolution of animal personalities. *Nature* **447,** 581–584.

Wootton, R. J. (1994). Energy allocation in the threespine stickle-back. In *The Evolutionary Biology of the Threespine Stickleback* (Bell, M. A. & Foster, S. A., eds), pp. 116–143. Oxford: Science Publications.

Wright, D. & Krause, J. (2006). Repeated measures of shoaling tendency in zebrafish (*Danio rerio*) and other small teleost fishes. *Nature Protocols* **1,** 1828–1831.

Electronic References

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2014). *lme4: Linear Mixed-effects Models Using Eigen and S4*. Vienna: R Foundation for Statistical Computing. Available at www.r-project.org.

Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. (2007). *Linear and Nonlinear Mixed Effects Models*. Vienna: R Foundation for Statistical Computing. Available at www.r-project.org.

Supporting Information

Table SI.

Distance moved				
Fixed factors	Estimate	*F*	d.f.$_2$, d.f.$_1$	*P*
L_T	-2.94	4.62	4,5	0.04
RMR	11.59	2.54	4,5	0.13
K	-21.46	0.37	4,5	0.60
Trial	-0.78	0.03	4,5	0.86
Random factor		*F*	d.f.$_2$, d.f.$_1$	*P*
Individual		-	6,7	<0.001
Variance components		V_{within} ± S.E.	V_{among} ± S.E.	Repeatability
L_T		710.9 ± 2.3	720.6 ± 2.3	0.50
RMR		710.9 ± 2.3	751.3 ± 2.3	0.51
K		710.9 ± 2.3	785.2 ± 2.4	0.53
L_T, RMR, K		710.9 ± 2.3	675.6 ± 2.2	**0.49**
Mean velocity				
Fixed factors	Estimate	*F*	d.f.$_2$, d.f.$_1$	*P*
L_T	-0.79	0.77	4,5	0.38
RMR	-0.16	<0.01	4,5	0.98
K	15.94	0.52	4,5	0.48
Trial	-5.17	1.58	4,5	0.21
Random factor		*F*	d.f.$_2$, d.f.$_1$	*P*
Individual		-	6,7	0.05
Variance components		V_{within} ± S.E.	V_{among} ± S.E.	Repeatability
L_T		575.6 ± 2.1	160.7 ± 1.1	0.22
RMR		575.6 ± 2.1	165.8 ± 1.1	0.22
K		575.6 ± 2.1	162.4 ± 1.1	0.22
L_T, RMR, K		575.6 ± 2.1	157.4 ± 1.1	**0.21**
Freezing				
Fixed factors	Estimate	*F*	d.f.$_2$, d.f.$_1$	*P*
L_T	7.89	6.79	4,5	0.01
RMR	-27.25	2.82	4,5	0.11
K	73.64	0.90	4,5	0.41
Trial	-2.67	0.08	4,5	0.78
Random factor		*F*	d.f.$_2$, d.f.$_1$	*P*
Individual		-	6,7	<0.001
Variance components		V_{within} ± S.E.	V_{among} ± S.E.	Repeatability
L_T		3112 ± 5	3770 ± 5	0.55
RMR		3112 ± 5	4058 ± 5	0.57
K		3112 ± 5	4209 ± 6	0.57
L_T, RMR, K		3112 ± 5	3487 ± 5	**0.53**

Swimming in open water				
Fixed factors	Estimate	*F*	d.f.$_2$, d.f.$_1$	*P*
L_T	-0.07	2.58	4,5	0.12
RMR	0.06	0.04	4,5	0.84
K	-1.51	1.84	4,5	0.19
Trial	<0.01	<0.01	4,5	0.97
Random factor		*F*	d.f.$_2$, d.f.$_1$	*P*
Individual		-	6,7	0.03
Variance components		V_{within} ± S.E.	V_{among} ± S.E.	Repeatability
L_T		1.32 ± 0.10	0.44 ± 0.06	0.25
RMR		1.32 ± 0.10	0.48 ± 0.06	0.27
K		1.32 ± 0.10	0.45 ± 0.06	0.25
L_T, RMR, *K*		1.32 ± 0.10	0.41 ± 0.05	**0.24**

Table SII.

Distance moved				
Fixed factors	Estimate	F	d.f.$_2$, d.f.$_1$	P
Body mass	-213.34	5.39	4,5	0.03
RMR	12.12	2.42	4,5	0.14
K	34.72	0.85	4,5	0.45
Trial	-0.78	0.03	4,5	0.86
Random factor		F	d.f.$_2$, d.f.$_1$	P
Individual		-	6,7	<0.001
Variance components		V_{within} ± S.E.	V_{among} ± S.E.	Repeatability
Body mass		710.9 ± 2.3	713.5 ± 2.3	0.50
RMR		710.9 ± 2.3	753.6 ± 2.4	0.51
K		710.9 ± 2.3	780.3 ± 2.4	0.52
Body mass, RMR, K		710.9 ± 2.3	660.4 ± 2.2	**0.48**
Mean velocity				
Fixed factors	Estimate	F	d.f.$_2$, d.f.$_1$	P
Body mass	-48.78	0.65	4,5	0.42
RMR	0.17	<0.01	4,5	0.92
K	31.67	1.64	4,5	0.20
Trial	-5.17	1.58	4,5	0.21
Random factor		F	d.f.$_2$, d.f.$_1$	P
Individual		-	6,7	0.05
Variance components		V_{within} ± S.E.	V_{among} ± S.E.	Repeatability
Body mass		575.6 ± 2.1	161.5 ± 1.1	0.22
RMR		575.6 ± 2.1	165.7 ± 1.1	0.22
K		575.6 ± 2.1	155.1 ± 1.1	0.21
Body mass, RMR, K		575.6 ± 2.1	150.9 ± 1.1	**0.21**
Freezing				
Fixed factors	Estimate	F	d.f.$_2$, d.f.$_1$	P
Body mass	588.11	8.40	4,5	<0.01
RMR	-28.49	2.71	4,5	0.13
K	-74.62	0.81	4,5	0.48
Trial	-2.67	0.08	4,5	0.78
Random factor		F	d.f.$_2$, d.f.$_1$	P
Individual		-	6,7	<0.001
Variance components		V_{within} ± S.E.	V_{among} ± S.E.	Repeatability
Body mass		3112 ± 5	3683 ± 5	0.54
RMR		3112 ± 5	4070 ± 5	0.57
K		3112 ± 5	4224 ± 6	0.58
Body mass, RMR, K		3112 ± 5	3399 ± 5	**0.52**
Swimming in open water				

Fixed factors	Estimate	*F*	d.f.$_2$, d.f.$_1$	*P*
Body mass	-7.34	6.21	4,5	0.01
RMR	-0.07	0.05	4,5	0.83
K	-0.07	<0.01	4,5	0.94
Trial	<0.01	<0.01	4,5	0.97
Random factor		*F*	d.f.$_2$, d.f.$_1$	*P*
Individual		-	6,7	0.03
Variance components		V_{within} ± S.E.	V_{among} ± S.E.	Repeatability
Body mass		1.32 ± 0.10	0.38 ± 0.05	0.23
RMR		1.32 ± 0.10	0.48 ± 0.06	0.27
K		1.32 ± 0.10	0.48 ± 0.06	0.27
Body mass, RMR, *K*		1.32 ± 0.10	0.38 ± 0.05	**0.22**

Journal of FISH BIOLOGY

fsbi An International Society for Fish Biology

Journal of Fish Biology (2016)
doi:10.1111/jfb.12933, available online at wileyonlinelibrary.com

Behaviour in a standardized assay, but not metabolic or growth rate, predicts behavioural variation in an adult aquatic top predator *Esox lucius* in the wild

K. L. Laskowski*†, C. T. Monk*, G. Polverino*, J. Alós*,
S. Nakayama*‡, G. Staaks*, T. Mehner* and R. Arlinghaus*‡

**Department of Biology & Ecology of Fishes, Leibniz-Institute for Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, 12587 Berlin, Germany and ‡Division of Integrative Fisheries Management, Department of Crop and Animal Sciences, Faculty of Life Sciences, Humboldt-Universität zu Berlin, Invalidenstrasse 42, 10115, Berlin, Germany*

(Received 3 July 2015, Accepted 27 January 2016)

This study tested for links among behaviour, state and life-history variables as predicted by the pace-of-life hypothesis in adult pike *Esox lucius*. First, a standardized open-field behavioural assay was developed to assess individual behaviour of wild-captured adult *E. lucius*. Behaviour within the standardized assay predicted swimming behaviour in the lake, providing an ecological validation of the assay. There was no relationship between standardized behaviour and any of the life-history and state variables, including metabolism, body condition, juvenile growth rate and adult growth rate in contrast to predictions from the pace-of-life hypothesis. This study demonstrates that it is possible to assess ecologically relevant behavioural variation in a large-bodied top predator using a standard open-field assay, but it is noteworthy that this standardized behaviour is not systematically related to standard metabolism or growth.

Key words: acoustic telemetry; behavioural type; *Esox lucius*; field validation; metabolism; pace-of-life.

INTRODUCTION

The presence of consistent among-individual differences in behaviour, or behavioural types, is now well documented across a wide range of taxa (Bell *et al.*, 2009). The ubiquity of consistent among-individual behavioural variation has led to a number of hypotheses about what might explain this limited behavioural plasticity in so many different animals (Dingemanse & Wolf, 2010). An increasingly popular, yet controversial (Adriaenssens & Johnsson, 2009; Niemelä *et al.*, 2013; Závorka *et al.*, 2015), hypothesis referred to as the ‘pace-of-life hypothesis’ predicts that variation in behavioural types is caused by variation in more slowly changing state variables, such as metabolic rate or body condition, which in turn consistently relates to life-history traits such as growth rate, reproductive effort and mortality rate (Biro & Stamps, 2008, 2010; Réale

---

†Author to whom correspondence should be addressed. Tel.: +49 30 64181 716; email: laskowski@igb-berlin.de

et al., 2010; Wolf & Weissing, 2010). In short, variation in state variables is predicted to couple different trait complexes together from metabolism to behaviour and life history leading to a suite of correlated traits at the individual level (Réale *et al.*, 2010). One prediction is that individuals with particularly high metabolic rates may need to be comparatively more active in order to find enough food to fuel their energy demands potentially also leading to faster growth rates when food is not limited. Another prediction is that individuals in poorer body condition are expected to engage in riskier behaviour to compensate for low energy reserves (Milinski, 1993). According to this hypothesis, an individual's metabolism may be a driver of many biological processes, including behavioural variation, which has found broad appeal in the recent literature (Brown *et al.*, 2004; Biro & Stamps, 2008; Burton *et al.*, 2011; Metcalfe *et al.*, 2016) in part because the metabolic theory is generalizable and not limited to one taxa or species of animal.

Teleosts have been popular models for investigating individual variation and co-variation in a number of traits including behaviour and physiology. A recent review by Metcalfe *et al.* (2016) summarizes how fish study systems have provided a fruitful testing ground for the pace-of-life hypothesis. For example, there is now firm evidence for a range of fishes, particularly salmonids, that metabolism is linked to some behaviours such as aggression or dominance (Metcalfe *et al.*, 1995; Cutts *et al.*, 1998; Sloat *et al.,* 2014; but Seppänen *et al.,* 2009 found no relationship between metabolism and aggression). Metcalfe *et al.* (2016), however, also point out that less is known about whether variation in state variables such as metabolic rate or body condition relate to other behaviours such as swimming performance in the wild, an ecologically relevant behaviour for most fishes (Wolter & Arlinghaus, 2003). Importantly, many of the studies that have investigated the links among metabolism, behaviour and life-history have been performed in juvenile animals (Nyqvist *et al.*, 2012, 2013; McGhee *et al.*, 2013). Recently, Tibblin *et al.* (2016) found that adult pike *Esox lucius* L. 1758 consistently differed in their migration timing and this was related to fitness in the wild; however, it is unknown whether and how this might relate to individual variation in metabolism. The lack of information on the links between metabolism and behaviour in adult fishes is of scientific importance because almost all fishes (Lorenzen, 2000), and especially *E. lucius* (Haugen *et al.*, 2007), are exposed to strong size-dependent mortality as juveniles. Given the fundamental growth–mortality trade-off governed by risk-taking behaviour (Stamps, 2007) surviving adult fishes most likely represent a non-random sub-set of the variation in behaviour and growth that was present at the early juvenile stages. Indeed, predation pressure is emerging as a key driver of individual behavioural variation in animals, and fishes in particular (Bell, 2005; Bell & Sih, 2007; Dingemanse *et al.*, 2007; Harris *et al.*, 2010; Archard & Braithwaite, 2011). It is therefore possible that the links among metabolism, behaviour and growth rate are more pronounced in adult fishes compared to juveniles.

The objectives of this study were two-fold: first, to develop an ecologically valid, logistically feasible and standardized assay to assess the behavioural variation present in an adult top predator fish, and second, to test whether variation in behaviour was related to variation in a number of physiological and life-history traits including metabolic rate, body condition and growth rate as predicted by the pace-of-life hypothesis (Biro & Stamps, 2010; Réale *et al.*, 2010). Tank-based behaviour was compared with behaviour in the wild using acoustic telemetry, thereby providing a test of ecological validation of standardized behavioural assays (Conrad *et al.*, 2011; Niemelä & Dingemanse, 2014). Adult *E. lucius* were used as a model, which is a large-bodied piscivorous fish that is an emerging model system for ecology and evolutionary biology (Forsman *et al.*, 2015). *Esox lucius* have previously been shown to exhibit behavioural types in the wild (Kobler *et al.*, 2009; Pieterek, 2014; Tibblin *et al.*, 2016). It was predicted that *E. lucius* with either high standard metabolic rates or low body condition would be more active and exploratory in a standardized behavioural assay and that this would also relate positively to both juvenile and adult growth rate. Tracking top aquatic predators such as *E. lucius* in the wild can be logistically difficult as it requires either extensive manual tracking or expensive automated tracking equipment. Thus, there is a need for rapid behavioural assays on wild-captured fishes that might quickly and easily assess natural variation in behaviour. To test whether the standardized behavioural assay was ecologically valid, acoustic transmitters were inserted in a sub-set of the experimental animals prior to release back into a wild, which allowed tracking of their swimming behaviour in a natural lake. *Esox lucius* is one of the most important target species for recreational fisheries around the globe and is subjected to intensive exploitation (Arlinghaus & Mehner, 2004; Arlinghaus *et al.*, 2009*a*; Crane *et al.*, 2015). Because a natural population of *E. lucius* was used in the present experiments, it was also possible to test whether capture technique predicted the behaviour of the *E. lucius* in order to disentangle whether behavioural types are a driver of the vulnerability of individual *E. lucius* to different gear types, such as angling and electrofishing (Wilson *et al.*, 1993; Biro & Dingemanse, 2009; Wilson *et al.*, 2015). Angling may select for bolder and more active individuals (Alós *et al.*, 2012; Klefoth *et al.*, 2013; Härkönen *et al.*, 2014), whereas a technique such as electrofishing may be selective for shyer, less active and slower growing fishes (Crane *et al.*, 2015) due to the confinement of electrofishing to the structured littoral zones, which serves as refuge for low active behavioural types in *E. lucius* (Kobler *et al.*, 2009).

MATERIALS AND METHODS

OVERVIEW

First, a newly developed standardized open-field behavioural assay was validated to confirm that it predicted natural behavioural variation of wild *E. lucius*. Second, it was tested whether behaviour in the open field was related to a set of physiological and life-history variables including standard metabolic rate, growth rate in both juvenile and adult life stages, body condition and capture technique (angling *v.* electrofishing). Briefly, *E. lucius* were captured with either rod-and-reel angling or electrofishing from the study lake, tested within the open-field arena on the same day, had their standard metabolic rate measured overnight and then released the next day. Each individual's body condition was estimated, and scale samples were obtained to estimate juvenile- and adult-life growth rates using validated methods (Pagel *et al.*, 2015). Acoustic transmitters were then inserted into a sub-set ($n = 6$) of *E. lucius* before release into the lake that was previously equipped with a validated automated acoustic telemetry array (Baktoft *et al.*, 2015) to test whether their behaviour in the open-field arena was related to their natural swimming behaviour in the lake. Invasive animal procedures were approved by the Landesamt für Umwelt, Gesundheit und Verbraucherschutz of Brandenburg Germany under the project 'Fishverhalten', project #23-2347-15-2010.

POPULATION CHARACTERISTICS

The study was conducted in Kleiner Döllnsee (25 ha surface area, mean depth 4·1 m, maximum depth 7·8 m), a natural, slightly eutrophic lake located in the north-eastern lowlands of Germany, *c.* 80 km northeast of Berlin (for full details of the study lake, see Klefoth *et al.*, 2008, 2011; Kobler *et al.*, 2009; Baktoft *et al.*, 2015). The experimental lake has no public access and hence no other fishing or artificial disturbance occurred on the lake during the study period. All *E. lucius* were captured and assayed from September to November 2012 to avoid any complicating effects of the breeding season in spring.

Two capture methods were used to catch *E. lucius*: angling and electrofishing. Angling was conducted using a variety of artificial lures and was performed opportunistically throughout the day during the entire study period (see details of angling sampling in Arlinghaus *et al.*, 2008; Kuparinen *et al.*, 2010). Electrofishing was performed using a DC electrofishing unit (Type EFGI 4000, 4 kW; Brettschneider Spezialelektronik; www.electric-fishing.de) with a 400 mm diameter ring anode and restricted to the reed-belt along the perimeter of the lake where it is most efficient as per Pagel *et al.* (2015). Electrofishing was performed once or twice a week during the study period on randomly selected days.

After capture, all *E. lucius* were immediately checked for a previous passive integrated transponder (PIT) tag from ongoing studies at the lake ($n = 17$ out of 60), and if untagged ($n = 43$ out of 60), a PIT tag was inserted in the dorsal musculature of the animal which is necessary for individual identification. The time and method of capture were recorded and the *E. lucius* was placed within the live well of a boat. Each *E. lucius* was kept in the live well until its behaviour was assayed in the open-field arena located at the field station next to the lake to avoid further handling-induced stress. All *E. lucius* were measured for their open-field behaviour on the same day as capture and were given at least 1 h rest in the live well before the assay began (range: 65–394 min, mean: 197 min). Pilot trials indicated that holding *E. lucius* outside of the lake in captivity prior to behavioural measurements resulted in excessive stress on the animals making results unreliable in agreement with previous research findings in adult wild captured *E. lucius* (Arlinghaus *et al.*, 2009*b*), hence *E. lucius* were only held in the live well (natural lake water) for a minimum amount of time.

OPEN-FIELD BEHAVIOURAL ASSAY

The behavioural protocol was designed to minimize the time each *E. lucius* spent in captivity both to minimize stress on the animals and to increase the number of animals to be assayed without large variation in ambient water temperature. The open-field arena was a large opaque fibreglass tank (3·3 m × 1·65 m × 1 m deep). The tank was located outside but within a black plastic tent to standardize conditions and minimize disturbance. Lighting was provided with overhead LED lights. Preliminary assays indicated that bright lighting eliminated any movement by *E. lucius*, so lighting was kept at the minimum (*c.* 6 lx) necessary for video observation. A video camera located over the arena recorded the behaviour of the individual after release. The tank was divided widthways into two equally sized sections: an open section and a covered section. The covered section contained nine artificial plants made out of black plastic to provide cover. The open section contained no plants or structures. A 0·55 m × 0·55 m grid drawn on the floor of the tank allowed the amount of movement each individual exhibited to be quantified (Fig. 1). The tank was filled with 30 cm of lake water of ambient temperature (mean: 11·5; range: 10–15° C over the duration of the study period) for the trials and was replaced in between each observation.

To begin an open-field assay, an individual *E. lucius* was removed from the live well and transferred to the open-field arena in a wet weigh sling (transfer time < 15 s). The *E. lucius* was immediately released into the centre of the arena between the open and covered sections and its behaviour was videotaped for the next 30 min. During the trial, four behavioural variables were measured: the proportion of time spent actively swimming (out of the 30 min trial), time spent in the covered section (s), swimming speed (squares min^{-1}) and the total number of jumps. Some *E. lucius* remained still without swimming or moving their fins for longer than 20 s at a time; therefore, the proportion of time each *E. lucius* spent actively swimming was recorded as a potential measure of exploration. Previous work has shown that *E. lucius* differ in their use of the littoral reed bed within Kleiner Döllnsee (Kobler *et al.,* 2009) so the amount of time spent

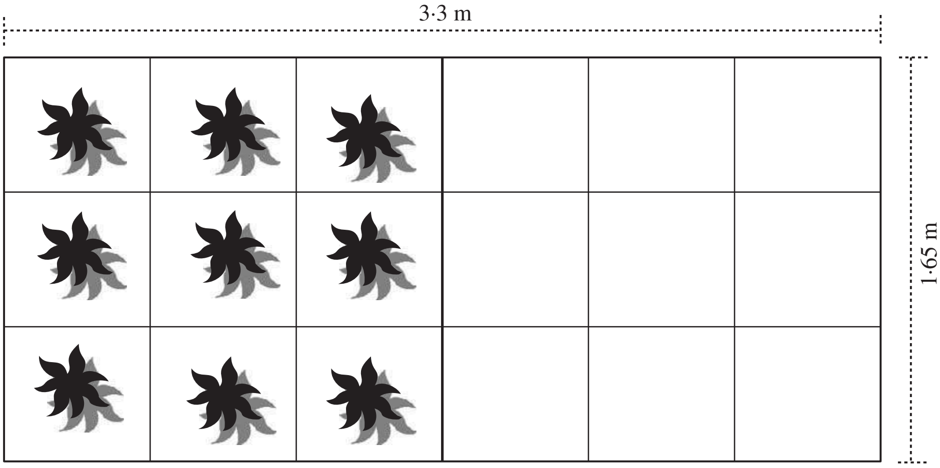


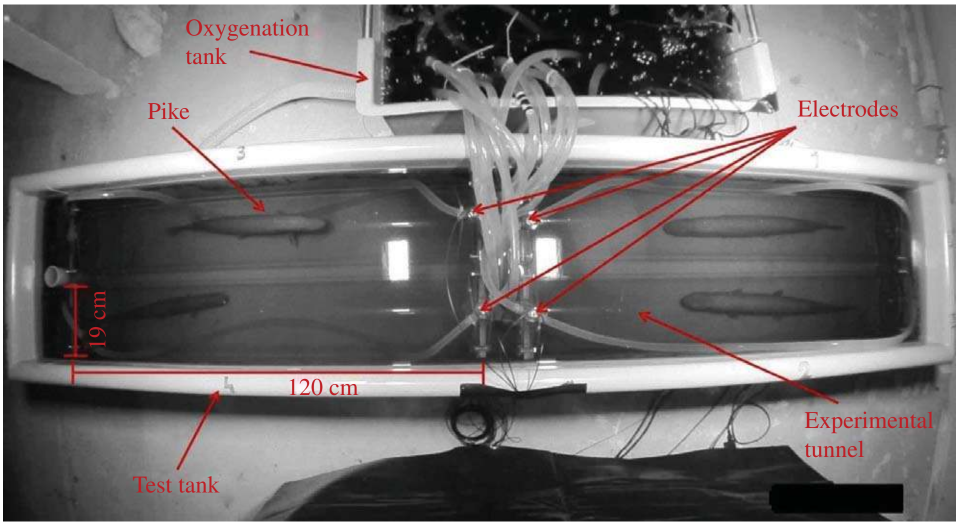
FIG. 1. Schematic of the open-field arena. The arena was divided width-ways into two equally sized areas: the covered section which contained nine artificial black plastic plants and the open section which contained no plants or structures. A grid was drawn on the floor to create nine equally sized grid-squares in each section. Lighting was provided by overhead LED lighting and an overhead camera recorded the trial. The plants are to signify the "nine artificial black plastic plants" that were located in the covered section of the open-field arena.

in the covered section was interpreted as being similar to reed bed use or use of underwater vegetation in the wild. Some *E. lucius* jumped out of the water while in the arena, which was interpreted as escape behaviour. After completion of the open-field assay, the individual was weighed to the nearest g and placed back into the live well where it remained until the evening when its metabolic rate was measured.

STANDARD METABOLIC RATE

The mass-specific standard metabolic rate (SMR, in mg O_2 kg^{-1} h^{-1}) was measured using the oxygen consumption method proposed by (Herskin, 1999). Briefly, it is based on 'computerized intermittent flow respirometry' (Loligo Systems; www.loligosystems.com) that allows repeated measurement of the oxygen consumption of a given individual during several hours in the night-time when this visual predator is often at rest (Steffensen *et al.*, 1984). Each *E. lucius* was transferred individually into 34 l glass experimental tunnels (Loligo Systems) for the metabolic measures the first night after its capture just before the sunset and remained there until the next morning. An array of four identical tunnels was used to perform measurements on up to four *E. lucius* simultaneously where each *E. lucius* was connected to an independent oxymeter sensor (Firesting 4-Channel oxygen meters; Pyroscience; www.pyro-science.com; Fig. 2). All individual tunnels were submersed in a test tank connected to a water bath (oxygen-saturated water) and were maintained at a fairly constant temperature of 11·5° C, range ± 1·5° C, which resembled current conditions in the lake. Each of the tunnels was connected to two submersible water pumps and a combination of fluxes allowed the water recirculation inside the tunnel for a known period of time to recover the saturated levels of oxygen. Specifically, the sequence of fluxes consisted of 210 s cycles formed by two phases: a 180 s phase of open re-circulation where the water inside the tunnel was replaced by water from the external reservoir to recover oxygen saturation inside the tunnel, and a 30 s phase of non-recirculation where the oxygen was consumed by the *E. lucius*. This cycle was repeated overnight and the concentration of oxygen (O_2 mg l^{-1}) inside the tunnel was monitored during the entire cycle (every s).

The oxygen consumption per cycle was determined using a custom routine in the R-package for identifying the starting and end point of every recirculation phase and to calculate the



[redacted]

FIG. 2. Set-up of the respirometer. The test tank was equipped with four experimental tunnels, each connected to an electrode and a closed-loop circulation system, and a valve to stabilize the water level. Oxygen concentration and temperature were controlled by using an oxygenation tank connected to the test tank. Two infrared lamps provided homogeneous illumination.

slope of the decrease in oxygen in the chamber for each specific cycle (Herskin, 1999). The oxygen consumption per cycle was calculated using the equation proposed by Herskin (1999), $V_{O_2} = V_T \left(\frac{dP_{O_2}}{dt}\right) \alpha W^{-1}$, where V_{O_2} is the mass-standardized oxygen consumption (mg O_2 kg^{-1} h^{-1}), V_T is the total volume of the chamber minus the volume of the *E. lucius* (*L*) estimated from the body mass, $\left(\frac{dP_{O_2}}{dt}\right)$ is the mean slope of the oxygen decrease during the recirculation phase (mmHg h^{-1}), α is the solubility of the oxygen for a given salinity and temperature [mg O_2 mmHg h^{-1} ($L\,H_2O$) $^{-1}$] and W is the mass of the individual (kg). Oxygen consumption was estimated each cycle when the r^2 of the slope of the regression assessing the decline in the oxygen in the chamber over time was >0·8. The distribution of oxygen consumption per individual was then used to estimate the mass-specific SMR for each individual.

The SMR is the amount of energy used by an animal in a post-absorptive inactive period, normally determined by the minimum value of oxygen consumption in an inactive period, after digestion and at a stable temperature (Burton *et al.,* 2011). The SMR can be altered by several factors such as spontaneous movements, stress or simply the individual circadian cycle. Herskin (1999) proposed that the distribution of the oxygen consumption per cycle for a given fish should, therefore, generate a bimodal distribution mainly composed of a combination of the oxygen consumptions from resting activity (lower mean) and spontaneous activity (larger mean). The function Mclust of the R-library mclust (Fraley & Raftery, 2002) was used to fit the data of each individual to a bimodal normal distribution to calculate the mean of the resting and spontaneous activity phases. The mean of the lower distribution was then used as the individual values of SMR (mg O_2 kg^{-1} h^{-1}) to test the relationship between the behavioural types, standard metabolic rate and growth rates in *E. lucius*.

SMR is closely related to body size and mass, and the mass-specific SMR is known to decline exponentially with size in *E. lucius* as in other fishes (Jobling, 1994). Hence, to be able to disentangle size effects on SMR from a potential relationship of SMR on behaviour, a size-independent measure of SMR was generated by regressing individual SMR on standard length (L_S) in a logarithmic scale and using the resulting residuals as a measure of size-corrected individual variation in SMR.

GROWTH RATE AND BODY CONDITION ESTIMATION

The morning following the metabolic measurements, the *E. lucius* were removed from the respirometer and anaesthetized using 1 ml l^{-1} 9:1 ethanol/clove oil solution in 50 l of lake-water as in previous work on *E. lucius* (Klefoth *et al.*, 2008). The sex (Casselman, 1974), total (L_T) and L_S of each *E. lucius* were recorded, and seven to 10 scales were removed dorsally to the lateral line to estimate juvenile and adult growth rates as per previously validated protocols (Pagel *et al.,* 2015).

To estimate growth rates, all scales collected from an individual were mounted on a slide and magnified using a Quick Scope vision measurement device (Quick Scope Manual; Mitutoyo; www.mitutoyo.co.jp/eng). For each individual, three scales were selected showing the clearest annuli, and the anterior distance was measured from the scale centre to each annulus and to the scale radius using the Quick Scope system. Results were averaged over the three scales. If there were fewer than three readable scales from an individual, that individual was excluded from the growth analysis. The Fraser-Lee equation (Francis, 1990) was used to backcalculate the size-at-age of each *E. lucius*, and the absolute increase in backcalculated size from age 1 to 2 years was used as a measurement of juvenile growth (Pagel *et al.*, 2015). The proportional increase in the backcalculated size during the last year (2011 annulus formation in spring minus 2012 annuli formation in spring) was used as a measurement of most recent adult growth rate. Experimental animals ranged from 24·6 to 70·4 cm L_S, and therefore the most recent adult growth might differ among animals based solely on age, given that growth rates decrease with age in fishes after maturation (Beverton & Holt, 1957; Lester *et al.*, 2004). Therefore, each individual's most recent adult growth rate was regressed on L_S and the resulting residuals were used as the measure of size-independent adult growth rate. No such regression was needed in the case of juvenile growth rate, which is linear in *E. lucius* and other temperate fishes (Lester *et al.,* 2004; Pagel *et al.,* 2015).

Finally, each individual's body condition was estimated using Fulton's K ($K = W L_T{}^{-3} \times 10^5$, where W is in g and L_T is in mm), which is appropriate for this within-population comparison (Pope & Kruse, 2007).

LAKE BEHAVIOURAL ASSAY: ACOUSTIC TAGGING

To test whether behaviour exhibited in the open-field arena was related to natural behaviour in the lake, acoustic transmitters were inserted into a sub-set ($n = 6$) of the experimental animals. These *E. lucius* were chosen in such a way as to minimize morphological differences among the individuals (in particular size because swimming activity is known to be positively related to size in *E. lucius*; Kobler *et al.,* 2008). Hence, all tagged *E. lucius* were adult females, appeared healthy and visibly free of parasites, were in a similar mass range (mean ± S.D = 1173 ± 260 g, range: 883–1449 g) and were similar in L_S (mean ± S.D. = 512 ± 37 cm, range 472–565 cm). The surgery was performed the morning following metabolic measurement while the *E. lucius* was anaesthetized following the methods of Lucas (1989) and Fredrich *et al.* (2003) which have been used previously for *E. lucius* in this study lake (Klefoth *et al.*, 2008; Kobler *et al.*, 2009). Briefly, the tag (MM-M-11-28-TP; burst rate = 9 s; Lotek Wireless Inc.; www.lotek.com) was inserted into the body cavity through a *c.* 5 cm incision. The incision was closed with three absorbable monofilament PDS-II sutures (size 3/0, FSL needle; Ethicon; www.ethicon.com). Total procedure time was less than 3 min. All animals were allowed to recover in a well-aerated, 250 l dark tank. After resuming normal swimming behaviour (<30 min), the *E. lucius* were released back into the lake at the point of capture. All animals were tagged within a 9 day period (21–30 October 2012). The Kleiner Döllnsee is equipped with an array of 20 wireless hydrophones (WHS 3050, Lotek Wireless Inc.) that covers the whole-lake providing an unprecedented level of detail and resolution (Baktoft *et al.,* 2015). The acoustic transmitters provided estimations of the position of each tagged individual with very high resolution of several fixes min^{-1} with data yields of over 40% and positional accuracy within 3·1 m when *E. lucius* was outside dense macrophytes (Baktoft *et al.*, 2015). From these position data, the daily swimming distance of each tagged individual was estimated.

STATISTICAL ANALYSES

The main research questions were to investigate the relationship between behaviour in the open field and natural swimming behaviour in adult *E. lucius* as a model of a top predatory freshwater fish species, and whether behavioural variation in the standardized open field was related to a number of potential state and life-history variables (SMR, juvenile and adult growth rates and body condition) and capture technique.

To summarize the behavioural variation exhibited in the open-field arena, a principal component analysis (PCA) was performed on the four behavioural variables (proportion of time spent swimming, time spent in the covered section, swimming speed and the total number of jumps). The first two principal components explained 81% of the behavioural variation and these PC scores were retained as a composite measure of each individual's open-field behaviour.

To determine the tagged individual's behaviour in the lake, the acoustic telemetry position data were extracted for a 30 day period starting 2 weeks after the final tagging to allow for surgery recovery as per previous work in *E. lucius* (Kobler *et al.,* 2009). All *E. lucius* were tagged during the fourth week of October and position data were collected for 30 days starting in the third week of November (11 November to 9 December 2012) to ensure that the animals experienced similar environmental conditions during the positioning period. From the position data, the daily swimming distance (km day^{-1}) was estimated. Using these daily measures, the repeatability of swimming behaviour was estimated to test whether there was evidence that the tagged *E. lucius* exhibited consistent individual differences in behaviour in the field (Dingemanse & Dochtermann, 2013). This was done using a linear mixed model with daily distance as the response variable. The number of observations per day, date and body size (L_S) were included as covariates, and individual was included as a random factor. The number of observations was included because when *E. lucius* is within the reed bed or dense macrophytes (which were rare in autumn in the study lake), successful detection by the hydrophones was difficult and the presence of missing data might artificially decrease the measurement of distance (Baktoft *et al.,* 2015). The resulting variance components (individual and residual variance) were used to estimate the proportion of variation attributable to individual differences in swimming activity (Nakagawa & Schielzeth, 2010). The significance of the individual effect was tested by comparing the log likelihoods of the full model described above to a model lacking the individual term (Zuur *et al.*, 2009). A significant repeatability estimate indicates that summarizing the daily behavioural measures provides a reasonable estimate of individual behaviour over time. The total swimming distance over the entire 30 day positioning period (11 November to 9 December 2012) was used to summarize the natural individual swimming behaviour of *E. lucius*. Because of the small sample size ($n = 6$), pair-wise Pearson's correlations were used to test for the relationship between behaviour in the lake (total swimming distance) and average behaviour in the open field (as estimated by PC1 and PC2).

To test whether behaviour in the open field was related to differences in state and life-history traits and capture technique, a linear model was used with the PC scores from the open-field arena as the response variable. The model included (size-independent) SMR, juvenile and (size-independent) adult growth rates, body condition (Fulton's K) and capture method as fixed effects of interest. L_S was included as an additional covariate given that length is known to influence the behaviour of adult *E. lucius* (Kobler *et al.*, 2008) and is also a strong correlate of age. Finally, time spent in the live well prior to the open-field assay was also included as holding time might affect the behaviour of the *E. lucius* due to captivity-induced physiological stress (Arlinghaus *et al.*, 2009*b*). Checks for co-linearity among the predictor variables were performed by estimating each predictor's variance inflation factor (F_{VI}): all F_{VI} were below three indicating it was appropriate to include them in the same model (Zuur *et al.,* 2009). In preliminary analyses, the possible effects of PIT-tagging (if the *E. lucius* was PIT-tagged from a previous study, or had a new PIT inserted immediately after capture), temperature in the arena and any differences in holding time between capture techniques were checked. None of these effects, however, had any influence on either behavioural axis, therefore in an effort to increase power, these effects were removed from the model (Zuur *et al.,* 2009). Separate models for the behavioural scores from PC 1 and 2 were performed. Finally, a linear model was used to test whether variation in size-independent SMR was predicted by sex, juvenile or adult growth rate, body condition and capture technique. W and L_S were not included in this model as the measure of SMR was already corrected for body size and standardized by mass. Models were then stepwise simplified by removing the least significant term and re-fitting the model (Zuur *et al.,* 2009).

All statistics were performed using R version 3.0.2 (R Core Team; www.r-project.org). PCA was performed using 'prcomp' in the 'stats' package. All linear models were performed with 'lme' using REML estimation in the 'nlme' package (Pinheiro *et al.*, 2013). A Gaussian error distribution was assumed, which was confirmed by visual inspection of the residuals and QQ plots (Zuur *et al.*, 2009).

RESULTS

A total of 60 adult *E. lucius* (43 females) were captured and assayed in the open-field arena. These *E. lucius* were caught using standard angling techniques (33) and electrofishing (27). *Esox lucius* were mean ± S.D. 42·6 ± 9·9 cm L_S (range: 24·6–70·4 cm) and 745·9 ± 523·7 g (range: 136–2498 g). Growth rates (juvenile and adult) were estimated for all *E. lucius*, and SMR was measured for 36 of these animals. In total, complete data (open-field behaviour, SMR and growth rates) were collected for 29 animals.

There was extensive variation in the behaviour exhibited in the open-field arena (Table I) and the PCA reduced the number of variables to two main principal components that were interpreted as measures of overall activity (54% of the total variation) and escape behaviour (27% of the total behavioural variance; Table I). There was also extensive variation in swimming behaviour in the lake upon the release of tagged *E. lucius*. Over the entire 30 day observation period, the six adult *E. lucius* swam mean ± S.D. 82·31 ± 36·44 km (range: 44·17–124·21 km) and daily swimming distance 3·07 ± 2·01 km (range: 1·83–4·44 km) was significantly repeatable over the sampling month (repeatability = 0·37, log likelihood = 42·30, $P < 0·001$) indicating that individual differences accounted for nearly 40% of the total behavioural variation. Importantly, despite the small sample size ($n = 6$), a significant negative correlation between total distance swam in the lake and activity (PC1) in the open-field arena was found [Fig. 3(a)]. Specifically, individuals that received higher activity scores in the open-field arena covered less distance over the month long sampling period in the lake. Additionally, there was a marginally significant correlation between escape behaviour (PC2) and total swimming distance with individuals that exhibited greater escape behaviour swimming further in the lake [Fig. 3(b)]. Altogether this indicates that an individual's behaviour in the open field provides some insight into that animal's swimming behaviour under natural conditions.

Contrary to the initial predictions, there was no relationship between behaviour in the open field and any of the state and life-history traits of metabolism, juvenile or adult growth rate or body condition (Table II). There was also no difference in open-field behaviour between *E. lucius* that were captured using angling or electrofishing (Table II). Finally, there was no effect of the additional covariates of body size, sex or time spent in captivity (Table II). Removing non-significant terms by model reduction did not change the results, therefore full models are reported (Table II).

There was no effect of sex, capture technique or juvenile growth rate on SMR, but there were significant effects of adult growth rate and body condition (Table II). Specifically, individuals that exhibited higher rates of adult growth also exhibited higher size-corrected SMR, while individuals in better body condition had lower SMR (Table II). Altogether these results suggest that while the state variables and

TABLE I. Mean ± S.D. values and principal component loadings of each behavioural measure of *Esox lucius* from the open-field arena assay

	Mean ± S.D.	PC1 loadings 'activity'	PC2 loadings 'escape behaviour'
Total time in cover (s)	1258 ± 505	−0·43	0·60
Swimming speed (squares min^{-1})	1·17 ± 1·10	0·60	−0·02
Total number of jumps	0·33 ± 0·83	0·29	0·80
Proportion time spent swimming	0·54 ± 0·38	0·60	−0·06
Eigenvalue	–	2·13	1·08
Total variance explained (%)	–	54	27

life-history traits were not related to the standardized behavioural measures in an open field, the state and life-history variables were coupled with each other.

DISCUSSION

There is considerable difficulty with measuring behaviour in large top predator species collected from the wild in a standardized fashion, which probably has contributed to the overall paucity of research on the personality (*i.e.* consistent individual differences in behaviour) (Tibblin *et al.*, 2016) and the relationship of metabolic phenotype and behaviour (Metcalfe *et al.,* 2016) in these animals. The present study demonstrated that a simple open-field arena can provide a rapid method to predict natural variation in adult *E. lucius* swimming behaviour in the wild. It is well argued on theoretical grounds that variation in behaviour may be a result of variation in underlying, slowly changing state variables, such as metabolism or body condition, and that behaviour should be related to life-history traits such as growth rate (Biro & Stamps, 2010; Réale *et al.*, 2010; Wolf & Weissing, 2010). Contrary to the predictions of the pace-of-life hypothesis, however, variation in adult *E. lucius* behaviour in the standardized open field was not explained by any of the predictor variables including mass-specific standard metabolic rate, juvenile and adult growth rates or body condition. Therefore, based on the data accumulated for the present work, there is no firm evidence for the pace-of-life hypothesis linking behaviour (in a standardized behavioural assay), metabolic physiology and life history in adult *E. lucius*.

Predation pressure has been implicated as driving correlations among different behaviours (*e.g.* boldness and aggression, leading to behavioural syndromes: Bell, 2005; Bell & Sih, 2007; Dingemanse *et al.*, 2007; Conrad *et al.*, 2011; Mittelbach *et al.*, 2014). Moreover, exposure of populations of fishes to environmental stressors has been found to strengthen correlations among metabolism and behaviour in several fish species (Killen *et al.*, 2013). Because all experimental animals were survivors of strong size-dependent predation in the juvenile stage due to cannibalism (Raat, 1988; Haugen *et al.*, 2007) and experimental procedures involving captivity are known to be perceived as stressful by *E. lucius* (Arlinghaus *et al.*, 2009*b*), it was initially expected to observe strong correlations among metabolism, behaviour and growth in adult *E. lucius*, which were not reported in juvenile *E. lucius* by other research groups (Nyqvist *et al.*, 2012, 2013). These expectations did not receive empirical support.

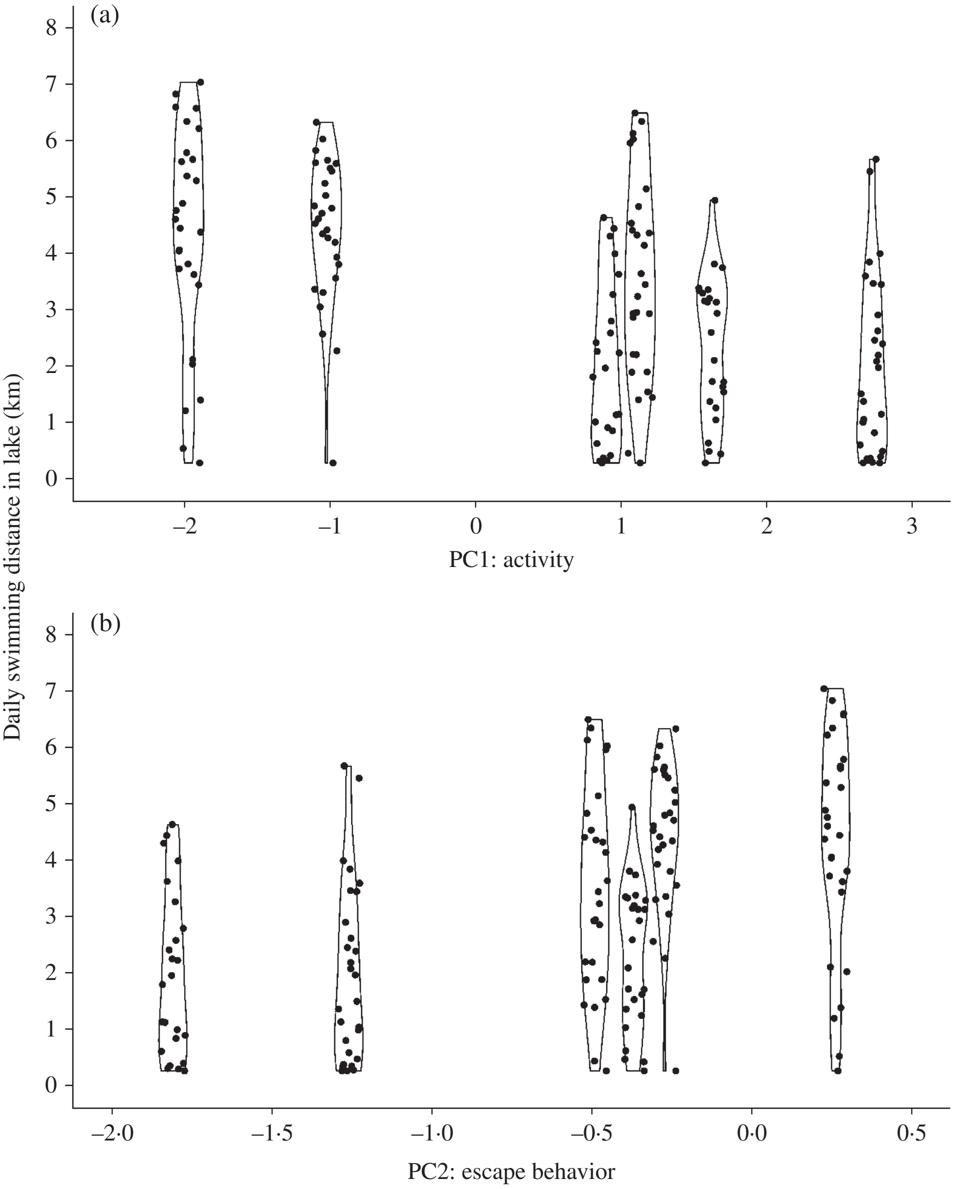


FIG. 3. Correlation between tagged *Esox lucius*'s total swimming behaviour in the lake and (a) their activity ($r = -0{\cdot}83$, $0{\cdot}05 > P > 0{\cdot}01$) and (b) escape behaviour ($r = 0{\cdot}80$, $0{\cdot}10 > P > 0{\cdot}05$) in the open field. Each violin represents one individual; data points within each violin are daily swimming distances.

Either correlations among metabolism, standardized behaviours and life-history do not exist in *E. lucius*, or available methods to assay standardized behaviour are not sufficient to reliably measure behavioural variation. It is also possible that once *E. lucius* mature to adults and are released from heavy predation pressure, correlations among behaviour and other traits weaken (Killen *et al.*, 2013); this would especially

TABLE II. Summary of fixed effects on behaviour of *Esox lucius* in the open-field arena and standard metabolic rate (SMR)

Effect	Estimate (95% C.I.)	t-value (d.f. = 1)	P-value
PC1*: 'activity' ($r^2 = 0{\cdot}06$, $F_{8,21} = 0{\cdot}36$, $P > 0{\cdot}05$)			
L_S (cm)	0·01 (−0·12, 0·10)	0·04	>0·05
Sex: male	−0·95 (−2·95, 1·28)	−0·91	>0·05
Holding time (min)	−0·001 (−0·01, 0·01)	−0·77	>0·05
Technique: electrofishing	1·12 (−0·95, 2·97)	1·16	>0·05
SMR	−0·88 (−6·56, 4·55)	−0·34	>0·05
Adult growth	2·26 (−3·22, 6·08)	0·78	>0·05
Juvenile growth	−0·10 (−0·31, 0·14)	−0·90	>0·05
Body condition	1·83 (−12·12, 15·89)	0·28	>0·05
PC2*: 'escape behaviour' ($r^2 = 0{\cdot}29$, $F_{8,21} = 1{\cdot}06$, $P > 0{\cdot}05$)			
L_S (cm)	0·05 (−0·03, 0·10)	1·22	>0·05
Sex: male	−0·54 (−1·79, 0·61)	−0·89	>0·05
Holding time (min)	0·001 (−0·003, 0·01)	0·56	>0·05
Technique: electrofishing	1·05 (−0·09, 2·13)	1·55	>0·05
SMR	−1·56 (−5·06, 1·27)	−1·01	>0·05
Adult growth	2·06 (−0·53, 4·77)	1·20	>0·05
Juvenile growth	−0·07 (−0·21, 0·04)	−1·06	>0·05
Body condition	−1·50 (−10·02, 5·93)	−0·38	>0·05
Standard metabolic rate†($r^2 = 0{\cdot}20$, $F_{2,27} = 3{\cdot}62$, $0{\cdot}05 > P > 0{\cdot}01$)			
Adult growth	0·22 (0·01, 0·43)	2·08	$0{\cdot}05 > P > 0{\cdot}01$
Body condition	−0·86 (−1·71, −0·01)	−2·07	$0{\cdot}05 > P > 0{\cdot}01$

L_S, standard length.

*For open-field behaviour (PC1 and PC2), no effects remained significant after model simplification so full models are reported.

†The initial model for SMR included sex, capture technique, adult growth rate, juvenile growth rate and body condition.

be the case if predation-induced behavioural syndromes are purely plastic rather than genetic in nature. Alternatively (or in addition), the heavy predation pressure as juveniles may be so strongly uni-directional that it removes so much variation so as to make any correlation difficult to uncover. Future studies investigating correlations among behaviour, metabolism and other life-history traits across the life span would help answer this question.

There are a number of factors that can be put forward to explain the lack of a relationship between metabolism, state, behaviour and life history in this population of *E. lucius*. As mentioned before, it may be that the pace-of-life syndrome is indeed weak, or even absent in adult *E. lucius*. Support for this assumption comes from two previous studies testing for the presence of behavioural syndromes (Nyqvist *et al.*, 2013) and the relationship between behaviour and body size and growth rate in a juvenile population of *E. lucius* (Nyqvist *et al.*, 2012). Collectively, both studies failed to find consistent evidence for behavioural syndromes in *E. lucius*, and there was no correlation of behaviour and juvenile growth rate, similar to the work presented here. This is in contrast to a more recent study that reported a relationship between foraging voracity of juvenile *E. lucius*, the size of the sensory organs related to vision and ventilation rate (as a surrogate for metabolic rate; McGhee *et al.*, 2013). Importantly, all the three studies cited above were conducted on juvenile *E. lucius* in the laboratory, highlighting the need for continued testing in adult animals, preferably using field validated behaviours. For example, Tibblin *et al.* (2015) found a relationship between juvenile growth rate and adult body size in migrating *E. lucius*, but how this relates to individual variation in behaviour or metabolism is unknown. In the pace-of-life literature, variables such as metabolism and body condition are often assumed to be fairly stable and slow-changing characteristics of animals (Nespolo & Franco, 2007), but more recent research has shown that metabolism may be more plastic than previously thought, and can change considerably and largely independent of mass and temperature, especially over longer periods of time (Seppänen *et al.*, 2010; Norin & Malte, 2011; DeLong *et al.*, 2014). A large plastic component to metabolism would be an obvious candidate explaining the lack of correlations of metabolic physiology and behaviour. It may also be that the metabolic rate measurements as well as the open-field assay conducted in the present study suffered from bias due to stress induced by capture and captivity (Arlinghaus *et al.*, 2009*b*). This could have altered the animals' metabolic rates and added noise to the behaviours so the measurements were no longer representative of the undisturbed metabolic rate and behaviour of an individual. Working with wild-caught animals also prevented standardizing time since last meal which could be influential on metabolic rates (Jobling, 1994). The relationship that was found between SMR and growth rate and body condition in these animals, however, suggests that the potential stress did not completely remove the signal of the individual's normal metabolic rate. Another possibility is that rather than metabolic rate driving, or causing variation in behaviour, metabolism may simply co-vary with behaviour as a result of links to other variables such as food intake (Glazier, 2015). Finally, it is possible that the open-field arena which is designed to represent a novel and potentially risky situation (Réale *et al.*, 2007) for the animal failed to elicit the most relevant behavioural variation for testing the pace-of-life hypothesis (Beckmann & Biro, 2013; Näslund *et al.*, 2015). Although risk-taking behaviours are predicted to be especially tightly associated with the pace-of-life syndrome, so are other behaviours such as aggression, dispersal and activity (Wolf *et al.*, 2007; Réale *et al.*, 2010). Therefore, the possibility remains that measuring more or different behaviours would have elucidated a link.

Despite the limited number of tagged individuals released into the wild, an individual's behaviour in the open-field arena was predictive of their future swimming behaviour in the lake. The direction of that relationship was on first impression counterintuitive: it was the least active or exploratory individuals in the open field that were more likely to cover greater distance in the lake. The relationship between the open-field behaviour and swimming behaviour, however, is consistent with variation in 'coping styles', which characterize an individual's response to stressful situations (Koolhaas *et al.*, 1999). Proactive individuals actively attempt to reduce or avoid stress (*e.g.* by escaping the stressful situation leading to high scores on the escape PC2 axis) and are generally considered to be superficial and cursory explorers in novel situations (leading to low scores on the activity PC1 axis). By contrast, reactive individuals respond to stress more passively and are active and thorough explorers in novel situations (Benus *et al.*, 1989, 1990; Réale *et al.*, 2000). Therefore in the lake, it appears that *E. lucius* that exhibit a more proactive coping style are the most active individuals. Active swimming in a lake outside refuges is potentially risky for *E. lucius* because even in large individuals there is the chance of attacks by cannibalistic *E. lucius*, fish-eating birds or other predators, *e.g.* European catfish *Silurus glanis* L. 1758, which are present in Kleiner Döllnsee. Hence, this study suggests that proactive *E. lucius* are also bolder. The present finding agrees well with a previous study on this particular population of *E. lucius* that found evidence for distinct behavioural types (Kobler *et al.*, 2009). Kobler *et al.* (2009) manually tracked radio-tagged *E. lucius* in Kleiner Döllnsee and found three distinct classes of individuals which utilized different parts of the available habitat: some individuals mostly occupied the emergent-reed belt of the lake, others mostly the area of submerged macrophytes and still others were opportunistic, switching between habitats regularly and being generally most active. Thus, it is possible that the most active *E. lucius* tagged here may have been following a similar opportunistic strategy, and that these *E. lucius* were the proactive individuals in the standardized behavioural assay. It should be noted, however, that the *E. lucius* tagged in this study were all relatively large, healthy, adult females representing a (necessarily) biased sample of the population. Investigating sex and age-related differences in field swimming behaviour would be a promising avenue for future work.

It is important to note that although this study failed to relate metabolism to behaviour, a link between metabolic phenotype and life-history productivity was revealed as predicted from theory (Biro & Stamps, 2008). Specifically, it was found that *E. lucius* with higher residual standard metabolic rates also exhibited faster adult growth rate. *Esox lucius* that had higher metabolic rates were also in lower body condition, however, suggesting that food may be limited in the lake and these animals cannot consistently meet their energy demands. Fast growth in a cannibalistic species such as *E. lucius* probably requires taking risks, such as engaging in an active swimming hunting strategy rather than purely in a sit-and-wait foraging strategy. Indeed, although not significant due to a small sample size, Kobler *et al.* (2009) reported that the more active habitat opportunistic adult *E. lucius* grew somewhat faster than the less explorative behavioural types. Other studies have found that angled *E. lucius* were also more likely to exhibit faster growth rates than those fished by electrofishing (Crane *et al.*, 2015) and to be more active and have larger home ranges (Pieterek, 2014). Therefore, it seems likely that fast-growing *E. lucius* vulnerable to angling gear probably encompass the bolder, more active and maybe more aggressive behavioural phenotypes, which have been linked to metabolism in several fish species (Metcalfe *et al.,* 2016). In the present study, however, there was no influence of metabolic phenotype or adult or juvenile growth rate on capture technique (data not shown). In addition, the sample size of tagged *E. lucius* was too small in this study to reliably test the relationship of all predictor variables to swimming behaviour in the wild, but the relationship between metabolic phenotype and adult growth rate and body condition found here suggests that links among metabolism, behaviour and life-history may be present in the wild. Further studies with larger samples sizes are needed to sort this issue out.

Logistical and ethical concerns prevent many researchers from maintaining large wild animals in captivity for extensive periods of time, increasing the need for rapid and relevant behavioural assays that can predict natural variation in other traits, including behaviour. The present work suggests that open-field assays may be a useful tool for rapidly assessing ecologically relevant behavioural variation in top predators. The initially counterintuitive direction of the relationship (least active *E. lucius* in the open field were the most active in the field) urges caution about assuming a one-to-one relationship between laboratory and field behaviours. For example, Klefoth *et al.* (2012) found no relationship between carp *Cyprinus carpio* L. 1758 behaviour in a laboratory and in a field setting in ponds, unless the *C. carpio* were exposed to simulated predation pressure in the laboratory. Standardized behavioural assays are also desirable if they can provide insight into variation in other traits, such as growth rates, or survival, however sometimes the relationships that are revealed are complex or unexpected. Adriaenssens & Johnsson (2011) reported that in juvenile resident brown trout *Salmo trutta* L. 1758, it was the least exploratory individuals that exhibited the fastest growth rates in the field; a result counter to their initial predictions. Závorka *et al.* (2015) validated that while laboratory and field-based personality-related behaviours are related in *S. trutta*, personality did not predict variation in growth rate in the wild, similar to the findings presented here for *E. lucius*. They hypothesized that resource availability may play a key role in mitigating the relationship between behaviour and growth rate, where low resource availability can limit phenotypic variation in growth rates and override any intrinsic relationship between behaviour and growth rate. Given the poor condition of the individuals with higher SMR in this study, such ecological effects may also have been at play in the current investigation. In general, there remains the need for continued validations of laboratory-assayed behaviours (Niemelä & Dingemanse, 2014) because while laboratory and field behaviour are sometimes correlated (Fraser *et al.*, 2001; Chapman *et al.*, 2011; White *et al.*, 2013), this is certainly not always the case (Klefoth *et al.*, 2012; Laskowski *et al.*, 2015).

In conclusion, the present work demonstrates that adult *E. lucius*, a keystone top predator of freshwater and brackish ecosystems in the northern hemisphere, exhibit consistent individual differences in natural swimming behaviour and that variation in this behaviour may be related to proactive and reactive stress coping styles as assessed in an open-field behavioural assay. Moreover, it was found that metabolism was related to body condition and adult growth in the wild, which very likely has an underlying behavioural component. There was no evidence, however, for a relationship between laboratory-based behaviour and metabolism, body condition and juvenile and adult growth rates demonstrating the continued need for investigations to understand the basis for individual variation in behaviour in this species and improvement in the architecture of the open-field assay or variants thereof. An alternative approach is to avoid running fishes through an experimental tank and instead measure metabolism on a large sample of wild captured fishes prior to release into the wild and assess the relationship of physiology, behaviour and fitness in its natural habitat to add ecological realism. There is now the tracking technology in place to 'mine the reality' of naturally thriving adult top predatory fishes (Krause *et al.*, 2013; Baktoft *et al.*, 2015) but this comes at the expense of losing the benefit of standardization and test–retest opportunities common in the personality literature in the laboratory. Hence, there is an unavoidable trade-off in adult wild fishes and a key decision to be made among the desire to control the test environment to assess personality of large bodied adult fishes collected from the wild and the analysis of unconstrained behaviour in the wild.

The authors would like to thank A. Türck, T. Klefoth and D. Hühn for valuable technical assistance and also thank B. Adriaenssens for valuable comments on an earlier version of this manuscript. This work was supported through the Leibniz Gemeinschaft ('B-types' SAW-2013-IGB-2).

References

Adriaenssens, B. & Johnsson, J. I. (2009). Personality and life-history productivity: consistent or variable association? *Trends in Ecology and Evolution* **24,** 179–180.

Adriaenssens, B. & Johnsson, J. I. (2011). Shy trout grow faster: exploring links between personality and fitness-related traits in the wild. *Behavioral Ecology* **22,** 135–143.

Alós, J., Palmer, M. & Arlinghaus, R. (2012). Consistent selection towards low activity phenotypes when catchability depends on encounters among human predators and fish. *PLoS One* **7,** e48030.

Archard, G. & Braithwaite, V. (2011). Increased exposure to predators increases both exploration and activity level in *Brachyrhaphis episcopi*. *Journal of Fish Biology* **78,** 593–601.

Arlinghaus, R. & Mehner, T. (2004). A management-orientated comparative analysis of urban and rural anglers living in a metropolis (Berlin, Germany). *Environmental Management* **33,** 331–344.

Arlinghaus, R., Klefoth, T., Kobler, A. & Cooke, S. J. (2008). Size selectivity, injury, handling time, and determinants of initial hooking mortality in recreational angling for northern pike: the influence of type and size of bait. *North American Journal of Fisheries Management* **28,** 123–134.

Arlinghaus, R., Matsumura, S. & Dieckmann, U. (2009a). Quantifying selection differentials caused by recreational fishing: development of modeling framework and application to reproductive investment in pike (*Esox lucius*). *Evolutionary Applications* **2,** 335–355.

Arlinghaus, R., Klefoth, T., Cooke, S. J., Gingerich, A. & Suski, C. (2009b). Physiological and behavioural consequences of catch-and-release angling on northern pike (*Esox lucius* L.) *Fisheries Research* **97,** 223–233.

Baktoft, H., Zajicek, P., Klefoth, T., Svendsen, J. C., Jacobsen, L., Pedersen, M. W., Morla, D. M., Skov, C., Nakayama, S. & Arlinghaus, R. (2015). Performance assessment of two whole-lake acoustic positional telemetry systems-is reality mining of free-ranging aquatic animals technologically possible? *PLoS One* **10,** e0126534.

Beckmann, C. & Biro, P. A. (2013). On the validity of a single (boldness) assay in personality research. *Ethology* **119,** 937–947.

Bell, A. (2005). Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*). *Journal of Evolutionary Biology* **18,** 464–473.

Bell, A. M. & Sih, A. (2007). Exposure to predation generates personality in threespined sticklebacks *(Gasterosteus aculeatus*). *Ecology Letters* **10,** 828–834.

Bell, A. M., Hankison, S. J. & Laskowski, K. L. (2009). The repeatability of behaviour: a meta-analysis. *Animal Behaviour* **77,** 771–783.

Benus, R., Bohus, B., Koolhaas, J. & Van Oortmerssen, G. (1989). Behavioural strategies of aggressive and non-aggressive male mice in active shock avoidance. *Behavioural Processes* **20,** 1–12.

Benus, R. F., den Daas, S., Koolhaas, J. M. & van Oortmerssen, G. A. (1990). Routine formation and flexibility in social and non-social behaviour of aggressive and non-aggressive male mice. *Behaviour* **112,** 176–193.

Beverton, R. J. & Holt, S. J. (1957). *On the Dynamics of Exploited Fish Populations*. London: Chapman & Hall.

Biro, P. A. & Dingemanse, N. J. (2009). Sampling bias resulting from animal personality. *Trends in Ecology and Evolution* **24,** 66–67.

Biro, P. A. & Stamps, J. A. (2008). Are animal personality traits linked to life-history productivity? *Trends in Ecology and Evolution* **23,** 361–368.

Biro, P. A. & Stamps, J. A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends in Ecology and Evolution* **25,** 653–659.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M. & West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology* **85,** 1771–1789.

Burton, T., Killen, S., Armstrong, J. & Metcalfe, N. (2011). What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings of the Royal Society B* **278,** 3465–3473.

Casselman, J. M. (1974). External sex determination of northern pike, *Esox lucius* Linnaeus. *Transactions of the American Fisheries Society* **103,** 343–347.

Chapman, B. B., Hulthén, K., Blomqvist, D. R., Hansson, L. A., Nilsson, J. Å., Brodersen, J., Anders Nilsson, P., Skov, C. & Brönmark, C. (2011). To boldly go: individual differences in boldness influence migratory tendency. *Ecology Letters* **14,** 871–876.

Conrad, J. L., Weinersmith, K. L., Brodin, T., Saltz, J. & Sih, A. (2011). Behavioural syndromes in fishes: a review with implications for ecology and fisheries management. *Journal of Fish Biology* **78,** 395–435.

Crane, D. P., Miller, L. M., Diana, J. S., Casselman, J. M., Farrell, J. M., Kapuscinski, K. L. & Nohner, J. K. (2015). Muskellunge and northern pike ecology and management: important issues and research needs. *Fisheries* **40,** 258–267.

Cutts, C., Betcalfe, N. & Caylor, A. (1998). Aggression and growth depression in juvenile Atlantic salmon: the consequences of individual variation in standard metabolic rate. *Journal of Fish Biology* **52,** 1026–1037.

DeLong, J. P., Hanley, T. C. & Vasseur, D. A. (2014). Competition and the density dependence of metabolic rates. *Journal of Animal Ecology* **83,** 51–58.

Dingemanse, N. J. & Dochtermann, N. A. (2013). Quantifying individual variation in behaviour: mixed-effect modelling approaches. *Journal of Animal Ecology* **82,** 39–54.

Dingemanse, N. J. & Wolf, M. (2010). Recent models for adaptive personality differences: a review. *Philosophical Transactions of the Royal Society B* **365,** 3947–3958.

Dingemanse, N. J., Wright, J., Kazem, A. J., Thomas, D. K., Hickling, R. & Dawnay, N. (2007). Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. *Journal of Animal Ecology* **76,** 1128–1138.

Forsman, A., Tibblin, P., Berggren, H., Nordahl, O., Koch-Schmidt, P. & Larsson, P. (2015). Northern pike *Esox lucius* as an emerging model organism for studies in ecology and evolutionary biology: a review. *Journal of Fish Biology* **87,** 472–479.

Fraley, C. & Raftery, A. E. (2002). Model-based clustering, discriminant analysis, and density estimation. *Journal of the American Statistical Association* **97,** 611–631.

Francis, R. (1990). Back-calculation of fish length: a critical review. *Journal of Fish Biology* **36,** 883–902.

Fraser, D. F., Gilliam, J. F., Daley, M. J., Le, A. N. & Skalski, G. T. (2001). Explaining leptokurtic movement distributions: intrapopulation variation in boldness and exploration. *American Naturalist* **158,** 124–135.

Fredrich, F., Ohmann, S., Curio, B. & Kirschbaum, F. (2003). Spawning migrations of the chub in the River Spree, Germany. *Journal of Fish Biology* **63,** 710–723.

Glazier, D. S. (2015). Is metabolic rate a universal 'pacemaker' for biological processes? *Biological Reviews* **90,** 377–407.

Härkönen, L., Hyvärinen, P., Paappanen, J. & Vainikka, A. (2014). Explorative behavior increases vulnerability to angling in hatchery-reared brown trout (*Salmo trutta*). *Canadian Journal of Fisheries and Aquatic Sciences* **71,** 1900–1909.

Harris, S., Ramnarine, I. W., Smith, H. G. & Pettersson, L. B. (2010). Picking personalities apart: estimating the influence of predation, sex and body size on boldness in the guppy *Poecilia reticulata*. *Oikos* **119,** 1711–1718.

Haugen, T. O., Winfield, I. J., Vøllestad, L. A., Fletcher, J. M., James, J. B. & Stenseth, N. C. (2007). Density dependence and density independence in the demography and dispersal of pike over four decades. *Ecological Monographs* **77,** 483–502.

Herskin, J. (1999). Effects of social and visual contact on the oxygen consumption of juvenile sea bass measured by computerized intermittent respirometry. *Journal of Fish Biology* **55,** 1075–1085.

Jobling, M. (1994). *Fish Bioenergetics*. London: Chapman and Hall.

Killen, S. S., Marras, S., Metcalfe, N. B., McKenzie, D. J. & Domenici, P. (2013). Environmental stressors alter relationships between physiology and behaviour. *Trends in Ecology and Evolution* **28,** 651–658.

Klefoth, T., Kobler, A. & Arlinghaus, R. (2008). The impact of catch-and-release angling on short-term behaviour and habitat choice of northern pike (*Esox lucius* L.) *Hydrobiologia* **601,** 99–110.

Klefoth, T., Kobler, A. & Arlinghaus, R. (2011). Behavioural and fitness consequences of direct and indirect non-lethal disturbances in a catch-and-release northern pike (*Esox lucius*) fishery. *Knowledge and Management of Aquatic Ecosystems* **403,** 11.

Klefoth, T., Skov, C., Krause, J. & Arlinghaus, R. (2012). The role of ecological context and predation risk-stimuli in revealing the true picture about the genetic basis of boldness evolution in fish. *Behavioral Ecology and Sociobiology* **66,** 547–559.

Klefoth, T., Pieterek, T. & Arlinghaus, R. (2013). Impacts of domestication on angling vulnerability of common carp, *Cyprinus carpio*: the role of learning, foraging behaviour and food preferences. *Fisheries Management and Ecology* **20,** 174–186.

Kobler, A., Klefoth, T., Wolter, C., Fredrich, F. & Arlinghaus, R. (2008). Contrasting pike (*Esox lucius* L.) movement and habitat choice between summer and winter in a small lake. *Hydrobiologia* **601,** 17–27.

Kobler, A., Klefoth, T., Mehner, T. & Arlinghaus, R. (2009). Coexistence of behavioural types in an aquatic top predator: a response to resource limitation? *Oecologia* **161,** 837–847.

Koolhaas, J., Korte, S., De Boer, S., Van Der Vegt, B., Van Reenen, C., Hopster, H., De Jong, I., Ruis, M. & Blokhuis, H. (1999). Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience and Biobehavioral Reviews* **23,** 925–935.

Krause, J., Krause, S., Arlinghaus, R., Psorakis, I., Roberts, S. & Rutz, C. (2013). Reality mining of animal social systems. *Trends in Ecology and Evolution* **28,** 541–551.

Kuparinen, A., Klefoth, T. & Arlinghaus, R. (2010). Abiotic and fishing-related correlates of angling catch rates in pike (*Esox lucius*). *Fisheries Research* **105,** 111–117.

Laskowski, K. L., Pearish, S., Bensky, M. & Bell, A. M. (2015). Predictors of individual variation in movement in a natural population of threespine stickleback (*Gasterosteus aculeatus*). *Advances in Ecological Research* **52,** 65–90.

Lester, N. P., Shuter, B. J. & Abrams, P. A. (2004). Interpreting the von Bertalanffy model of somatic growth in fishes: the cost of reproduction. *Proceedings of the Royal Society B* **271,** 1625–1631.

Lorenzen, K. (2000). Allometry of natural mortality as a basis for assessing optimal release size in fish-stocking programmes. *Canadian Journal of Fisheries and Aquatic Sciences* **57,** 2374–2381.

Lucas, M. (1989). Effects of implanted dummy transmitters on mortality, growth and tissue reaction in rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Biology* **35,** 577–587.

McGhee, K. E., Pintor, L. M. & Bell, A. M. (2013). Reciprocal behavioral plasticity and behavioral types during predator-prey interactions. *American Naturalist* **182,** 704–717.

Metcalfe, N. B., Taylor, A. C. & Thorpe, J. E. (1995). Metabolic rate, social status and life-history strategies in Atlantic salmon. *Animal Behaviour* **49,** 431–436.

Metcalfe, N. B., Van Leeuwen, T. E. & Killen, S. S. (2016). Does individual variation in metabolic phenotype predict fish behaviour and performance? *Journal of Fish Biology* **88,** 298–321. doi: 10.1111/jfb.12699

Milinski, M. (1993). Predation risk and feeding behaviour. In *Behaviour of Teleost Fishes* (Pitcher, T. J., ed), pp. 285–305. London: Chapman & Hall.

Mittelbach, G. G., Ballew, N. G., Kjelvik, M. K. & Fraser, D. (2014). Fish behavioral types and their ecological consequences. *Canadian Journal of Fisheries and Aquatic Sciences* **71,** 927–944.

Nakagawa, S. & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biological Reviews* **85,** 935–956.

Näslund, J., Bererhi, B. & Johnsson, J. I. (2015). Design of emergence test arenas can affect the results of boldness assays. *Ethology* **121,** 556–565.

Nespolo, R. F. & Franco, M. (2007). Whole-animal metabolic rate is a repeatable trait: a meta-analysis. *Journal of Experimental Biology* **210,** 2000–2005.

Niemelä, P. T. & Dingemanse, N. J. (2014). Artificial environments and the study of 'adaptive' personalities. *Trends in Ecology and Evolution* **29,** 245–247.

Niemelä, P. T., Dingemanse, N. J., Alioravainen, N., Vainikka, A. & Kortet, R. (2013). Personality pace-of-life hypothesis: testing genetic associations among personality and life history. *Behavioral Ecology* **24,** 935–941.

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. *Journal of Experimental Biology* **214,** 1668–1675.

Nyqvist, M. J., Gozlan, R. E., Cucherousset, J. & Britton, J. R. (2012). Behavioural syndrome in a solitary predator is independent of body size and growth rate. *PLoS One* **7,** e31619.

Nyqvist, M. J., Gozlan, R. E., Cucherousset, J. & Robert Britton, J. (2013). Absence of a context-general behavioural syndrome in a solitary predator. *Ethology* **119,** 156–166.

Pagel, T., Bekkevold, D., Pohlmeier, S., Wolter, C. & Arlinghaus, R. (2015). Thermal and maternal environments shape the value of early hatching in a natural population of a strongly cannibalistic freshwater fish. *Oecologia* **178,** 951–965.

Pieterek, T. (2014). Determinanten der anglerischen Fangbarkeit von Hechten (*Esox lucius*). MSc Thesis, Humboldt-Universität zu Berlin & Leibniz-Institut für Gewässerökologie und Binnenfischerei, Berlin. Available at http://besatz-fisch.de/images/stories/Papers/thesis_msc_pieterek.pdf/

Pope, K. & Kruse, C. (2007). Condition. In *Analysis and Interpretation of Freshwater Fisheries Data* (Guy, C. S. & Brown, M. L., eds), pp. 423–471. Bethesda, MD: American Fisheries Society.

Raat, A. J. (1988). Synopsis of biological data on the northern pike*: Esox lucius* Linnaeus, 1758. *FAO Fisheries Synopsis* **30.**

Réale, D., Gallant, B. Y., Leblanc, M. & Festa-Bianchet, M. (2000). Consistency of temperament in bighorn ewes and correlates with behaviour and life history. *Animal Behaviour* **60,** 589–597.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T. & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews* **82,** 291–318.

Réale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V. & Montiglio, P.-O. (2010). Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions of the Royal Society B* **365,** 4051–4063.

Seppänen, E., Tiira, K., Huuskonen, H. & Piironen, J. (2009). Metabolic rate, growth and aggressiveness in three Atlantic salmon *Salmo salar* populations. *Journal of Fish Biology* **74,** 562–575.

Seppänen, E., Piironen, J. & Huuskonen, H. (2010). Consistency of standard metabolic rate in relation to life history strategy of juvenile Atlantic salmon *Salmo salar*. *Comparative Biochemistry and Physiology A* **156,** 278–284.

Sloat, M. R., Reeves, G. H. & Jonsson, B. (2014). Individual condition, standard metabolic rate, and rearing temperature influence steelhead and rainbow trout (*Oncorhynchus mykiss*) life histories. *Canadian Journal of Fisheries and Aquatic Sciences* **71,** 491–501.

Stamps, J. A. (2007). Growth-mortality tradeoffs and ‘personality traits’ in animals. *Ecology Letters* **10,** 355–363.

Steffensen, J. F., Johansen, K. & Bushnell, P. G. (1984). An automated swimming respirometer. *Comparative Biochemistry and Physiology A* **79,** 437–440.

Tibblin, P., Forsman, A., Koch-Schmidt, P., Nordahl, O., Johannessen, P., Nilsson, J. & Larsson, P. (2015). Evolutionary divergence of adult body size and juvenile growth in sympatric subpopulations of a top predator in aquatic ecosystems. *American Naturalist* **186,** 98–110.

Tibblin, P., Forsman, A., Borger, T. & Larsson, P. (2016). Causes and consequences of repeatability, flexibility and individual fine-tuning of migratory timing in pike. *Journal of Animal Ecology* **85,** 136–145.

White, J. R., Meekan, M. G., McCormick, M. I. & Ferrari, M. C. (2013). A comparison of measures of boldness and their relationships to survival in young fish. *PLoS One* **8,** e68900.

Wilson, D. S., Coleman, K., Clark, A. B. & Biederman, L. (1993). Shy-bold continuum in pumpkinseed sunfish (*Lepomis gibbosus*): an ecological study of a psychological trait. *Journal of Comparative Psychology* **107,** 250.

Wilson, A. D., Brownscombe, J. W., Sullivan, B., Jain-Schlaepfer, S. & Cooke, S. J. (2015). Does angling technique selectively target fishes based on their behavioural type? *PLoS One* **10,** e0135848.

Wolf, M. & Weissing, F. J. (2010). An explanatory framework for adaptive personality differences. *Philosophical Transactions of the Royal Society B* **365,** 3959–3968.

Wolf, M., van Doorn, G. S., Leimar, O. & Weissing, F. J. (2007). Life-history trade-offs favour the evolution of animal personalities. *Nature* **447,** 581–584.

Wolter, C. & Arlinghaus, R. (2003). Navigation impacts on freshwater fish assemblages: the ecological relevance of swimming performance. *Reviews in Fish Biology and Fisheries* **13,** 63–89.

Závorka, L., Aldvén, D., Näslund, J., Höjesjö, J. & Johnsson, J. I. (2015). Linking lab activity with growth and movement in the wild: explaining pace-of-life in a trout stream. *Behavioral Ecology* **26,** 877–884.

Zuur, A. F., Ieno, E. N., Walker, N. J., Savaliev, A. A. & Smith, G. M. (2009). *Mixed Effects Models and Extensions in Ecology with R*. New York, NY: Springer.

Electronic Reference

Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. (2013). *R Development Core Team (2012) nlme: Linear and Nonlinear Mixed Effects Models. R Package Version 3.1-103*. Vienna: R Foundation for Statistical Computing. Available at http://CRAN.R-project.org/package=nlme/

Chapter 5

Life-history strategies and pace-of-life syndrome hypothesis: unmatched predictions and varying correlation structures

This chapter consists of the following publication:

G Polverino, F Santostefano, C Diaz, T Mehner, unpublished. Life-history strategies and pace-of-life syndrome hypothesis: unmatched predictions and varying correlation structures.

> Authors' contributions (*i.e., entire, large, predominant, partial):*
>
> **GP** (**large**) and TM developed the concept; **GP** (**entire**) did the literature research; **GP** (**large**) and CD developed the methods; **GP** (**entire**) collected the data; **GP** (**partial**), FS, and CD analyzed the data; **GP** (**large**), FS, CD, and TM discussed the results; **GP** (**entire**) wrote the manuscript; **GP** (**large**), FS, CD, and TM revised the manuscript.

UNPUBLISHED MANUSCRIPT

Life-history strategies and pace-of-life syndrome hypothesis: unmatched predictions and varying correlation structure

Giovanni Polverino[1,*], Francesca Santostefano[2], Carlos Díaz-Gil[3,4], Thomas Mehner[1]

ABSTRACT

Life-history adaptations to ecological conditions have been widely observed in the animal kingdom, with fast and slow life histories that are respectively favored under unstable and stable environments. The Pace-of-Life Syndrome (POLS) hypothesis expands those predictions by including metabolic costs for body maintenance and behavior into this evolutionary framework. Yet, empirical support for POLS is scarce and the generality of its predictions controversial. In this study, we tested whether first-generation offspring from different fish populations differed on average in life histories, behaviors, and metabolic rates. Secondly, we tested whether correlations among those traits were maintained within each population. We observed that life histories diverged on average between fish populations, with individuals adapted to unstable and human-disturbed conditions that matured earlier, were more fecund, and less incline to develop large propulsion-devoted muscles (i.e., "fast" life history) than fish from stable contexts (i.e., "slow" life history). Yet, contrary to what expected, fish with slow life histories had, on average, higher risk-taking attitudes, activity rates, and mass-specific metabolic costs than fish with a faster life history. Traits correlations also differed between populations, with repeatable among-individual differences in behavior, metabolic rate, and size correlated only in slow life history fish. Our results suggest predictions from the POLS might not be universal and that ecological adaptations can play a relevant role in driving resources allocation and correlated evolution of phenotypic traits.

Keywords: gambusia; life history; metabolic rate; ontogeny; pace of life; personality; selection; trade-off.

---

*Author for correspondence

Giovanni Polverino
Postal address: Müggelseedamm 310,
Berlin 12587, Germany
Tel.: +49 (30) 64181609
E-mail: polverino@igb-berlin.de

[1] *Department of Biology and Ecology of Fishes, Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Germany*

[2] *Research Group Evolutionary Ecology of Variation, Max Planck Institute for Ornithology, Germany*

[3] *Department of Ecology and Marine Resources, Instituto Mediterráneo de Estudios Avanzados IMEDEA (CSIC-UIB), Spain*

[4] *Laboratori d'Investigacions Marines i Aqüicultura (LIMIA), Balearic Government, Spain*

INTRODUCTION

Evolutionary biologists are typically devoted to explain why animals differ from each other in life-history traits (LHTs), such as morphology, fecundity, and lifespan, both among and within species. The classic life-history theory (Roff, 1993; Stearns, 1992) suggests that variation in these traits among species and populations can be explained based on the trade-off between investments on survival and reproduction across different environments (Garland, 2014). Indeed, the proportion of energy that animals allocate to survival and reproduction typically varies among environmental contexts in response to natural selection (Stearns, 1989; Stearns, 2000). In particular, energetic investments in reproduction should be favored over survival under disturbed and unstable environments (Stearns, 1977), whereby selection acts in favor of early sexual maturation, high fecundity, low immune investment, and short life span (i.e., r-strategy; (MacArthur and Wilson, 2015; Stearns, 1989; Stearns, 2000; Woodhams et al., 2016)). On the contrary, permanent and stable environments should favor survival over reproduction (Stearns, 1977), encouraging delayed maturation, large body sizes, high immune investments, and low reproductive rates over a long life span (i.e., K-strategy; (MacArthur and Wilson, 2015; Stearns, 1989; Stearns, 2000; Woodhams et al., 2016)). Animals can thus adaptively adjust their life-history strategies in response to selection pressures from the environment. The magnitude of those ecological adaptations may ultimately lead to genetic divergence and reproductive isolation between animal populations (Schluter, 2009).

Inspired by classic life-history approaches (Bielby et al., 2007; Gaillard et al., 1989; Stearns, 1989), recent theories have proposed a comprehensive framework to predict how adaptation should drive the allocation of resources across life-history, behavioral, and metabolic traits in animals (Réale et al., 2010). The Pace-of-Life Syndrome (POLS) hypothesis expands predictions from the life-history theory by including behavior and metabolic rate into the evolutionary framework (Réale et al., 2010; Sih et al., 2015; Wolf et al., 2007; Wolf and Weissing, 2010). Hence, animals on the slow side of the life-history continuum, in which survival is favored over reproduction, should also exhibit low energetic requirements for their body maintenance (Brown et al., 2004), in turn allowing for reduced activity levels (reviewed by (Biro and Stamps, 2010; Metcalfe et al., 2016)) and risk-taking attitudes in the attempt to find food under predation threat (Killen et al., 2011; Krause et al., 1998) compared to individuals with fast life histories (Biro and Stamps, 2008, 2010; Careau et al., 2010; Réale et al., 2010). The allocation of energy within the organism is thus constrained among competing functions (Careau et al., 2008; Garland, 2014), in which behavioral and metabolic investments affect, and are in turn affected, by life-history trajectories (Ghalambor et al., 2004; Reznick and Travis, 1996; Stamps, 2007; Werner and Anholt, 1993; Wolf et al., 2007). A corollary of the POLS is that animals display consistent among-individual variation (i.e., repeatable differences) in labile traits such as behaviors (i.e., personality; (Réale et al., 2007)) and metabolism, assuming that individual variation in those traits is maintained through their association with LHTs, both within and between species (Réale et al., 2010).

While empirical work has extensively and unambiguously verified the assumptions behind the life-history theory (reviewed by (Stearns, 2000)), experimental supports for the POLS is still controversial (Careau and Garland Jr, 2015; Hille and Cooper, 2015; Mathot and Dingemanse, 2015; Santostefano et al., (submitted)) and approximately 50% of the studies have rejected its predictions (Dammhan, Reale, et al. in prep.). The reasons behind this controversy may rely on both methodological issues, related to empirically testing the POLS, and the generality of the theory itself, which might suffer from providing precise causal predictions. For example, methodological challenges in testing the POLS might be especially related to the reliability of the metabolic estimates (Mathot and Dingemanse, 2015). In this direction, Mathot and Dingemanse (2015) have suggested that behavioral influences associated to metabolic estimates can mask or even generate unauthentic relationships between behavioral and metabolic traits. Furthermore, metabolic rates are often assumed to be more stable than behavioral traits (Nespolo and Franco, 2007) and, thus, metabolic rates of individuals are typically estimated based on single measurements (see for example (Rønning et al., 2015)). However, a recent meta-analysis concluded that the repeatability of metabolic rates declines over time (White et al., 2013), most probably as a consequence of metabolic adjustments to varying environmental conditions (Killen et al., 2013). As a result, spurious metabolic estimates could impede testing effectively POLS assumptions.

Conversely, the POLS may fail in providing universal predictions on the strength and sign of phenotypic correlations (Réale et al., 2010) and we might not expect its predicted correlations in all contexts. For example, the relationship between repeatable among-individual differences in behavior and metabolic rate may vary between organisms as a function of fixed or variable energy budgets (Mathot and Dingemanse, 2015), that is, if the increase/decrease in one trait affects or not the energy available for the other trait. Moreover, relationships between phenotypes may diverge from POLS predictions especially under ecological and evolutionary scenarios in which selection alters or suppresses trade-offs between specific traits (Hille and Cooper, 2015). In their review, Hille and collaborators (2015) have indeed provided convincing evidence that relationships between life-history, behavioral, and metabolic traits may vary among animals as a function of climatic and ecological challenges associated to latitude and altitude (Hille and Cooper, 2015). Unstable and chronically-disturbed environments may especially disfavor the evolution of phenotypic correlations. For example, in the case of selection acting against a specific phenotype (e.g., large body size) that is strongly correlated with another one not under selection (e.g., boldness), the ability of individuals to adapt rapidly (e.g., to size-selected fishing; (Enberg et al., 2012)) would reduce substantially (Sih et al., 2012). Under this scenario, deleterious correlations should be disfavored by natural selection and traits expected to evolve freely when those selective pressures are maintained over time (Sih et al., 2004)).

The primary goal of this study was to empirically verify predictions from the life-history theory and POLS within an evolutionary framework. Indeed, if abiotic and/or biotic factors shape the life-history trajectories of animal populations, then do fast and slow life-history trajectories reflect fast and slow trajectories in behavior and metabolic demands? Then secondly, do populations with different life histories exhibit similar phenotypic correlations? We tested whether offspring of wild caught Eastern mosquitofish (*Gambusia holbrooki,* Girard 1859), obtained from populations adapted to different environmental conditions, differed on average in their age and size at sexual maturity, fecundity, and morphology, despite being maintained under identical conditions since birth. Then, we tested whether mean differences in LHTs were associated with different mean behaviors and metabolic rates between populations. Lastly, we tested whether among-individual variation in behavior and metabolism were consistent over lifetime in both populations, with repeatable traits correlated with each other as suggested by the POLS hypothesis. We expected that a) different ecological and evolutionary pressures present in natural environments shape diverse life-history strategies between fish populations that are detectable in first-generation laboratory reared individuals; b) boldness, activity, and metabolic rates are enhanced in fast-growing fish; and c) repeatable phenotypic differences among individuals are correlated with each other in both populations, as expected by the POLS (null hypothesis).

MATERIALS AND METHODS

The experimental procedure was approved through an animal care permit (G 0074/15) granted by the Landesamt für Gesundheit und Soziales Berlin (LAGeSo). Both housing and experimental procedures were designed to minimize stress in the tested animals. All experiments were performed at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) in Berlin, Germany.

Study organism and maintenance

Fish utilized in this study were first-generation progeny of wild caught Eastern mosquitofish (*Gambusia holbrooki*) from Torre Castiglione (Italy, (Ariani et al., 2000)) and Maccarese (Italy, (Missiroli, 1930)). Environmental conditions for Torre Castiglione's population are extremely stable, that is, temperature fluctuates of only 3°C over the year, flooded caves and permanent macrophytes offer protected nurseries to fries, and aquatic predator species are absent (SP2 site described in (Ariani et al., 2000)). Conversely, fish from the artificial water body in Maccarese have adapted to unstable and human-disturbed environments, including large temperature fluctuations over the year (> 20°C), absence of nursery areas, and intense size-related selection over the past twenty years (i.e., approximately 80% of adults have been removed with dip nets every year; personal communication).

Wild caught fish from each of the two populations were housed separately in 50-l aquaria, with a maximum density of 0.4 fish/l, for a minimum of five months after capture as described in (Polverino et al., 2016b). Then, pregnant females from each population were removed from their housing tanks and individually transferred into 10-l aquaria until giving birth, following the procedure described in (Polverino et al., 2016b). In particular, the 10-l aquaria were inspected twice a day until newly-born fish were found. Soon after, the adult female was transferred back into its housing tank, while the newly-born fish (approximately 20 individuals per clutch) were maintained in their native aquarium for 15 days after birth and were fed twice a day with live and frozen *Artemia salina* nauplii.

Subsequently, *N*=80 fish (40 per each population) born on the same day were randomly selected, with individuals showing physical anomalies and/or malformations excluded *a priori* from the pool of experimental individuals. Experimental fish were randomly assigned to four identical experimental housing tanks (25 cm wide x 25 cm high x 120 cm long each; Figure 1), described in detail in (Polverino et al., 2016a). Juvenile fish from the two populations were housed separately, with two experimental housing tanks dedicated to each population. Each experimental housing tank hosted two parallel rows of 10 transparent Plexiglas cylinders (18 cm high and 10 cm diameter) confined on their bottom surface with stainless-steel net and submerged in water for 10 cm (Figure 1). A single fish was housed in each of the transparent Plexiglas cylinders (i.e., 20 fish per experimental housing tank) and maintained in it for all the duration of the study, that is, approximately five months. This setup allowed the exchange of visual and chemical cues among individuals (Ward and Mehner, 2010), but prevented competition for resources and sexual harassment (Dadda et al., 2005; Pyke, 2005). Moreover, marking each Plexiglas cylinder with a unique identification code allowed to identify each fish even at small sizes, when common marking procedures typically cause high mortality rates (Frederick, 1997).

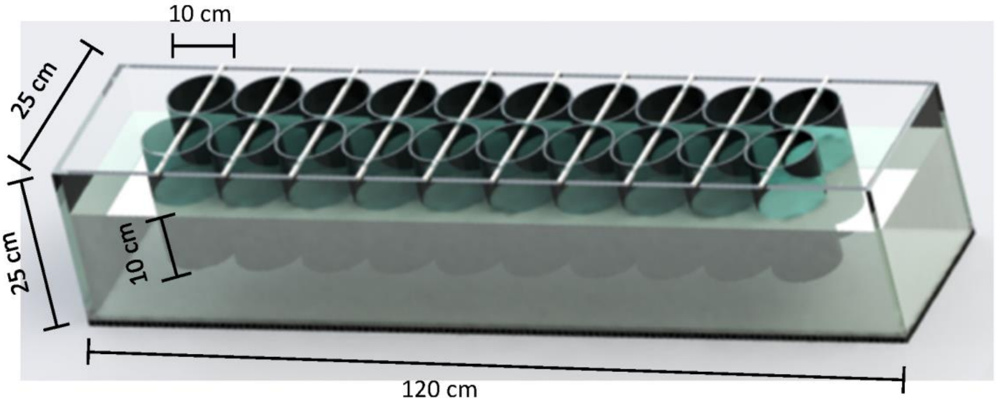


Figure 1. Scheme of an experimental housing tank. Two experimental housing tanks were used for each population. Each tank hosted two parallel arrays of ten transparent Plexiglas cylinders. Juvenile mosquitofish were housed individually in the transparent Plexiglas cylinders (i.e., 20 fish per tank).

We periodically randomized the position of the cylinders within each experimental housing tank so that all fish experienced different neighbor compositions, as suggested by (Polverino et al., 2016a).

We measured the behavior, Standard metabolic rate, and LHTs on each experimental fish at the onset of sexual maturation (i.e., immature fish) and after that the full sexual maturity was reached (i.e., adult stage). Notably, eight experimental fish from Torre Castiglione and one fish from Maccarese died over the experiment and, thus, a total of 32 and 39 fish were respectively tested at both ontogenetic stages. Sexual maturation was detected according with the morphogenesis of the anal fin in mosquitofish males, as suggested by (Polverino et al., 2016a; Polverino et al., 2016b).

Water temperature was maintained consistently at 23 ± 0.5°C and artificial illumination on a 12-h cycles (Pyke, 2005) across all housing conditions described above. Fish were fed twice a day at 9 a.m. and at 7 p.m with *Artemia salina* nauplii and flake food, respectively, before their maturation, while *Artemia salina* nauplii, blood worms, and flake food were used for adults.

Behavioral assay

Behavioral tests were performed in a rectangular open field (60 cm long, 42 cm wide, and 30 cm high), with a capacity of 75 l (Figure 2). As described in detail by (Polverino et al., 2016b), an experimental refuge and its lid were used to acclimate each fish before the initiation of the test, while the squared area around the closed refuge represented the only shelter available to the fish once in the open field (Figure 2). The open field was filled with 7 cm of conditioned water to offer natural shallow-water conditions to mosquitofish (Pyke, 2005), while minimizing their activity along the water column.

The behavioral response of each fish was tested in the open field twice at the immature and twice at the adult stage (i.e., four trials per each fish), with trials within each stage one week apart from each other. All behavioral tests were performed in a temperature-controlled chamber (Feutron Temperaturzelle, Feutron Klimasimulation GmbH, Greiz, Germany) between 11 a.m. and 6 p.m. from Monday to Friday. Hence, external disturbances were minimized and water temperature was maintained constantly at 23°C. A high resolution camera (Bosch Dinion HD 1080p, Bosch, Grasbrunn, Germany) was placed 1 m above the open field for a complete top view of the apparatus and it recorded the fish motion on the x-y plane. Two lateral lights provided homogeneous illumination of the open field. Fish were tested in a randomized order to exclude consistent differences in

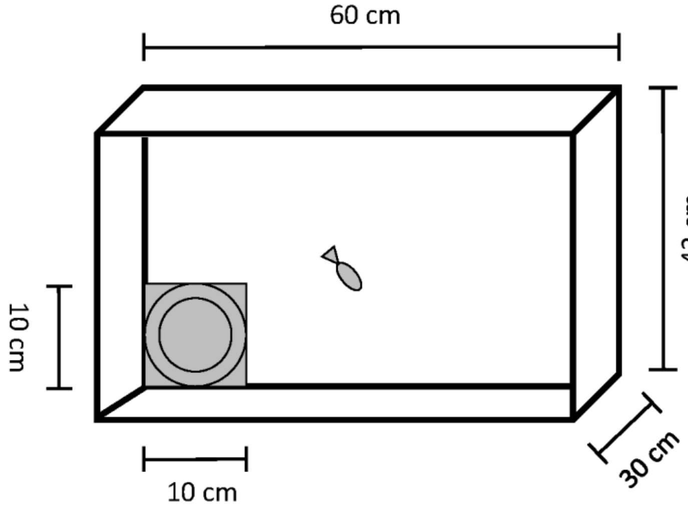


Figure 2. Top-view schematic of the open field. The two concentric circles represent the refuge with its sliding lid (used for acclimating the fish before the test), while the grey-squared area around the closed refuge represents the only shelter available to the fish during the test. Both hiding area and refuge are here illustrated as grey to facilitate their identification but they were both colored in white, consistently with the background of the open field.

their behavioral outcome caused by hunger (Krause et al., 1998).

Following the procedure in (Polverino et al., 2016b), for each behavioral trial a single fish was netted from its transparent Plexiglas cylinder and assayed as follows: 1) the fish was gently introduced into the closed refuge and left to acclimatize for 5 min; 2) the lid of the refuge was opened and the time interval before the fish exited the refuge was assessed manually with a stopwatch ("Emergence latency", in s); 3) soon after the fish left the refuge, the lid was automatically closed and fish behavior in the open field was video recorded for 10 min; and 4) the fish transferred back to its Plexiglas cylinder within its original experimental housing tank. The video-tracking software (EthoVision XT Version 9.0; Noldus Information Technologies Inc., www.noldus.com) was then utilized to calculate the inactivity time ("Freezing time", in s), "Distance moved" (in cm), and the time spent within the shelter area ("Hiding time", in s) for each fish in each video.

We interpreted short Emergence latency or Hiding time as fish's willingness to take risks in exploring open spaces that were unfamiliar and potentially dangerous (i.e., Boldness; (Krause et al., 1998; Réale et al., 2007)), while short Freezing time and long Distance moved characterized high Activity levels of the individuals (Polverino et al., 2016a; Réale et al., 2007).

Standard metabolic rate

Soon after the conclusion of the behavioral assay (at both immature and adult stages, respectively), a fish was fasted for 24 h before its metabolic rate was measured overnight for a 12 h period (8 pm to 8 am). Thus, the metabolic rate was tested twice on each fish.

A computerized intermittent flow respirometer (DAQ-PAC-WF8, Loligo Systems, Tjele, Denmark, www.loligosystems.com) was utilized to perform the metabolic measurements. An array of eight cylindrical glass chambers (5 ml each) was utilized to perform measurements on eight fish simultaneously. The chambers were submerged in an aerated water bath maintained at a constant temperature of 23 ± 0.1 °C, consistently with the water temperature experienced in both housing tanks and open field. An ultraviolet sterilizer (EHEIM reeflex-UV-350, EHEIM GmbH, Germany, www.eheim.com) was connected to the water bath through a closed recirculation system to maintain low bacteria concentrations in the water during night. Nevertheless, seven fish were measured each night and the oxygen consumption related to bacteria respiration was consistently measured in one of the chamber in absence of fish. Water oxygen content within the chambers was measured once every second using eight independent oximeter sensors (Firesting 8-Channel oxygen meters, Pyroscience, Aachen, Germany, www.pyro-science.com). The entire apparatus was located within a second temperature-controlled room comparable with the one described for the behavioral assays, which remained closed and undisturbed during measurements.

The oxygen-saturated water from the water bath was periodically flushed into each chamber of the respirometer through an external pump that was set to turn on and off for alternating 10 min periods. After 1 min since the flow of oxygen-saturated water into the chambers was interrupted (i.e., waiting phase), the decrease in oxygen content in the closed chambers was measured once every second for a 9 min period (i.e., closed phase). Subsequently, each chamber was automatically flushed with aerated water for 10 min (i.e., flushing phase) before the start of the next measurement.

The mass-specific Standard metabolic rate (specific SMR, mg O_2 kg^{-1} h^{-1}) was estimated for each fish at each ontogenetic stage from the decrease in oxygen concentration over time (i.e., respiration rate; (Herskin, 1999)). Specifically, a mixture distribution composed of two normal distributions was fitted to the respiration rates obtained from the closed phases for a given individual (R package 'mixtools' v. 1.0.4; (Benaglia et al., 2009)) and the lower mean of the two normal distributions was used as its mass-specific SMR, as per (Herskin, 1999). Therefore, the metabolic estimates measured here separated the extra oxygen consumption beyond SMRs caused by spontaneous motion activity (i.e., higher mean of the two normal distributions) and, thus, referred specifically to the individual cost of self-maintenance measured at a particular temperature and post-absorptive and inactive state (i.e., SMR, (Burton et al., 2011)). To accurately measure oxygen consumption, we discarded closed phases in which oxygen did not decrease linearly over time, that is, when the slope of the regression assessing the decline in oxygen over time for a given chamber was lower than 0.95. In addition, the first and last two minutes were excluded from each measure (i.e., closed phase) such that only the linear component of O^2 degradation was captured (Dupont-Prinet et al., 2010). Pilot trials, determined that SMR of mosquitofish stabilized after 1 h. Thus, measurements from the first hour represented here the acclimation period and were not included into the estimate of SMRs.

Life-history assessment

Soon after the conclusion of the metabolic assay (at both immature and adult stages), fish were anesthetized in a solution of 2-phenoxiethanol (0.3 ml per 250 ml H_2O) and measured for Standard size (to the nearest 0.1 mm) and Body weight (to the nearest 0.01 g). The Fulton's condition factor *K* ((g mm^{-3} 10^4); (Froese, 2006)) was then calculated as a proxy of the nutritional state of each fish at each ontogenetic stage. Thus, Standard size, Body weight, and Fulton's condition factor were measured twice on each fish.

Morphometry and fecundity measurements were performed on each fish at the adult stage only (Figures S1 and S2 in the Electronic Supplementary Material). In particular, the region of the fish tail devoted to locomotor performances was measured at adulthood (i.e., Swimming muscle, mm^2), according to the landmarks described by (Langerhans et al., 2004). Gonopodium length (mm) was measured for all adult males (Langerhans et al., 2004), while the Number of eggs, their Dry weight (mg), and the Mean dry weight per egg (mg) were measured for adult females after they were euthanized in a concentrated solution of 2-phenoxiethanol. Details on morphometric and fecundity measurements are in the Electronic Supplementary Material.

Statistical Analysis

As a first step we tested whether offspring of mosquitofish belonging to two distinct wild populations differed in mean LHTs, although all individuals were maintained under standardized conditions since birth.

After this was confirmed, we tested whether mean differences in behavioral traits and metabolic demands were also present between populations. Next, we estimated the repeatability of these traits separately for each population. As a final step, we used a multivariate extension of this framework to estimate patterns of trait covariance among and within individuals for each population separately as a test of the POLS.

Prior to all analyses, Emergence latency was log-transformed, while Hiding time, Freezing time, and mass-specific SMR were squared-root transformed to normalize error distribution. The explanatory variables Age and Sex were transformed as numeric variables, both coded as -0.5 (immature and females, respectively) and 0.5 (adults and males, respectively), as suggested by (Gelman, 2008). All models described below were fitted using restricted maximum likelihood, whereby dependent variables were mean-centered and their variance standardized to facilitate comparison of variance components across traits, as suggested by (Dingemanse and Dochtermann, 2013). Throughout, we assumed a Gaussian error distribution, which was confirmed for all response variables after visual inspection of model residuals.

In the first set of analysis, we tested whether captive-bred fish from two wild populations differed on average in their LHTs. Average differences between populations were calculated for each of the following traits separately with lineal models (LMs) in which the trait was included as the dependent variable and Population as a fixed factor: Age at sexual maturation (days), Standard size at sexual maturation (mm), Body weight at sexual maturation (g), Standard size at adulthood (mm), Body weight at adulthood (g), Swimming muscle at adulthood (mm^2), Gonopodium length at adulthood (for males only; mm), Number of eggs at adulthood (for females only), Dry weight of eggs at adulthood (for females only; mg), and Mean dry weight per egg at adulthood (for females only; mg). Since the area of the caudal fin devoted to propulsion might vary across fish depending on their size, we included Standard size as a fixed factor into the model for Swimming muscle to account for mean differences in size among fish populations. Similarly, Body weight was included as a fixed factor into females' fecundity models (i.e., Number of eggs Dry weight of eggs, and Mean dry weight per egg) to account for mean differences in body mass among adult females.

Secondly, we tested whether fish populations differed, on average, also in their behavioral traits and mass-specific SMRs. To do that, we tested each dependent variable (i.e., Emergence latency, Hiding time, Distance moved, Freezing time, and mass-specific SMR) separately with a linear mixed-effects model (LMM) with Population, Age, Sex, Standard size, Fulton's *K*, and Trial included as fixed factors. All other traits were either significantly correlated with Standard size and Fulton's *K* (Table S1 in the Electronic Supplementary Material) or characterized only one of the two ontogenetic stages or sexes (Table 1) and, thus, were excluded from these general models. The individual was specified as a random effect (i.e., random intercepts) to account for repeated measures.

As a next step, we estimated repeatabilities of all traits (i.e., behaviors, SMR, and Standard size) over the ontogeny for both populations separately. Since among-individual correlations are expected only between repeatable traits (Réale et al., 2010), we included in the model below only those traits that were repeatable for both populations. To measure repeatability, we ran LMMs separately for each population with each of the four behavioral traits included one-by-one as the dependent variable, the individual as a random effect (i.e., random intercepts), and Age, Sex, and Trial as fixed factors. The very same model structure was also used for the dependent variables mass-specific SMR and Standard size, with the fixed factor Trial that was however excluded from the models since those variables were not measured repeatedly within a given ontogenetic stage. The adjusted repeatability (Nakagawa and Schielzeth, 2010) was estimated for each of those traits by calculating the proportion of the total phenotypic variance not attributable to fixed effects that was explained by among-individual variance ($r = V_{Among} / V_{Among} + V_{Within}$). Then, we tested the statistical significance of fixed effects using numerator and denominator degrees of freedom (*df*) estimated from the algebraic algorithm in ASReml 3.0 (Gilmour et al., 2009). We used likelihood ratio tests (LRTs) to evaluate the statistical significance of random effects (i.e., to test whether a given trait was significantly repeatable over the ontogeny for a given population). This χ^2-distributed test statistic was calculated as twice the difference in log-likelihood between a model in which a target random effect was fitted versus not fitted (Shaw, 1991). Variances were bound to be positive, therefore probability (*P*) of a LRT applied to a variance was calculated assuming an equal mixture of P (χ^2, df=0) and P (χ^2, df=1), i.e. df=0.5 (Pinheiro and Bates, 2000; Self and Liang, 1987; Visscher, 2006).

Finally, we estimated patterns of trait covariance, between repeatable traits only, at the among-individual and residual level for both populations separately. By expanding the same structure of the univariate models above to multivariate LMMs, we fitted all repeatable traits (i.e., behaviors, SMR, and Standard size) together as dependent variables. Residual variances for (and covariances between) all traits were modelled. Covariances (correlations) were not bound to be positive and their probability was therefore calculated assuming P (χ^2, df=1). LRTs involving one variance and one covariance were tested assuming an equal mixture of P (χ^2, df=1) and P (χ^2, df=2), i.e. df=1.5. Significant differences between populations in covariances (correlations) were then tested by constraining each pairwise covariance (correlation) to be the same between the two fish populations. We then applied a LRT to compare the unconstrained model to the one where corresponding covariances between the two populations were constrained to be the same.

Data exploration and mean comparisons between populations were performed in R-3.1.1 version (R Core Team, 2013), while repeatabilities and covariances among traits were performed in ASReml 3.0 (Gilmour et al., 2009).

RESULTS

Overall, we found that mosquitofish descending from separate wild populations differed in mean life-history traits (LHTs), despite being maintained under identical conditions (Table 1). Sexual maturation started synchronically in all fish within a population either after one month (fast-growing population, FG, adapted to human-disturbed and unstable contexts) or two months (slow-growing population, SG, adapted to more stable conditions) since birth. Accordingly, FG fish grew faster and reached sexual maturity in half the time than SG fish (Table 1). Then, FG fish maintained smaller sizes at adulthood than SG fish, with FG females investing more in reproduction (i.e., Number of eggs and their Dry weight) than SG females. However, the eggs size was comparable among populations (i.e., Mean dry weight per egg; Table 1). On the contrary, SG fish invested more in swimming performance (i.e., larger Swimming muscles) than FG ones (Table 1), with the whole body shape differing on average between the two fish populations (Figure S3 in the Electronic Supplementary Material).

Furthermore, we found that the fixed factor Population explained significant differences in three of the four behavioral traits and also explained differences in mass-specific SMRs between fish populations (Table 2). In particular, fish from the slow-growing population (SG fish) were on average bolder (i.e., emerged faster from a refuge), were more active (longer distance travelled and shorter time

Table 1. Mean differences between populations for a given life-history trait (± SE). * refers to mean difference between populations for a given life-history trait after accounting for differences in Standard size (*****^{1}) or Body weight (*****^{2}), that is, Standard size or Body weight were included as fixed effects into the models.

Life-history traits	SG Mean (SE)	FG Mean (SE)	*P*
Age at sexual maturation (days)	60 ± 1	30 ± 1	-
Standard size at sexual maturation (mm)	22.06 ± 0.38	20.37 ± 0.10	**<0.01**
Body weight at sexual maturation (g)	0.19 ± 0.01	0.16 ± <0.01	**<0.01**
Standard size at adulthood (mm)	27.32 ± 0.55	26.62 ± 0.27	0.09
Body weight at adulthood (g)	0.39 ± 0.02	0.34 ± 0.01	**<0.01**
Swimming muscle at adulthood (mm^2)	112.12 ± 10.35	94.27 ± 8.55	**<0.01***^{1}
Gonopodium length at adulthood (mm; ♂)	7.66 ± 0.09	7.47 ± 0.11	0.07
Num. of eggs at adulthood (♀)	49.42 ± 5.41	60.54 ± 5.95	**0.01***^{2}
Dry weight of eggs at adulthood (mg; ♀)	80.53 ± 8.21	99.02 ± 8.90	**0.02***^{2}
Mean dry weight per egg at adulthood (mg; ♀)	1.75 ± 0.15	1.66 ± 0.09	0.18*^{2}

Table 2. Parameter estimates (± SE) of fixed effects derived from univariate models fitted to partition variation in boldness (i.e., Emergence latency and Hiding time), activity (i.e., Distance moved and Freezing time), and mass-specific Standard metabolic rate (SMR). Values printed in bold face represent significant effects based on Wald F tests.

	Emergence latency	Hiding time	Distance moved	Freezing time	SMR
Fixed effects	β (SE)	β (SE)	β (SE)	β (SE)	
Intercept	-0.109 (0.237)	-2.972 (2.068)	200.76 (127.94)	-0.927 (1.166)	**16.46 (5.20)**
Population	**0.358 (0.148)**	**-4.256 1.343**	-23.74 (86.23)	**3.037 (0.728)**	**-7.089 (3.608)**
Age	0.028 (0.297)	4.770 (2.596)	162.66 (158.00)	-2.637 (1.463)	**-25.37 (6.224)**
Sex	-0.024 (0.156)	-0.454 (1.438)	45.75 (94.21)	0.134 (0.768)	1.400 (3.969)
Standard size	-0.029 (0.046)	-0.580 (0.409)	**-55.36 (24.99)**	0.141 (0.229)	**-7.277 (0.993)**
Fulton's *K*	0.710 (0.454)	4.169 (4.015)	**557.08 (247.93)**	3.853 (2.235)	**-54.78 (9.853)**
Trial	-0.080 (0.121)	**2.341 (1.029)**	**-191.55 (60.61)**	0.251 (0.600)	-

spent being inactive), and had higher mass-specific SMRs than fast-growing fish (FG), even after accounting for mean differences in fixed effects between populations (Figure 3). However, once in the open field, FG fish spent on average less time hiding around the refuge than their SG counterparts (Table 2; Figure 3). Furthermore, mass-specific SMRs decreased on average with increasing age, size, and nutritional state (Table 2) for both populations. Distance moved decreased on average with increasing size, but it increased with increasing nutritional state (Table 2).

When moving from populations comparisons to among-individual differences within a given population, significant adjusted repeatabilities were observed over the ontogeny for both populations with respect to Boldness (Hiding time), Activity (Distance moved), mass-specific SMR, and Standard size (referred below to as “Size”; Table 3). On the contrary, Emergence latency and Freezing time were not repeatable over ontogeny of fish (data not shown) and, thus, were not included into the correlation structure within each population. Moreover, Activity decreased with age in FG fish, but not in SG fish (Table 3). FG males were smaller and showed higher SMRs than their female counterpart, while no differences in Standard size and SMR were present between males and females from the SG population (Table 3). Nevertheless, males and females did not differ in their average behaviors in both populations (Table 3).

By using a multivariate approach, we tested predictions from the POLS at the population level, that is, among-individual correlations in repeatable traits. Tests of significance on SG and FG matrixes revealed that correlations between Boldness,

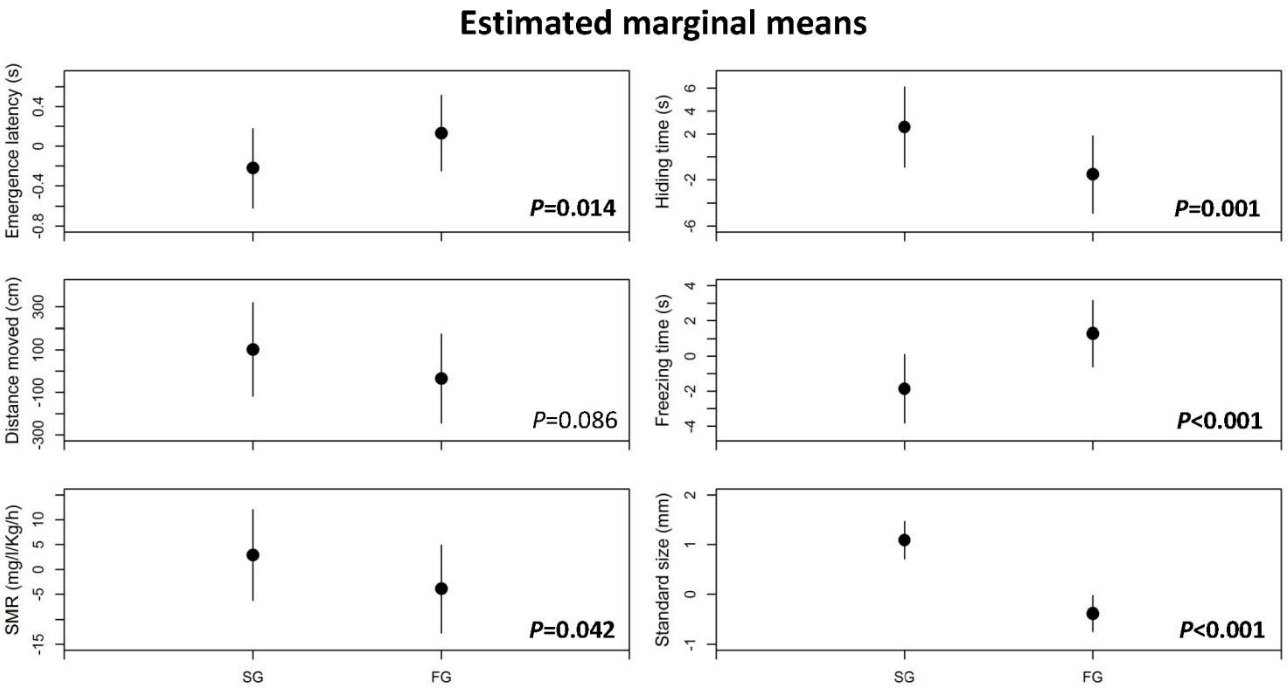


Figure 3. Estimated marginal means (± CI) based on univariate models (Table 2) between fish from the slow-growing (SG) and the fast-growing (FG) population with respect to boldness (i.e., Emergence latency and Hiding time), activity (i.e., Distance moved and Freezing time), mass-specific Standard metabolic rate (i.e., SMR), and Standard size. Estimated marginal means represent adjusted means of a given trait for each fish population, once the contribution from fixed effects (i.e., Age, Sex, Trial, Standard size, and Fulton’s *K*) is accounted for. Notably, the fixed factor Standard size was excluded from the estimated marginal mean for Standard size.

Table 3. Parameter estimates (± SE) of fixed and random effects derived from univariate models fitted to partition variation in Boldness, Activity, Standard size (size), and mass-specific Standard metabolic rate (SMR) with respect to fish from the fast-growing (FG) and the slow-growing (SG) population. Random effects are expressed as the proportion of total phenotypic variation not attributable to fixed effects. Values printed in bold represent significant effects based either on Wald F tests (for fixed effects) or likelihood ratio tests (for random effects).

	SG				FG			
	Boldness	Activity	Size	SMR	Boldness	Activity	Size	SMR
Fixed effects	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)
Intercept	0.314 (0.262)	**0.903** **(0.241)**	0.186 (0.115)	-0.049 (0.149)	**-0.825** **(0.226)**	0.181 (0.223)	**-0.108** **(0.030)**	-0.018 (0.149)
Age	0.168 (0.158)	0.093 (0.136)	**1.389** **(0.058)**	**-1.536** **(0.077)**	0.113 (0.134)	**-0.481** **(0.131)**	**1.820** **(0.046)**	**-1.475** **(0.088)**
Sex	-0.235 (0.228)	-0.081 (0.260)	-0.263 (0.234)	0.237 (0.190)	0.139 (0.206)	0.351 (0.207)	**-0.440** **(0.060)**	**0.438** **(0.142)**
Trial	-0.077 (0.158)	**-0.613** **(0.136)**	-	-	**0.452** **(0.134)**	-0.138 (0.131)	-	-
Random effects	σ^2 (SE)	σ^2 (SE)	σ^2 (SE)	σ^2 (SE)	σ^2 (SE)	σ^2 (SE)	σ^2 (SE)	σ^2 (SE)
Individual	0.196 (0.106)	0.361 (0.134)	0.382 (0.107)	0.222 (0.071)	0.226 (0.096)	0.240 (0.097)	0.013 (0.008)	0.114 (0.045)
Residual	0.775 (0.115)	0.575 (0.085)	0.106 (0.016)	0.185 (0.027)	0.721 (0.094)	0.691 (0.090)	0.085 (0.011)	0.300 (0.040)
Repeatability	**0.202** **(0.097)**	**0.386** **(0.100)**	**0.783** **(0.055)**	**0.546** **(0.090)**	**0.238** **(0.086)**	**0.258** **(0.087)**	**0.137** **(0.080)**	**0.275** **(0.089)**

Activity, SMR, and Standard size were overall present in slow-growing fish (i.e., SG fish), but were largely suppressed among fish from the fast-growing population (i.e., FG fish; Figure 4). In particular, large SG fish were more active while large FG fish were less active than their smaller siblings, respectively (Figure 4; Table S2). Moreover, large SG fish had lower SMRs compared to their smaller siblings, while Boldness and Activity were negatively correlated in SG fish (i.e., lower Boldness scores correspond to bolder attitudes because of the nature of the variable; (Figure 4; Table S2).

We also found that overall pairwise covariances (correlations) between traits differed significantly between fish from the SG and the FG population ($P<0.001$; $\chi2$= 38.891, df=6), confirming that the two populations differed from each other in terms of trait correlations.

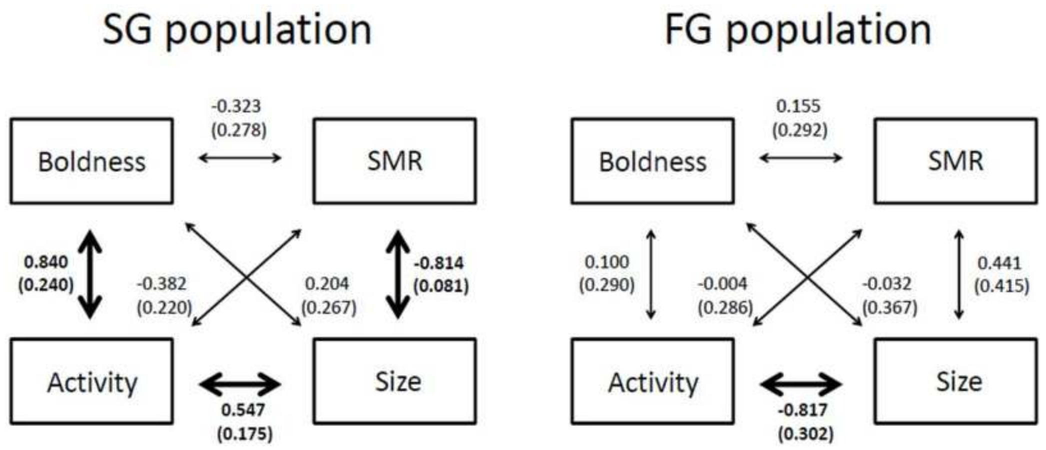


Figure 4. Estimated among-individual correlations (with SE) between two behaviors (Boldness and Activity), Standard size, and mass-specific Standard metabolic rate (SMR) in slow-growing (SG) and fast-growing (FG) fish. Because of the nature of the behavioral variable, lower Boldness scores correspond to bolder attitudes. Correlations printed in bold are significant (*P*<0.05) based on likelihood ratio tests derived from the multivariate model as detailed in the main text.

DISCUSSION

In this study, we aimed to test predictions from both the life-history theory and the POLS within an evolutionary framework. We observed that first-generation progenies from two distinct wild mosquitofish populations differed on average in LHTs, although maintained under identical controlled conditions since birth. Yet, contrary to what expected, slow-growing fish (SG) were on average bolder (i.e., more willing to take risks by leaving a submerged refuge faster), more active, and had on average higher mass-specific metabolic costs for their body maintenance than fish from the fast-growing population (FG). Finally, correlations between repeatable traits differed between fish from the two populations, with Boldness, Activity, SMR, and Size that were overall correlated in slow-growing fish, while correlations were inhibited in fish from the fast-growing population.

The main hallmark of the POLS is that species and/or populations adapted to different ecological conditions should differ in a suite of traits related to life history, behavior, and metabolism that are interconnected with each other as pieces of the same puzzle (Réale et al., 2010). Accordingly, at the fast end of the POLS continuum bold and exploratory individuals should have higher SMRs to fuel their high muscular activity and cellular machinery (Careau et al., 2008; White and Kearney, 2013), resulting in higher productivity rates (e.g., growth and fecundity) and a shorter lifespan (Biro and Stamps, 2008; Wolf et al., 2007) than conspecifics at the slow end of the continuum. Within the last decade, a multitude of studies have experimentally tested those assumptions. Yet, empirical evidences on those correlations are controversial (Hille and Cooper, 2015; Mathot and Dingemanse, 2015). Our findings contribute to this literature by rejecting predictions from POLS at the inter-population level, whereby fast and slow life histories mismatched with fast and slow trajectories on behavior and metabolism. Indeed, we found that, on average, slower life histories (SG fish) were coupled with bolder attitudes in an open field, higher activity levels, and higher mass-specific metabolic costs (mass-specific SMR) than fast-growing fish (FG). SG fish spent, however, more time hiding around the refuge than FG fish, probably because SG fish were more active and explorative overall and, thus, they may had higher chances to pass through the hiding region of the open field than their FG counterpart.

Allocation of resources typically entails trade-offs between competing functions (Reznick and Travis, 1996), whereby adaptation favors diverse strategies to allocate energy in animals evolved to different ecological contexts (Stearns, 1989). Here, adaptation to unstable climatic conditions and intense predator pressures, in which the life expectancy is compromised by size-selective removal of adult individuals, may have resulted in resources being allocated mainly to reproduction (i.e., growth and fecundity), as suggested by (Enberg et al., 2012). On the contrary, slow life histories might have favored the energy allocation to running costs of the organism (i.e., SMR, activity, and morphology functional to self-protection; (Enberg et al., 2012)) in favor of survival under reasonably stable environmental conditions (Stearns, 1977). In support of this interpretation, we verified that activity rates measured in the open field matched the routine activity rates observed in fish within their housings (Figure S4 in the Electronic Supplementary Material), expanding behavioral outcomes measured in this study outside of the open field test.

At the among-individual level, the POLS hypothesis suggests that repeatable differences in behavior (i.e., personality) and metabolic rate (SMR) among individuals mediate life-history trade-offs (Réale et al., 2010; Stearns, 2000), thus explaining why behavioral and metabolic variations are maintained within populations. For example, higher metabolic rates should allow bold individuals to sustain a greater muscular activity (White and Kearney, 2013) to be more successful in competing for resources and grow faster than their conspecifics (Biro and Stamps, 2008). However, results from this study do not support the generality of POLS predictions and may suggest, instead, that correlations between those phenotypic traits may vary between animal populations (see also (Heinen-Kay et al., 2016; Rønning et al., 2015)). In particular, we found that Boldness, Activity, SMR, and Standard size were repeatable over ontogeny in both fish populations, but correlations among those traits differed between populations. In particular, phenotypes were overall correlated only in fish from the slow-growing population (SG), while among-individual correlations in FG fish were consistently low and had high standard errors. Similarly, Rønning and colleagues (2015) found different correlation patterns between metabolic and LHTs between free-living populations of house sparrow (*Passer domesticus*) from distinct Norwegian islands. Heinen-Kay and her colleagues have also observed that correlations among behavioral traits varied between populations of Bahamas mosquitofish (*Gambusia hubbsi*) evolved in presence or absence of aquatic predators (Heinen-Kay et al., 2016). In agreement with our findings, Hille and Cooper (2015) have recently suggested that predictions from the POLS may diverge under different geographic gradients, as a consequence of varying trade-offs among phenotypic traits dictated by environmental adaptations (Sih et al., 2004). Under this perspective, it reasonable to believe that fish adapted to intense fishing pressures on large individuals, in which risk-aversive behaviors do not result in a higher survival of the individuals, might lack of the trade-off between survival and boldness or SMR. In support of this interpretation, animals living under strong environmental challenges (i.e., high elevations) were found to lack of the trade-off between survival and metabolic rates (reviewed by (Hille and Cooper, 2015)), while severe environmental challenges have been suggested to collapse the SMR variation between individuals and thus mask the link between energy metabolism and boldness in animals (Killen et al., 2013). Similarly, we observed opposite correlations between Activity and Standard size between fish populations, most probably caused by different trade-offs between those traits under the diverse ecological conditions in which the two populations have evolved. Large SG fish were more active than their smaller siblings, probably because less vulnerable to predation from large conspecifics (Polverino et al., 2016b; Pyke, 2005) and more successful in competing for food resources (Rudin and Briffa, 2012; Schoener, 1983). On the contrary, large FG fish were less active than small FG fish, probably because more vulnerable to size-selected predation than smaller ones (i.e., fishing on large individuals; (Arlinghaus et al., 2016)). Interestingly, FG fish decreased substantially their activity levels from the immature to the adult stage while SG fish did not, supporting our interpretation that evolutionary adaptations to size-selected fishing might have induced FG fish to be less active once they reached large sizes, that is, when they become fishing targets.

Natural selection typically favors fast and slow life-history adaptations depending by levels and stability of environmental factors such as predator pressure (Crowl and Covich, 1990; Reznick et al., 2001), resources availability (Grether et al., 2001; Wilbur et al., 1974), and environmental temperature (Pankhurst and Munday, 2011; Pepin, 1991). In our case, it is reasonable to assume that evolutionary adaptations to size-selected fishing (Enberg et al., 2012) might have especially favored increased reproductive investments in fast-growing fish (FG) in the form of early sexual maturation (reviewed by (Dieckmann and Heino, 2007)), reduced size-at-age (Pardoe et al., 2009), slim body shape (Hay et al., 1986), and increased fecundity (Law, 1979) than SG fish. Nevertheless, other unmeasured factors may also have contributed to shape different life-history strategies between FG and SG fish (e.g., temperature, food quality, etc.). Whether those differences in life-history strategies were genetically inherited or generated via parental effects (Skinner, 2015) is beyond the interest of this study.

The present study does not support the hypothesized link between classical life-history adaptation, behavior, and energetic demand for body maintenance between and within animal populations. Instead, our findings suggest that selective evolutionary pressures may constrain phenotypic correlations under less predictable routes than as predicted by the POLS, especially under unstable and chronically disturbed environments in which those correlations might compromise the capacity of individuals to adapt to changes.

ACKNOWLEDGEMENTS

The authors would like to acknowledge all members of the B-Types group and the research group Evolutionary Ecology of Variation for the important insights on data analysis. This work was part of the B-Types project (http://www.b-types.igb-berlin.de/) funded through the Wissenschaftsgemeinschaft Leibniz (WGL, SAW-2013-IGB-2). CD-G funding was provided by FPI-INIA-2012.

REFERENCES

Ariani AP, Camassa MM, Wittmann KJ, 2000. The dolinas of Torre Castiglione (Gulf of Tarent, Italy): environmental and faunistic aspects of a semi-hypogean water system. Mémoires de Biospéologie 27:1-14.

Arlinghaus R, Laskowski KL, Alós J, Klefoth T, Monk CT, Nakayama S, Schröder A, 2016. Passive gear-induced timidity syndrome in wild fish populations and its potential ecological and managerial implications. Fish and Fisheries:000.

Benaglia T, Chauveau D, Hunter D, Young D, 2009. Mixtools: An R package for analyzing finite mixture models. Journal of Statistical Software 32:1-29.

Bielby J, Mace GM, Bininda-Emonds OR, Cardillo M, Gittleman JL, Jones KE, Orme CDL, Purvis A, 2007. The fast-slow continuum in mammalian life history: an empirical reevaluation. The American Naturalist 169:748-757.

Biro PA, Stamps JA, 2008. Are animal personality traits linked to life-history productivity? Trends in Ecology & Evolution 23:361-368.

Biro PA, Stamps JA, 2010. Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? Trends in Ecology & Evolution 25:653-659.

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB, 2004. Toward a metabolic theory of ecology. Ecology 85:1771-1789.

Burton T, Killen SS, Armstrong JD, Metcalfe NB, 2011. What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? Proceedings of the Royal Society of London Series B: Biological Sciences 278:3465-3473.

Careau V, Garland Jr T, 2015. Energetics and behavior: many paths to understanding. Trends in ecology & evolution 30:50-60.

Careau V, Réale D, Humphries MM, Thomas DW, 2010. The pace of life under artificial selection: personality, energy expenditure, and longevity are correlated in domestic dogs. The American Naturalist 175:753-75Careau V, Thomas D, Humphries MM, Réale D, 2008. Energy metabolism and animal personality. Oikos 117:641-653.

Crowl TA, Covich AP, 1990. Predator-induced life-history shifts in a freshwater snail. Science (Washington) 247:949-951.

Dadda M, Pilastro A, Bisazza A, 2005. Male sexual harassment and female schooling behavior in the eastern mosquitofish. Animal Behaviour 70:463–471.

Dieckmann U, Heino M, 2007. Probabilistic maturation reaction norms: their history, strenghts and limitations. Marine Ecology Progress Series 335:253-269.

Dingemanse NJ, Dochtermann NA, 2013. Quantifying individual variation in behaviour: mixed-effect modelling approaches. Journal of Animal Ecology 82:39-54.

Dupont-Prinet A, Chatain B, Grima L, Vandeputte M, Claireaux G, McKenzie DJ, 2010. Physiological mechanisms underlying a trade-off between growth rate and tolerance of feed deprivation in the European sea bass (*Dicentrarchus labrax*). The Journal of experimental biology 213:1143-1152.

Enberg K, Jørgensen C, Dunlop ES, Varpe Ø, Boukal DS, Baulier L, Eliassen S, Heino M, 2012. Fishing-induced evolution of growth: concepts, mechanisms and the empirical evidence. Marine Ecology 33:1-25.

Frederick JL, 1997. Evaluation of fluorescent elastomer injection as a method for marking small fish. Bulletin of Marine Science 61:399-408.

Froese R, 2006. Cube law, condition factor and weight–length relationships: history, meta-analysis and recommendations. Journal of Applied Ichthyology 22:241-253.

Gaillard JM, Pontier D, Allaine D, Lebreton JD, Trouvilliez J, Clobert J, 1989. An analysis of demographic tactics in birds and mammals. Oikos 56:59-76.

Garland T, 2014. Trade-offs. Current Biology 24:R60-R61.

Gelman A, 2008. Scaling regression inputs by dividing by two standard deviations. Statistics in Medicine 27:2865-2873.

Ghalambor CK, Reznick DN, Walker JA, 2004. Constraints on adaptive evolution: The functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). American Naturalist 164:38-50.

Gilmour AR, Gogel BJ, Cullis BR, Thompson R, Butler D, 2009. ASReml user guide release 3.0. VSN International Ltd, Hemel Hempstead, UK.

Grether GF, Millie DF, Bryant MJ, Reznick DN, Mayea W, 2001. Rain forest canopy cover, resource availability, and life history evolution in guppies. Ecology 82:1546-1559.

Hay DE, Cooke KD, Gissing CV, 1986. Experimental studies of Pacific herring gillnets. Fisheries Research 4: 191-211.

Heinen-Kay JL, Schmidt DA, Stafford AT, Costa MT, Peterson MN, Kern EMA, Langerhans RB, 2016. Predicting multifarious behavioural divergence in the wild. Animal Behaviour 121:3-10.

Herskin J, 1999. Effects of social and visual contact on the oxygen consumption of juvenile sea bass measured by computerized intermittent respirometry. Journal of Fish Biology 55:1075-1085.

Hille SM, Cooper CB, 2015. Elevational trends in life histories: revising the pace-of-life framework. Biological Reviews 90:204-213.

Killen SS, Marras S, McKenzie DJ, 2011. Fuel, fasting, fear: routine metabolic rate and food deprivation exert synergistic effects on risk-taking in individual juvenile European sea bass. Journal of Animal Ecology 80:1024-1033.

Killen SS, Marras S, Metcalfe NB, McKenzie DJ, Domenici P, 2013. Environmental stressors alter relationships between physiology and behaviour. Trends in Ecology & Evolution 28:651-658.

Krause J, Loader SP, McDermott J, Ruxton GD, 1998. Refuge use by fish as a function of body length–related metabolic expenditure and predation risks. Proceedings of the Royal Society of London B: Biological Sciences 265:2373-2379.

Langerhans RB, Layman CA, Shokrollahi AM, DeWitt TJ, 2004. Predator-driven phenotypic diversification in Gambusia affinis. Evolution 58:2305-2318.

Law R, 1979. Harvest optimization in populations with age distributions. The American Naturalist 114:250-259.

MacArthur RH, Wilson EO, 2015. Theory of island biogeography (MPB-1): Princeton University Press.

Mathot KJ, Dingemanse NJ, 2015. Energetics and behavior: unrequited needs and new directions. Trends in ecology & evolution 30:199-206.

Metcalfe NB, Van Leeuwen TE, Killen SS, 2016. Does individual variation in metabolic phenotype predict behaviour and performance in fish? Journal of Fish Biology 88:298-321.

Missiroli A, 1930. Large-Scale Bonification with Regard to Biology and Hygiene. Rivista di Malariologia 9.

Nakagawa S, Schielzeth H, 2010. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biological Reviews 85:935-956.

Nespolo RF, Franco M, 2007. Whole-animal metabolic rate is a repeatable trait: a meta-analysis. Journal of Experimental Biology 210:2000-2005.

Pankhurst NW, Munday PL, 2011. Effects of climate change on fish reproduction and early life history stages. Marine and Freshwater Research 62:1015-1026.

Pardoe H, Vainikka A, Thordarson G, Marteinsdottir G, Heino M, 2009. Temporal trends in probabilistic maturation reaction norms and growth of Atlantic cod (*Gadus morhua*) on the Icelandic shelf. Canadian Journal of Fisheries and Aquatic Sciences 66:1719-1733.

Pepin P, 1991. Effect of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. Canadian Journal of Fisheries and Aquatic Sciences 48:503-518.

Pinheiro JC, Bates DM, 2000. Linear mixed-effects models: basic concepts and examples. Mixed-effects models in S and S-Plus:3-56.

Polverino G, Cigliano C, Nakayama S, Mehner T, 2016a. Emergence and development of personality over the ontogeny of fish in absence of environmental stress factors. Behavioral Ecology and Sociobiology 0:1-11.

Polverino G, Ruberto T, Staaks G, Mehner T, 2016b. Tank size alters mean behaviours and individual rank orders in personality traits of fish depending on their life stage. Animal Behaviour 115:127-135.

Pyke GH, 2005. A review of the biology of *Gambusia affinis* and *G. holbrooki*. Reviews in Fish Biology and Fisheries 15:339-365.

R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

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. Philosophical Transactions of the Royal Society B: Biological Sciences 365:4051-4063.

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ, 2007. Integrating animal temperament within ecology and evolution. Biological Reviews 82:291-318.

Reznick D, Butler IV MJ, Rodd H, 2001. Life-history evolution in guppies. VII. The comparative ecology of high-and low-predation environments. The American Naturalist 157:126-140.

Reznick DN, Travis J, 1996. The empirical study of adaptation in natural populations. In: Rose MR, Lauder GV, editors. Adaptation San Diego, California: Academic Press. p. 243-289.

Roff D, 1993. Evolution of life histories: theory and analysis. New York: Chapman & Hall.

Rønning B, Broggi J, Bech C, Moe B, Ringsby TH, Pärn H, Hagen IJ, Sæther BE, Jensen H, 2015. Is basal metabolic rate associated with recruit production and survival in free-living house sparrows? Functional Ecology 30:1140-1148.

Rudin FS, Briffa M, 2012. Is boldness a resource-holding potential trait? Fighting prowess and changes in startle response in the sea anemone, *Actinia equina*. Proceedings of the Royal Society of London B: Biological Sciences 279:1904-1910.

Santostefano F, Wilson AJ, Niemelä PT, Dingemanse NJ, (submitted). Behavioral mediators of genetic life-history trade-offs in field crickets. XXX X:000.

Schluter D, 2009. Evidence for ecological speciation and its alternative. Science 323:737-741.

Schoener TW, 1983. Field experiments on interspecific competition. American Naturalist 122:240-285.

Self SG, Liang KY, 1987. Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under nonstandard conditions. Journal of the American Statistical Association 82:605-610.

Shaw RG, 1991. The comparison of quantitative genetic parameters between populations. Evolution:143-151.

Sih A, Bell AM, Johnson JC, Ziemba RE, 2004. Behavioral syndromes: an integrative overview. The Quarterly Review of Biology 79:241-277.

Sih A, Cote J, Evans M, Fogarty S, Pruitt J, 2012. Ecological implications of behavioural syndromes. Ecology Letters 15:278-289.

Sih A, Mathot KJ, Moiron M, Montiglio PO, Wolf M, Dingemanse NJ, 2015. Animal personality and state–behaviour feedbacks:

a review and guide for empiricists. Trends in Ecology & Evolution 30:50-60.

Skinner MK, 2015. Environmental epigenetics and a unified theory of the molecular aspects of evolution: a neo-Lamarckian concept that facilitates neo-Darwinian evolution. Genome Biology and Evolution 7:1296-1302.

Stamps JA, 2007. Growth-mortality tradeoffs and ‘personality traits’ in animals. Ecology Letters 10:355-363.

Stearns SC, 1977. The evolution of life history traits: a critique of the theory and a review of the data. Annual Review of Ecology and Systematics 8:145-171.

Stearns SC, 1989. Trade-offs in life-history evolution. Functional Ecology 3:259-268.

Stearns SC, 1992. The evolution of life histories. Oxford: Oxford University Press.

Stearns SC, 2000. Life history evolution: successes, limitations, and prospects. Naturwissenschaften 87:476-486.

Visscher PM, 2006. A note on the asymptotic distribution of likelihood ratio tests to test variance components. Twin research and human genetics 9:490-495.

Ward AJW, Mehner T, 2010. Multimodal mixed messages: the use of multiple cues allows greater accuracy in social recognition and predator detection decisions in the mosquitofish, *Gambusia holbrooki*. Behavioral Ecology 21:1315-1320.

Werner EE, Anholt BR, 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. American Naturalist:242-272.

White CR, Kearney MR, 2013. Determinants of inter-specific variation in basal metabolic rate. Journal of Comparative Physiology B 183:1-26.

White CR, Schimpf NG, Cassey P, 2013. The repeatability of metabolic rate declines with time. Journal of Experimental Biology 216:1763-1765.

Wilbur HM, Tinkle DW, Collins JP, 1974. Environmental certainty, trophic level, and resource availability in life history evolution. The American Naturalist 108:805-817.

Wolf M, van Doorn GS, Leimar O, Weissing FJ, 2007. Life-history trade-offs favour the evolution of animal personalities. Nature 447:581-584.

Wolf M, Weissing FJ, 2010. An explanatory framework for adaptive personality differences. Philosophical Transactions of the Royal Society B: Biological Sciences 365:3959-3968.

Woodhams DC, Bell SC, Bigler L, Caprioli RM, Chaurand P, Lam BA, Reinert LK, Stalder U, Vazquez VM, Schliep K, Hertz A, 2016. Life history linked to immune investment in developing amphibians. Conservation Physiology 4:000.

MATERIALS AND METHODS

Life-history assessment

Body shape of each individual was analyzed using the geometric morphometry-landmarks method (Rohlf and Marcus, 1993). Eleven (males) and ten (females) different landmarks were selected similarly to previous studies on mosquitofish (Langerhans et al., 2004). As described in detail by Langerhans et al. (2004), fixed landmarks were chosen to characterize especially the region of the fish tail devoted to locomotor performances (i.e., Swimming muscle, mm^2), according to the area marked between landmarks 4-8 (Figure S1). Gonopodium length (mm) was also measured in male fish and it was represented by the segment defined by landmarks 9 and 10 (Figure S1). The outline of female individuals was instead characterized by 10 landmarks only (i.e., absence of landmark 9).

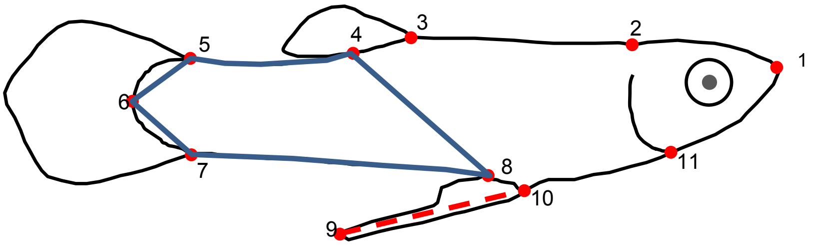


Figure S1. Outline of an adult male *Gambusia holbrooki* based on 11 fixed landmarks. Landmarks were defined as follows: 1, tip of the snout; 2, depression on the end of the head; 3 and 4, insertions of the dorsal fin; 5 and 7, insertions of the caudal fin; 6, end of the caudal muscle; 8 and 10, insertions of the anal fin; 9 (only in males), tip of the gonopodium; and 11, insertion of the operculum. Polygon in shade represents the "Swimming muscle", following the description by (Langerhans et al., 2004). The red dashed line represents the Gonopodium length.

Figure S2. Snapshot of the abdominal cavity of an adult female *Gambusia holbrooki* used for fecundity measurements (number of eggs, their Dry weight, and Mean dry weight per egg).

The xy coordinates of those landkmarks were digitalized using tpsDig2 software (Rohlf, 2004) and superimposed using a Generalized Procrustes Analysis (GPA) as implemented in the function *procGPA* from the *shapes* library (Dryden and Dryden, 2012) of the R-3.1.1 software (R Core Team, 2013). The superimposed landmarks were used as input for a MANOVA using redundancy analysis (RDA) to test for average differences in the total morphometry of adult individuals between the two populations. The test was performed on males and females separately since the number of landmarks varied between genders. Furthermore, the individual size (i.e., Standard length) was standardized to remove differences in size between individuals.

The total Number of eggs and their Dry weight (mg) were measured per each female separately with an electronic microscope (Modular stereo microscope MZ8, Leica Microsystems, Wetzlar, Germany, www.leica-microsystems.com) and a laboratory-weight scale (Secura 124-1CCH analytical balance, Sartorius AG, Goettingen, Germany, www.sartorius.com) respectively, while the Mean dry weight per egg (mg) was estimated indirectly as the total Number of eggs for a given female divided by their total Dry weight (mg).

RESULTS

Morphometric measurements

RDA results offered significant whole body shape differences between fish from the slow-growing (SG) and the fast-growing (FG) population in both males and females (F_1=3.2858, P= 0.033; and F_1=4.1952 P= 0.001, respectively; Figure S3).

Correlation between Activity in the open field and in the housing Plexiglas cylinder

Two weeks after the conclusion of the behavioral assay, we have selected three groups of six adult fish each (i.e., 18 fish total) that respectively spent the lower, medium, and higher time swimming (i.e., Swimming time, s) within the open field. Then, we have tested them again for their Swimming time, but within their housing Plexiglas cylinders. As for the tests in the open field, the behavior of each fish was measured across two 10-min trials, two days apart from each other.

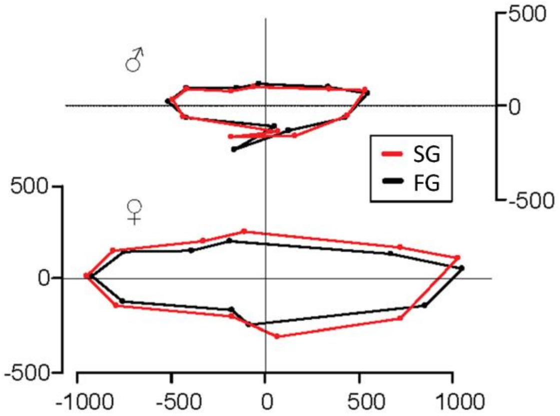


Figure S3. Schematic of the average body shape outlined for both males (above) and females (below) separately for each population (SG and FG). Differences are magnified (x3) to facilitate the graphical identification of the landmarks driving those differences. The units are based on the adimensional redimensioning carried out by the GPA analysis (the coordinate 0, 0 is the centroid center). Notably, Standard body size was standardized to favor a comprehensive interpretation of differences between populations for each gender.

We ran a Pearson product-moment correlation to test whether Swimming time in the open field and in the housing Plexiglas cylinders were correlated with each other, that is, whether among-individual differences in activity rates measured in the open field reflected different activity rates among fish within their housings. We observed that Swimming time measured in the open field was a valid proxy for the Swimming time observed in fish within their housing Plexiglas cylinders (Figure S4).

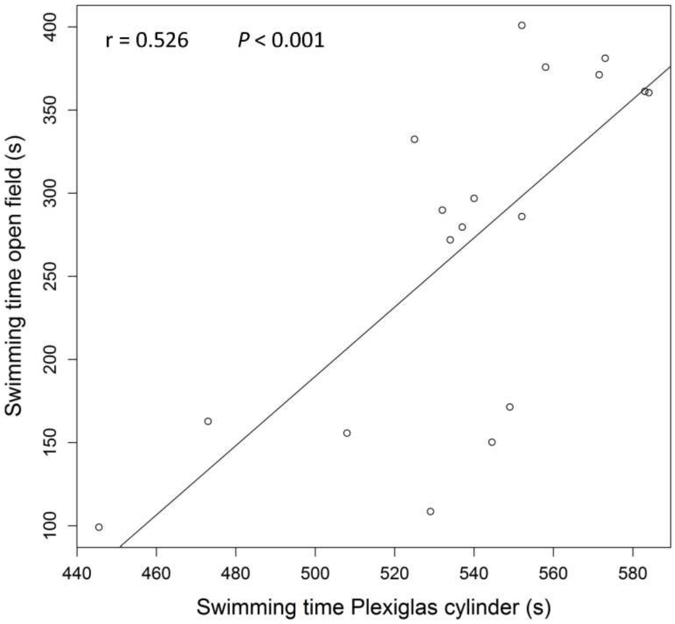


Figure S4. Correlation between Swimming time in the open field and Swimming time in the housing Plexiglas cylinder

Univariate LMMs: correlations between pairs of fixed effects

We estimated the phenotypic correlation (i.e., the overall correlation jointly contributed by among- and within-individual correlations; (Dingemanse and Dochtermann, 2013)) between each pair of life-history traits (LHTs) measured. To do this, we used bivariate linear mixed-effects models (LMMs), as suggested by (Dingemanse and Dochtermann, 2013), using Markov Chain Monte Carlo techniques, while including individuals as a random effect (i.e., random intercepts) to account for repeated measures of the same individuals over time. The bivariate LMMs were performed using MCMC sampling methods under a Bayesian framework (R package ‘MCMCglmm’; (Hadfield, 2010)). The parameters were estimated using a non-informative prior, with 1 500 000 resamplings, 500 000 burn-ins and 100 thinnings.

Table S1. Phenotypic-correlation estimates between pairs of LHTs. The best estimate of correlation coefficients (i.e. values above the diagonal) and their 95% credible intervals (i.e. values below the diagonal) are represented for each pair of LHTs. Significant results are represented in bold and correspond to correlation coefficients whose confidence intervals do not overlap with zero.

	Standard size	Fulton's K	Body weight
Standard size	-	-0.065	**0.597**
Fulton's K	-0.155 0.029	-	0.058
Body weight	**0.521** **0.669**	-0.099 0.219	-

Covariances and correlations between repeatable traits for each population

Table S2. Estimated among-individual covariances and correlations (± SE) between two behaviors (Shyness and Activity), Standard size, and specific Standard metabolic rate (SMR) in slow-growing (SG) and fast-growing (FG) fish. We present covariances (lower-off diagonals) and correlations (upper-off diagonals) for each set of traits. Correlations printed in bold-face are significant ($P<0.05$) based on likelihood ratio tests derived from the multivariate model detailed in the main text.

	SG				**FG**			
	Shyness	Activity	Size	SMR	Shyness	Activity	Size	SMR
Shyness	-	**0.840** **(0.240)**	0.204 (0.267)	-0.323 (0.278)	-	0.100 (0.290)	-0.032 (0.367)	0.155 (0.292)
Activity	**0.223** **(0.093)**	-	**0.547** **(0.175)**	-0.382 (0.220)	0.023 (0.069)	-	**-0.817** **(0.302)**	-0.004 (0.286)
Size	0.056 (0.075)	**0.203** **(0.093)**	-	**-0.814** **(0.081)**	-0.002 (0.020)	**-0.046** **(0.022)**	-	0.441 (0.415)
SMR	-0.067 (0.062)	-0.108 (0.071)	**-0.237** **(0.078)**	-	0.024 (0.046)	-0.001 (0.046)	0.017 (0.014)	-

REFERENCES

Dingemanse NJ, Dochtermann NA, 2013. Quantifying individual variation in behaviour: mixed-effect modelling approaches. Journal of Animal Ecology 82:39-54.

Dryden I, Dryden MI, 2012. Shapes package. Vienna, Austria: R Foundation for Statistical Computing.

Hadfield JD, 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. Journal of Statistical Software 33:1-22.

Langerhans RB, Layman CA, Shokrollahi A, DeWitt TJ, 2004. Predator-driven phenotypic diversification in *Gambusia affinis*. Evolution 58:2305-2318.

R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Rohlf FJ, 2004. tpsdig2 Stony Brook. NY: Department of Ecology and Evolution, State.

Rohlf FJ, Marcus LF, 1993. A revolution morphometrics. Trends in Ecology & Evolution 8:129-132.

Chapter 6

General discussion

Research on animal personality has flourished in recent years, but methodological and conceptual controversies within this young research field still undermine its progress (Carter et al. 2013). This thesis aims to offer novel insights on the mechanisms behind the emergence and development of personality over lifetime of animals, the context dependency of animal personality, and the relationship between personality, metabolic, and life-history traits. In this chapter, I discuss the current state of knowledge, synthesize the thesis findings, state their contribution to current knowledge, and suggest new perspectives for future research.

EMERGENCE AND DEVELOPMENT OF ANIMAL PERSONALITY OVER LIFETIME

Genetic differences, parental effects, and early life experiences are considered to be some of the main sources of behavioral differences among animals (Réale et al. 2007; Stratmann and Taborsky 2014). The consistency of those differences over time plays a key role in the animal personality concept (Réale et al. 2007). However, most empirical studies have concentrated on relatively short time intervals compared to the lifespan of an animal (Biro and Stamps 2015) and studies on animal personality that follow individuals over longer periods of time are scarce (Trillmich and Hudson 2011). As a consequence, surprisingly little attention was given to developmental origins of animal personality (Trillmich and Hudson 2011). Longitudinal studies might then provide new insights on underlying mechanisms that generate or strengthen personality in animals (Stamps and Groothuis 2010; Trillmich et al. 2015). Under this perspective, theoretical studies have moved further ahead than empirical evidence (Fischer et al. 2014; Fawcett and Frankenhuis 2015; Panchanathan and Frankenhuis 2016).

In chapter 2, I tested whether personality differences were present and consistently maintained over lifetime of fish once their main sources of behavioral variation were minimized. Surprisingly, I observed that personality was present in mosquitofish despite minimizing genetic, maternal, and environmental differences among individuals. More interestingly, I observed that personality differences were initially low and increased during lifetime as a function of consistent declines in behavioral plasticity. Results from my study confirm theoretical predictions on the decline of behavioral plasticity in animals with increasing age (Fischer et al. 2014; Fawcett and Frankenhuis 2015; Panchanathan and Frankenhuis 2016), suggesting that behavior in adult animals might be more predictable than in juveniles. Similar results have been widely observed in the human-personality literature (reviewed by (Baltes and Baltes 1993)), but this study represents the very first evidence of a loss in behavioral plasticity during the lifetime of animals. The development of animal personality over lifetime contrasts with cornerstone predictions that personality is consistent over time and mainly caused by genetic, maternal, and environmental differences among individuals (see (Réale et al. 2007)). Results from chapter 2 provide the first evidence that animal personality indeed develops over ontogeny, despite controlling for its typical triggers.

Overall, results from chapter 2 suggest that animal personality might be the inevitable outcome of development. In particular, the decline in behavioral plasticity might be capable of magnifying small initial differences in the behavior of animals, thus representing an intrinsic mechanism for their behavioral differentiation.

AGE-DEPENDENT VULNERABILITY OF PERSONALITY ESTIMATES TO EXPERIMENTAL BIASES

Differences in behavior among animals from the same population have been found to be more repeatable in the wild than in laboratory contexts (Bell et al. 2009). As a consequence, it has been suggested that animal personality, which is by definition consistent between contexts (Réale et al. 2007), might be compromised or misrepresented under laboratory settings (Carter et al. 2013; Niemelä and Dingemanse 2014). Examples of the context dependency of animal personality are well documented in laboratory studies (see (van Oers et al. 2005) and references included therein). With respect to fishes, small variations in water temperature (Biro et al. 2010) or different gate sizes where individuals entered an open field (Näslund et al. 2015) have been found to alter the rank order in personality traits of individuals. However, the current literature mainly refers to context dependency of personality in juvenile animals, especially in fishes.

Building on results from chapter 2, in chapter 3 I explored whether personality estimates on juvenile mosquitofish were more vulnerable to context variations compared to adults. As expected, I observed that changes in the size of the test tank, common in fish personality literature (see references in (Polverino et al. 2016b), compromised the consistency of personality ranks only in juvenile fish. In contrast, personality rank orders were always maintained in adult fish across treatments and I observed intermediate results for individuals at the onset of sexual maturation (i.e., subadults). In short, results from chapter 3 indicate no or only a weak context dependency of behavior in adult and subadult individuals, while personality estimates in young fish were remarkably altered by varying experimental settings.

Findings from chapter 3 thus support the idea that low behavioral repeatabilities can affect the experimental determination of personality primarily in young animals. More specifically, changes in laboratory contexts (i.e., the size of the test tank) might be sufficient to compromise personality estimates in immature animals, but not in adults.

PACE-OF-LIFE SYNDROME IN LABORATORY *VERSUS* WILD CONTEXTS

The coexistence of personality types within animal populations has been related to consistent individual variation in maintenance metabolism (Wolf and Weissing 2010; Sih et al. 2015) and life history trade-offs (Stamps 2007; Wolf et al. 2007). This concept is commonly expressed as the pace-of-life syndrome hypothesis (POLS; (Réale et al. 2010)). Accordingly, animals should differ from each other in a suite of traits related to behavior, metabolism, and life history that are correlated at the individual level (Réale et al. 2010). With respect to fishes, higher metabolic demands are expected to promote foraging (Krause et al. 1998), resulting in increased exposure to risky situations (Ahrens et al. 2012). If not fatal, risk propensity should result in greater abilities to compete for limited resources and higher productivity rates (e.g., growth and fecundity) of individuals (Biro and Stamps 2008). Nevertheless, approximately 50 percent of the empirical studies do not support predictions from the POLS (Dammhahn et al. in prep). The discrepancy between theoretical predictions and empirical evidence might be partially explained by methodological issues when testing the POLS (Mathot and Dingemanse 2015). In this view, laboratory settings might be inadequate to capture meaningful variations in labile traits, such as behavior, outside their evolutionary context (Niemelä and Dingemanse 2014). Moreover, the lack of environmental challenges typical of laboratory environments (e.g., absence of predators and/or risk of starvation) could mask the latent link between behavior, metabolic, and life-history traits of individuals (Killen et al. 2013) and, therefore, impede the actual testing of the POLS assumptions. For example, predation threat is expected to reveal individual differences in risk-taking behavior depending on individual differences in energetic demands.

Based on the methodological uncertainties above, in chapter 4 of this thesis I tested the POLS hypothesis in both laboratory and wild conditions through two independent studies. I selected two distinct fish species, namely zebrafish (laboratory) and Northern pike (wild), each one presenting its own strengths under the given experimental conditions. Results from both studies supported the presence of personality in fishes, independent of the experimental setting adopted for the tests. In addition, personality types of wild pikes measured in a standardized assay reflected the ones from the same individuals expressed in their natural habitat. Nevertheless, neither of the studies supported the overall predictions from the POLS. In fact, behavioral, metabolic, and life-history traits were overall not correlated at the individual level, under both laboratory and natural settings. However, I observed that laboratory zebrafish with a smaller body size were more active and potentially risk prone compared to their larger siblings (i.e., personality types and size at a given age were negatively correlated). Differently, personality in wild pikes was unrelated to other traits and individual variation in metabolic rates was instead associated to body condition and adult growth.

Findings from the two studies in chapter 4 do not support POLS predictions, independent of whether testing laboratory or wild animals under standardized or more natural conditions. However, these results point out that animal personality can be successfully tested under standardized settings. Therefore, results from chapter 4 suggest that methodological issues linked to artificial environments may not fully explain the discrepancy between theoretical predictions and empirical evidence on the POLS.

PACE-OF-LIFE SYNDROME UNDER DIFFERENT SELECTION PRESSURES

Building upon results from chapter 4 (and many other studies before), new efforts are currently focused on revising certain aspects of the POLS hypothesis in an attempt to solve its conceptual controversies (see (Careau et al. 2011; Hille and Cooper 2015; Mathot and Dingemanse 2015)). For example, the presence, sign, and strength of correlations between metabolic and personality traits may vary depending on divergent trade-offs between those traits (Mathot and Dingemanse 2015), as observed between mammal and fish species (see (Careau et al. 2011) and references therein). Under a more general evolutionary perspective, it is reasonable to believe that opposite selection pressures might favor or suppress trade-offs between specific traits described in the POLS, as reviewed by (Hille and Cooper 2015) across latitudinal and altitudinal gradients. In this vein, Sih and coauthors (2004) suggested that stable selective pressures might act against deleterious correlations, with traits expected to evolve freely under those conditions (Sih et al. 2004).

In the last study included in this thesis (chapter 5), I tested the POLS predictions in two separated and geographically isolated fish populations adapted to different biotic and abiotic conditions. By utilizing first-generation individuals kept under standardized conditions since birth, I tested whether evolutionary adaptations might alter phenotypic correlations in animals. At the between-population level, I observed that slower life histories were coupled with bolder and more active behaviors and higher mass-specific metabolic costs compared to fast life-history trajectories. This result contrasts with the concept of fast and slow POLS (i.e., "performance model"; (Careau et al. 2008)). Instead, results from this study suggest that in an energy-limited environment adaptation might favor diverse strategies for allocating energy between competing functions (i.e., "allocation model"; (Vaanholt et al. 2007)). When testing the POLS at the among-individual level, repeatable differences in personality, metabolic rates, and life-history traits were present in both populations. Nevertheless, I observed that those traits were overall correlated only in the fish population with the slower life history. In contrast with POLS predictions, correlations among traits were marginal in the population with the faster life history.

In summary, results from chapter 5 bring novel insights on the POLS and suggest that its rules might not apply to all animal populations. Correlations between traits included in the POLS may, therefore, differ among animal populations that evolved under divergent selection pressures.

SHIFT IN MEAN BEHAVIORS DURING ONTOGENY OF FISH

Activity and risk-taking behaviors typically vary over the ontogeny of fishes (Dial et al. 2008), especially in response to varying foraging and risk-taking optimal strategies (Clark 1994; Biro et al. 2005). In fact, fish size is a key predictor to discriminate who eats whom in most aquatic ecosystems (Lundvall et al. 1999). However, predictions for changes in behavioral strategies during ontogeny of fishes are contradictory. On one side, predator-prey theories suggest that adult and large fishes should be more inclined to take risks compared to juveniles because they are less vulnerable to predation by gape-limited predators (Sogard 1997; Nilsson and Brönmark 2000). On the other side, adult and large individuals should be less willing to take risks compared to juveniles because high reproductive values of adults tend to be highly protected (Clark 1994; Biro et al. 2005).

In chapters 2, 3, and 5, I explored whether and how behavioral strategies varied, on average, over the lifetime of the Eastern mosquitofish (i.e., the model species utilized in all ontogenetic studies included in this thesis). Accordingly, I observed that both activity and risk taking varied over ontogeny of mosquitofish. In particular, young fish were, on average, less risk prone and their activity pattern was dominated by rapid accelerations compared to adults (chapter 3). However, mosquitofish tended to move less and for shorter distances with increasing age (chapters 2 and 5). It is reasonable to believe that juvenile mosquitofish were less prone to take risks and were more reactive in their swimming than adults because they are more susceptible to gape-limited predation (Nilsson and Brönmark 2000). This approach should be especially valid for cannibalistic species, such as mosquitofish, in which adult individuals represent a main threat for newborns (Pyke 2005). Nevertheless, lower energy requirements of larger individuals compared to smaller ones may have required juvenile fish to be more explorative in the attempt to look for food.

Despite my keen interest on the results above, I refrained from elaborating more detailed interpretations of those results. Indeed, the strength of these studies (chapters 2, 3, and 5) consists in their ability to finely control various aspects of the experimental environment and I am aware that they are not well suited to make ecological inferences. The exact ecological reason for varying behavioral strategies during the lifetime of mosquitofish has a marginal interest in this thesis. Nevertheless, the variable behavioral strategies detected here during the lifetime of mosquitofish support the choice of this species for investigating how personality traits were, instead, maintained over time at the individual level.

OUTLOOK AND AVENUES FOR FUTURE RESEARCH

Until recently, most of the empirical studies have concentrated on confirming the existence of animal personality, with examples across all of the animal kingdom. Such a descriptive approach has unquestionably brought attention to this young research field, with growing interest in its potential consequences at the ecological and evolutionary level. Nevertheless, the current understanding of the mechanisms behind the emergence, persistence, and correlation of animal personality with other ecologically-relevant polymorphisms is still scarce. Both methodological and conceptual controversies have delayed the progress of this field and mechanistic approaches are increasingly necessary to explore its core aspects. In my view, the surface of this emerging research field has just been scratched.

Results from this thesis bring attention to two immediate research questions: (1) Do environmental challenges speed up the behavioral differentiation of animals by acting, in opposite directions, on both variance components of behavior? In other words, does plasticity of individuals decline while differences in behavior among individuals increase over lifetime in presence of environmental challenges?; and (2) Are metabolic rates in young animals more plastic than in adults as observed in their behaviors (i.e., chapters 2 and 3)? In my opinion, answering these two questions would provide a better understanding of animal personality. On one hand, the first research question could help clarify how behavioral differences in animals develop in a more "realistic" ecological scenario, without losing the benefit of standardization. On the other hand, by exploring the behavioral and metabolic response of individuals along ontogenetic transitions, the second research question could help uncover the relative role of a physiological basis behind behavioral variation and ultimately animal personality.

In general, uncovering the role of age on personality expression may add new insight into the understanding of personality variation in animals and may inform other fields that can build on animal personality research, such as population dynamics.

Finally, I believe that more studies testing POLS predictions across ecological gradients are needed. By following the classical life-history literature, new studies should investigate whether selective factors (e.g., predation) impact correlations between behavioral, metabolic, and life-history traits in animals.

Acknowledgements

Time flies people say and I feel it now more than ever. I want to thank all people who contributed in making the last three years a tremendous experience for me to grow both as a scientist and as a person.

Firstly, I am grateful to the B-Type group for funding my PhD and all of the conferences and workshops I attended during this time. I will never forget the day of my first colloquium at the IGB… I was terrified to present my ideas in front of successful scientists that I presumed to be very severe (I am not sure why I presumed it actually). Nothing could be more wrong than that! I did not stop laughing during the first dinner with the B-type Pis. The familiar atmosphere within the B-Type group (and the whole IGB in general) made this whole experience both extremely productive and enjoyable for me.

I especially thank my supervisor Thomas Mehner for his extraordinary support, for encouraging me, and being available every time I needed it. His role in the progress of my PhD has been invaluable. He helped to improve my writing skills (renouncing sometimes to a florid and articulated style in favor of clarity and logic), improve the scientific reasoning, and to make plans that can be reasonably accomplished within a given time interval. My sincere thanks also go to Georg Staaks for his support especially in the first stage of my PhD and for allowing me to use all of the facilities from his lab. Robert Arlinghaus has also been a great source of inspiration for my studies. His critical and logic way of thinking about science helped me a lot when I designed my experiments and I acknowledge him for involving me in some of the research activities developed within his group, which are in part included in this thesis. I am thankful to Max Wolf for his help with the experimental designs and for

revising some of the manuscripts included in this thesis. His help and expertise has been fundamental for me to gain an in depth understanding of the animal personality theory and for formulating my own research. The contagious enthusiasm from Jens Krause has been a great source of inspiration and I truly thank him for introducing me to the social network theory. Working with him has been really enjoyable and he has also been a terrific travel companion during the best conference I have ever attended (Behaviour 2015 in Cairns, Australia).

Countless are the reasons why I want to thank David Bierbach, Ralf Kurvers, Kate Laskowski, Christopher Monk, and Shinnosuke Nakayama. They have been the demonstration that being good friends and having fun together is a refreshing medicine against the sometimes boring aspects of science. I learned a lot from each of them and their support with my studies contributed to improve their quality. I am proud of the results that we have obtained together. The constructive discussions I had with Arne Schröder and Gregor Kalinkat have also contributed to improve my research skills and I thank them both for that. A special thank you goes to Ralf, Christopher, and Maria Elena Miletto Petrazzini for reviewing my thesis, to David who also reviewed my thesis and translated the German summary, and to Tanya Shadbolt who did the proofreading and also took care of this wonderful layout. A very special thank you goes to all my coauthors for their patience and help.

I am grateful to Christian Baal, Marcus Herbert, Susanne Joop, Karena Kuntze, David Lewis, Asja Vogt, Henrik Zwadlo and to all the student assistants and Okis for their technical assistance and for helping with the data collection.

I would also like to thank my former supervisors Enrico Alleva, Maurizio Porfiri, and Simone Macrì for their precious support before and during my PhD, for visiting me in Berlin, and having fun together.

Finally, I thank my mother, father, sister, future wife, and friends for their support during the whole PhD process. Some of them helped me capture my experimental fish from their native ponds, some helped me make fancy 3D figures for my manuscripts, many of them kept my motivation up, and with others I had a lot of fun when it was time to stop thinking. A big thank you goes to all of them. Nevertheless, the biggest among all of my thanks go to my future wife for filling my life with her love that soon will have a name, the name of a little girl.

References

Ahrens RN, Walters CJ, Christensen V (2012) Foraging arena theory. Fish and Fisheries 13:41-59

Arlinghaus R, Laskowski KL, Alós J, Klefoth T, Monk CT, Nakayama S, Schröder A (2016) Passive gear induced timidity syndrome in wild fish populations and its potential ecological and managerial implications. Fish and Fisheries:000

Baltes PB, Baltes MM (1993) Successful aging: Perspectives from the behavioral sciences, vol 4. Cambridge University Press, UK

Bell AM, Hankison SJ, Laskowski KL (2009) The repeatability of behaviour: a meta-analysis. Animal Behaviour 77:771-783

Bell AM, McGhee KE, Stein LR (2016) Effects of mothers' and fathers' experience with predation risk on the behavioral development of their offspring in threespined sticklebacks. Current Opinion in Behavioral Sciences 7:28-32

Biro PA, Beckmann C, Stamps JA (2010) Small within-day increases in temperature affects boldness and alters personality in coral reef fish. Proceedings of the Royal Society B: Biological Sciences 277:71e77

Biro PA, Post JR, Abrahams MV (2005) Ontogeny of energy allocation reveals selective pressure promoting risk-taking behaviour in young fish cohorts. Proceedings of the Royal Society B: Biological Sciences 272:1443-1448

Biro PA, Stamps JA (2008) Are animal personality traits linked to life-history productivity? Trends in Ecology & Evolution 23:361-368

Biro PA, Stamps JA (2015) Using repeatability to study physiological and behavioural traits: ignore time-related change at your peril. Animal Behaviour 105:223-230

Boon AK, Réale D, Boutin S (2007) The interaction between personality, offspring fitness and food abundance in North American red squirrels. Ecology Letters 10:1094-1104

Careau V, Réale D, Humphries MM, Thomas DW (2010) The pace of life under artificial selection: personality, energy expenditure, and longevity are correlated in domestic dogs. The American Naturalist 175:753-758

Careau V, Thomas D, Humphries MM, Réale D (2008) Energy metabolism and animal personality. Oikos 117:641-653

Careau V, Thomas D, Pelletier F, Turki L, Landry F, Garant D, Réale D (2011) Genetic correlation between resting metabolic rate and exploratory behaviour in deer mice (*Peromyscus maniculatus*). Journal of Evolutionary Biology 24:2153-2163

Carere C, Eens M (2005) Unravelling animal personalities: how and why individuals consistently differ. Behaviour 142:1149-1157

Carter AJ, Feeney WE, Marshall HH, Cowlishaw G, Heinsohn R (2013) Animal personality: what are behavioural ecologists measuring? Biological Reviews 88:465-475

Clark CW (1994) Antipredator behavior and the asset-protection principle. Behavioral Ecology 5:159-170

Costa PT, McCrae RR (1992) Four ways five factors are basic. Personality and Individual Differences 13:653-665

Dial KP, Greene E, Irschick DJ (2008) Allometry of behavior. Trends in Ecology & Evolution 23:394-401

Dingemanse NJ, Kazem AJ, Réale D, Wright J (2010) Behavioural reaction norms: animal personality meets individual plasticity. Trends in Ecology & Evolution 25:81-89

Dochtermann NA, Schwab T, Sih A (2015) The contribution of additive genetic variation to personality variation: heritability of personality. Proceedings of the Royal Society B: Biological Sciences 282:20142201

Edenbrow M, Croft DP (2013) Environmental and genetic effects shape the development of personality traits in the mangrove killifish *Kryptolebias marmoratus*. Oikos 122:667-681

Enberg K, Jørgensen C, Dunlop ES, Varpe Ø, Boukal DS, Baulier L, Eliassen S, Heino M (2012) Fishing-induced evolution of growth: concepts, mechanisms and the empirical evidence. Marine Ecology 33:1-25

Evans JP, Pilastro A, Schlupp I (2011) Ecology and evolution of poeciliid fishes. University of Chicago Press., Chicago

Favati A, Zidar J, Thorpe H, Jensen P, Løvlie H (2015) The ontogeny of personality traits in the red junglefowl, *Gallus gallus*. Behavioral Ecology:arv177

Fawcett TW, Frankenhuis WE (2015) Adaptive explanations for sensitive windows in development. Frontiers in Zoology 12:1

Fischer B, van Doorn GS, Dieckmann U, Taborsky B (2014) The evolution of age-dependent plasticity. The American Naturalist 183:108-125

Fisher DN, David M, Tregenza T, Rodríguez-Muñoz R (2015) Dynamics of among-individual behavioral variation over adult lifespan in a wild insect. Behavioral Ecology:arv048

Freund J, Brandmaier AM, Lewejohann L, Kirste I, Kritzler M, Krüger A, Sachser N, Lindenberger U, Kempermann G (2013) Emergence of individuality in genetically identical mice. Science 340:756-75

Gerlai R (2010) High-throughput behavioral screens: the first step towards finding genes involved in vertebrate brain function using zebrafish. Molecules 15:2609-2622

Goldberg LR (1990) An alternative "description of personality": the big-five factor structure. Journal of Personality and Social Psychology 59:1216

Gorski JN, Dunn-Meynell AA, Hartman TG, Levin BE (2006) Postnatal environment overrides genetic and prenatal factors influencing offspring obesity and insulin resistance. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 291:R768-R778

Gosling SD (2001) From mice to men: what can we learn about personality from animal research? Psychological Bulletin 127:45

Gosling SD, John OP (1999) Personality dimensions in nonhuman animals a cross-species review. Current Directions in Psychological Science 8:69-75

Greven H (2011) Gonads, genitals, and reproductive biology. In: Ecology and Evolution of Poeciliid Fishes. University of Chicago Press., Chicago, pp 3-17

Heinen-Kay JL, Schmidt DA, Stafford AT, Costa MT, Peterson MN, Kern EMA, Langerhans RB (2016) Predicting multifarious behavioural divergence in the wild. Animal Behaviour 121:3-10

Herborn KA, Macleod R, Miles WT, Schofield AN, Alexander L, Arnold KE (2010) Personality in captivity reflects personality in the wild. Animal Behaviour 79:835-843

Hille SM, Cooper CB (2015) Elevational trends in life histories: revising the pace-of-life framework. Biological Reviews 90:204-213

Killen SS, Marras S, Metcalfe NB, McKenzie DJ, Domenici P (2013) Environmental stressors alter relationships between physiology and behaviour. Trends in Ecology & Evolution 28:651-658

Krause J, Loader SP, McDermott J, Ruxton GD (1998) Refuge use by fish as a function of body length-related metabolic expenditure and predation risks. Proceedings of the Royal Society B: Biological Sciences 265:2373-2379

Krause J, Winfield AFT, Deneubourg JL (2011) Interactive robots in experimental biology. Trends in Ecology & Evolution 26:369-375

Laskowski KL, Monk CT, Polverino G, Alós J, Nakayama S, Staaks G, Mehner T, Arlinghaus R (2016) Behaviour in a standardized assay, but not metabolic or growth rate, predicts behavioural variation in an adult aquatic top predator *Esox lucius* in the wild. Journal of Fish Biology 88:1544-1563

Lienart GDH, Mitchell MD, Ferrari MCO, McCormick MI (2014) Temperature and food availability affect risk assessment in an ectotherm. Animal Behaviour 89:199-204

Lowe S, Browne M, Boudjelas S, De Poorter M (2000) 100 of the world's worst invasive alien species a selection from the global invasive species database. Aliens 12:1-12

Lundvall D, Svanbäck R, Persson L, Byström P (1999) Size-dependent predation in piscivores: interactions between predator foraging and prey avoidance abilities. Canadian Journal of Fisheries and Aquatic Sciences 56:1285-1292

Mathot KJ, Dingemanse NJ (2015) Energetics and behavior: unrequited needs and new directions. Trends in Ecology & Evolution 30:199-206

Miklósi Á, Andrew RJ (2006) The zebrafish as a model for behavioral studies. Zebrafish 3:227-234

Näslund J, Bererhi B, Johnsson JI (2015) Design of emergence test arenas can affect the results of boldness assays. Ethology 121:556-565

Nettle D, Bateson M (2015) Adaptive developmental plasticity: what is it, how can we recognize it and when can it evolve? Proceedings of the Royal Society B: Biological Sciences 282:20151005

Niemelä PT, Dingemanse NJ (2014) Artificial environments and the study of 'adaptive'personalities. Trends in Ecology & Evolution 29:245-247

Nilsson PA, Brönmark C (2000) Prey vulnerability to a gape-size limited predator: behavioural and morphological impacts on northern pike piscivory. Oikos 88:539-546

Palkovacs EP, Dalton CM (2012) Ecosystem consequences of behavioural plasticity and contemporary evolution. In: Candolin U, Wong BB (eds)

Behavioural responses to a changing world: mechanisms and consequences. Oxford University Press.

Panchanathan K, Frankenhuis WE (2016) The evolution of sensitive periods in a model of incremental development. Proceedings of the Royal Society B: Biological Sciences 283:20152439

Polverino G, Cigliano C, Nakayama S, Mehner T (2016a) Emergence and development of personality over the ontogeny of fish in absence of environmental stress factors. Behavioral Ecology and Sociobiology 0:1-11

Polverino G, Ruberto T, Staaks G, Mehner T (2016b) Tank size alters mean behaviours and individual rank orders in personality traits of fish depending on their life stage. Animal Behaviour 115:127-135

Pyke GH (2005) A review of the biology of *Gambusia affinis* and *G. holbrooki*. Reviews in Fish Biology and Fisheries 15:339-365

Raat AJ (1988) Synopsis of biological data on the northern pike: *Esox lucius* Linnaeus, 1758. Food and Agriculture Organization of the United Nations, Rome

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. Philosophical Transactions of the Royal Society B: Biological Sciences 365:4051-4063

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ (2007) Integrating animal temperament within ecology and evolution. Biological Reviews 82:291-318

Roff D (1993) Evolution of life histories: theory and analysis. Chapman & Hall, New York

Sih A, Bell AM, Johnson JC, Ziemba RE (2004) Behavioral syndromes: an integrative overview. The Quarterly Review of Biology 79:241-277

Sih A, Cote J, Evans M, Fogarty S, Pruitt J (2012) Ecological implications of behavioural syndromes. Ecology Letters 15:278-289

Sih A, Mathot KJ, Moiron M, Montiglio PO, Wolf M, Dingemanse NJ (2015) Animal personality and state–behaviour feedbacks: a review and guide for empiricists. Trends in Ecology & Evolution 30:50-60

Sogard SM (1997) Size-selective mortality in the juvenile stage of teleost fishes: a review. Bulletin of Marine Science 60:1129-1157

Stamps JA (2007) Growth-mortality tradeoffs and 'personality traits' in animals. Ecology Letters 10:355-363

Stamps JA, Groothuis TG (2010) Developmental perspectives on personality: implications for ecological and evolutionary studies of individual differences. Philosophical Transactions of the Royal Society B: Biological Sciences 365:4029-4041

Stamps JA, Krishnan VV (2014) Combining information from ancestors and personal experiences to predict individual differences in developmental trajectories. The American Naturalist 184:647-657

Stearns SC (1989) Trade-offs in life-history evolution. Functional Ecology 3:259-268

Stearns SC (1992) The evolution of life histories, vol 249. Oxford University Press., Oxford

Stearns SC (2000) Life history evolution: successes, limitations, and prospects. Naturwissenschaften 87:476-486

Stratmann A, Taborsky B (2014) Antipredator defences of young are independently determined by genetic inheritance, maternal effects and own early experience in mouthbrooding cichlids. Functional Ecology 28:944-953

Taborsky B (2006) Mothers determine offspring size in response to own juvenile growth conditions. Biology Letters 2:225-228

Toms CN, Echevarria DJ, Jouandot DJ (2010) A methodological review of personality-related studies in fish: focus on the shy-bold axis of behavior. International Journal of Comparative Psychology 23:1-25

Trillmich F, Günther A, Müller C, Reinhold K, Sachser N (2015) New perspectives in behavioural development: adaptive shaping of behaviour over a lifetime? Frontiers in Zoology 12:S1

Trillmich F, Hudson R (2011) The emergence of personality in animals: the need for a developmental approach. Developmental Psychobiology 53:505-509

Vaanholt LM, De Jong B, Garland T, Daan S, Visser GH (2007) Behavioural and physiological responses to increased foraging effort in male mice. Journal of Experimental Biology 210:2013-2024

van Oers K, Klunder M, Drent PJ (2005) Context dependence of personalities: risk-taking behavior in a social and a nonsocial situation. Behavioral Ecology 16:716-723

White CR, Kearney MR (2013) Determinants of inter-specific variation in basal metabolic rate. Journal of Comparative Physiology B 183:1-26

Wolf M, van Doorn GS, Leimar O, Weissing FJ (2007) Life-history trade-offs favour the evolution of animal personalities. Nature 447:581-584

Wolf M, Weissing FJ (2010) An explanatory framework for adaptive personality differences. Philosophical Transactions of the Royal Society B: Biological Sciences 365:3959-3968

Wolf M, McNamara JM (2012) On the evolution of personalities via frequency-dependent selection. The American Naturalist 179:679-692

Wolf M, Weissing FJ (2012) Animal personalities: consequences for ecology and evolution. Trends in Ecology & Evolution 27:452-461

Woodhams DC, Bell SC, Bigler L, Caprioli RM, Chaurand P, Lam BA, Reinert LK, Stalder U, Vazquez VM, Schliep K, Hertz A (2016) Life history linked to immune investment in developing amphibians. Conservation Physiology 4:000

Declaration of authorship

I hereby declare that this PhD thesis has been written only by the undersigned and that no sources other than those indicated have been used. This thesis has not been submitted for a doctor's degree at any other institution. I am aware of the underlying doctorate regulations of the faculty this thesis is submitted to, i.e., Faculty of Life Sciences of the Humboldt-University at Berlin.

Selbstandigkeitserklärung

Hiermit erkläre ich, die Dissertation selbständig und ohne unerlaubte Hilfe angefertigt zu haben. Ich habe mich nicht anderwärts als Doktorand beworben. Ich erkläre die Kenntnisnahme der dem Verfahren zugrunde liegenden Promotionsordnung der Lebenswissenschaftlichen Fakultät der Humboldt-Universität zu Berlin.

Berlin, den 28.09.2016
Giovanni Polverino

Curriculum vitae

Personal data removed for online publication

SELECTED PARTICIPATION IN CONFERENCES AND WORKSHOPS

2017 (Aug 28-31)	Oral presentation, as the first author, titled "Life-history strategies and pace-of-life syndrome hypothesis: unmatched predictions and varying correlation structures" at the 7th Congress of the Italian Society for Evolutionary Biology, Rome, Italy.
(Jul 30-Aug 4)	Oral presentation, as the first author, titled "Unexpected variability of pace-of-life syndrome in two populations of Eastern mosquitofish (*Gambusia holbrooki*) with opposing life history" at the conference Behaviour 2017, Estoril, Portugal.
2016 (Nov 23-25)	Participation to the workshop Invasion Dynamics Network, Freie University, Berlin, Germany.
(Nov 9-11)	Participation to the workshop Leibniz-Kolleg for young researchers: chances and challenges of interdisciplinary research, Leibniz Association, Berlin, Germany.
(Jul 28-Aug 4)	Poster presentation, as the first author, titled "Testing the pace-of-life syndrome hypothesis in juvenile zebrafish" at the 16th congress of the International Society for Behavioural Ecology, Exeter, UK.
(Feb 25-26)	Oral presentation, as the first author, titled "Personality emerges during the ontogeny of sibling fish in absence of genetic and extrinsic-stress factors" at the conference Behavioural Ecology Meeting 2016, Chioggia, Italy.
2015 (Aug 9-14)	Oral presentation, as the first author, titled "Ontogeny of personality in *Gambusia holbrooki*: a longitudinal study" at the conference Behaviour 2015, Cairns, Australia.
2014 (Sept 29-Oct 1)	Participation to the workshop New perspectives in behavioural development: adaptive shaping of behaviour over a lifetime?, Center for Interdisciplinary Research (ZiF), Bielefeld University, Bielefeld, Germany.
2013 (Apr 15-17)	Poster presentation, as the first author, at the online conference Ethology Investigates: invasive species.
(Feb 28)	Participation, with an oral presentation, to the workshop Ecological consequences of fish behavioural types, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany.
2012 (Jul 9-11)	Presentation, as co-author, of multiple posters in the National Science Foundation CMMI Research and Innovation Conference 2012, Boston, USA.

(Jun 3)	Participation, as representative of the Dynamical System Laboratory (New York University), to the event World Science Festival, New York City, USA.
2010 (May 20-22)	Participation to the National conference for biodiversity, Ministry for the Environment and the Protection of Land of Italy and Sea & Sapienza University of Rome, Italy.
(Jun 7-9)	Participation to the Summer school of higher education in philosophy and human ethology: animal's culture and ecological dimension, University of Cassino, Cassino, Italy.
2008 (Nov 7-8)	Participation to the course Understanding of individual strategies for the preparation of a scientific international work, Sapienza University of Rome & Institute of Health (ISS) of Rome, Italy.

HONORS & AWARDS

2017 (Sept 12)	"Forrest Research Fellowship" (2018-2021; **with scholarship** of 344.131 $ AUD) for the project "Integrating phenotypic plasticity in biological invasions and climate change to predict species response to a changing world", The University of Western Australia, Australia.
2013 (May 17)	Award for the "contribution to the scientific excellence in the USA with the outstanding achievement in the field of Natural Sciences", Honor Center of Italian Universities, USA.
(Apr 30)	"18th degree Award Marco Marchesini" (**with scholarship** of 1.000 euro) for the best Italian M.Sc. thesis in Animal Behaviour in 2012, Istituto Veneto di Scienze, Lettere ed Arti of Venice, Italy.
2011 (Sept 01)	"Fellowship for research and educational exchanges between Italy and USA" through a free housing (duration of 12 months), Honor Center of Italian Universities, USA.
(Apr 04)	Award (**with scholarship** of 500 euro) for the "best M.Sc. thesis in 2010", Banca di Credito Cooperativo, Italy.
(Jan 01)	"Fellowship for research and educational exchanges between Italy and USA" through a free housing (duration of 3 months), Honor Center of Italian Universities, USA.

UNPUBLISHED JOURNAL ARTICLES

1) **Polverino G.** Santostefano F. Diaz C. & Mehner T. Pace-of-life syndrome differs predictably between two populations of Eastern mosquitofish with opposing life history. *Proceedings of the Royal Society B* (**accepted with major revision**).

PEER-REVIEWED JOURNAL ARTICLES

18) Ruberto T. **Polverino G.** & Porfiri M. **2017** How different is a 3D-printed replica from a conspecific in the eyes of a zebrafish? *Journal of the Experimental Analysis of Behavior*, 107: 279-293.

17) **Polverino G.** Cigliano C. Nakayama S. & Mehner T. **2016** Emergence and development of personality over the ontogeny of fish in absence of environmental stress factors. *Behavioral Ecology and Sociobiology*, 70: 2027-2037.

16) **Polverino G.** Bierbach D. Killen S.S. Uusi-Heikkilä S. & Arlinghaus R. **2016**. Body length rather than routine metabolic rate and body condition correlates with risk-taking behaviour in juvenile zebrafish (*Danio rerio*). *Journal of Fish Biology*, 89: 2251-2267.

15) **Polverino G.** Ruberto T. Staaks G. & Mehner T. **2016**. Tank size alters mean behaviours and individual rank orders in personality traits of fish depending on their life stage. *Animal Behaviour, 115: 127-135.*

14) Laskowski K.L. Monk C.T. **Polverino G**. Alós J. Nakayama S. Staaks G. Mehner T. Arlinghaus R. **2016**. Behaviour in a standardized assay, but not metabolic or growth rate, predicts behavioural variation in an adult aquatic top predator Esox lucius in the wild. *Journal of Fish Biology*, 88: 1544-1563.

13) **Polverino G.** Manciocco A. Vitale A. & Alleva E. **2015**. Stereotypic behaviours in *Melopsittacus undulatus*: behavioural consequences of social and spatial limitations. *Applied Animal Behaviour Science*, 165: 143-155.

12) Uusi-Heikkilä S. Whiteley A.R. Kuparinen A. Matsumura S. Venturelli P.A. Wolter C. Slate J. Primmer C.R. Meinelt T. Killen S.S. Bierbach D. **Polverino G.** Ludwig A. & Arlinghaus R. **2015**. The evolutionary legacy of size-selective harvest extends from genes to populations. *Evolutionary Applications*, 8: 597-620.

11) Butail S. **Polverino G.** Phamduy P. Del Sette F. & Porfiri M. **2014.** Influence of robotic shoal size, configuration, and activity on zebrafish behavior in a free-swimming environment. *Behavioural Brain Research*, 275: 269-280.

10) Phamduy P. **Polverino G.** Fuller B. & Porfiri M. **2014**. Fish and robot dancing together: bluefin killifish females respond differently to the courtship of a robot with varying color morphs. *Bioinspiration and Biomimetics*, 9: 36021.

9) DeLellis P. **Polverino G.** Ustuner G. Abaid N. Macrì S. Bollt E.M. & Porfiri M. **2014**. Collective behavior across animal species. *Scientific Reports*, 4.

8) **Polverino G.** Phamduy P. & Porfiri M. **2013**. Fish and robots swimming together in a water tunnel: robot colour and tail-beat frequency influence fish behaviour. *PLoS ONE*, 8: e77589.

7) **Polverino G.** & Porfiri M. **2013**. Zebrafish (*Danio rerio*) behavioural response to bioinspired robotic fish and mosquitofish (*Gambusia affinis*). *Bioinspiration and Biomimetics*, 8: 044001.

6) **Polverino G.** & Porfiri M. **2013**. Mosquitofish (*Gambusia affinis*) responds differentially to a robotic fish of varying swimming depth and aspect ratio. *Behavioural Brain Research*, 250: 133-138.

5) Kopman V. Laut J. W. **Polverino G.** & Porfiri M. **2013**. Closed-loop control of zebrafish response using a bioinspired robotic-fish in a preference test. *Journal of the Royal Society Interface*, 10: 20120540.

4) **Polverino G.** Liao J. C. & Porfiri M. **2013**. Mosquitofish (*Gambusia affinis*) preference and behavioral response to animated images of conspecifics altered in their color, aspect ratio, and swimming depth. *PLoS ONE*, 8: e54315.

3) **Polverino G.** Abaid N. Kopman V. Macrì S. & Porfiri M. **2012**. Zebrafish response to robotic fish: preference experiments on isolated individuals and small shoals. *Bioinspiration and Biomimetics*, 7: 036019.

2) **Polverino G.** Manciocco A. & Alleva E. **2012**. Effects of spatial and social restrictions on the presence of stereotypies in the budgerigars (*Melopsittacus undulatus*): a pilot study. *Ethology Ecology and Evolution*, 24: 46-60.

1) **Polverino G.** & Alleva E. **2011**. Book reviews "Il Pappagallo dal Ventre Arancio. Un'Intelligenza Animale" and "Manual of Parrot Behavior". Massa R. & Luescher A., *Annali Istituto Superiore di Sanità*, 47: 236-237.

CONFERENCE PROCEEDINGS

5) Ruberto T. **Polverino G.** & Porfiri M. Comparison of live stimuli and 3D printed replicas: preference tests for zebrafish, in the Proceedings of SPIE's 10162, Bioinspiration, Biomimetics, and Bioreplication, Portland, Oregon, USA, March 25-29, **2017**.

4) Butail S. **Polverino G.** Phamduy P. Del Sette F. & Porfiri M. Fish-robot interactions in a free-swimming environment: Effects of speed and configuration of robots on live fish, in the Proceedings of SPIE's 9055, Bioinspiration, Biomimetics, and Bioreplication, San Diego, California, USA, March 9-13, **2014**.

3) **Polverino G.** Phamduy P. Facci A.L. Drago M. Khamran K. Yang L. & Porfiri M. Analysis of fish and bioinspired robotic fish swimming together in a water tunnel, in the Proceedings of SPIE's 8686, Bioinspiration, Biomimetics, and Bioreplication, San Diego, California, USA, March 10-14, **2013** *(Invited for oral presentation 40 minutes long).*

2) Kopman V. **Polverino G.** Laut J.W. & Porfiri M. Using a bioinspired robotic-fish for closed-loop control of zebrafish response in a preference test, in the ASME 5th Annual Dynamic Systems and Control Conference joint with the JSME 2012 11th Motion and Vibration Conference, Fort Lauderdale, Florida, USA, October 17-19, **2012**.

1) Abaid A. Bartolini T. Kopman V. Macrì S. **Polverino G.** & Porfiri M. And the zebrafish said: I like biomimetic robots, in The Fourth IEEE RAS/EMBS International Conference on Biomedical Robotics and Biomechatronics, Rome, Italy, June 24-27, **2012**.