

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

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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.

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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

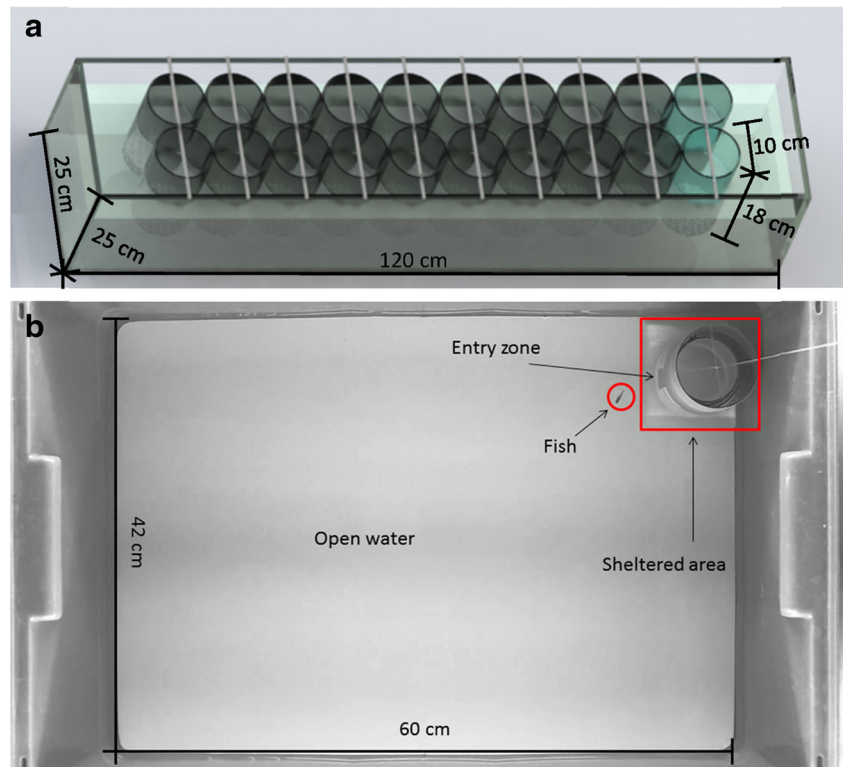
Fish utilized in this study were first-generation progeny of wild-caught individuals from Torre Castiglione (Italy, +40° 17' 20.44", +17° 49' 24.30" (Ariani et al. 2000)). Notably, fish from this isolated population are characterized by an extremely low heterozygosity compared to other wild populations 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

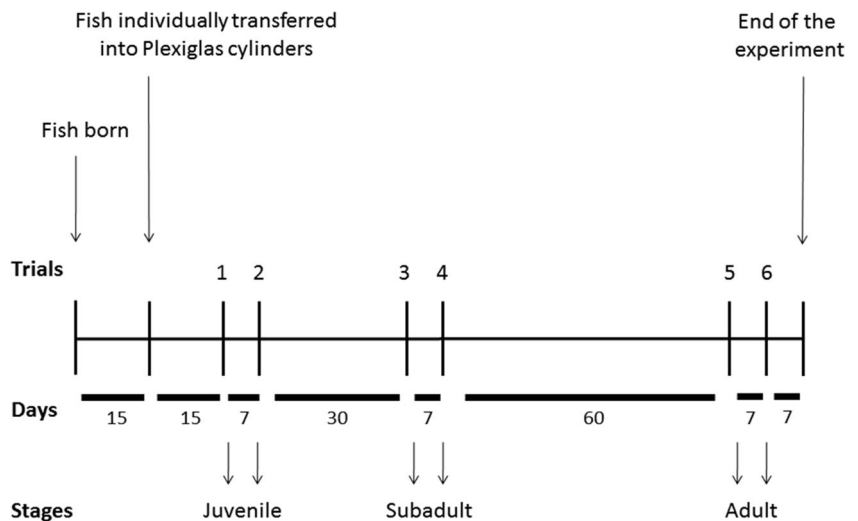
Fig. 1 Scheme of an experimental housing tank (a) and snapshot of the experimental arena (b). Each of the two experimental housing tanks (a) hosted two parallel arrays of ten transparent Plexiglas cylinders. Sibling mosquitofish were housed individually in the cylinders (i.e., 20 fish per tank), facilitating 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 semiformal 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 criteria (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

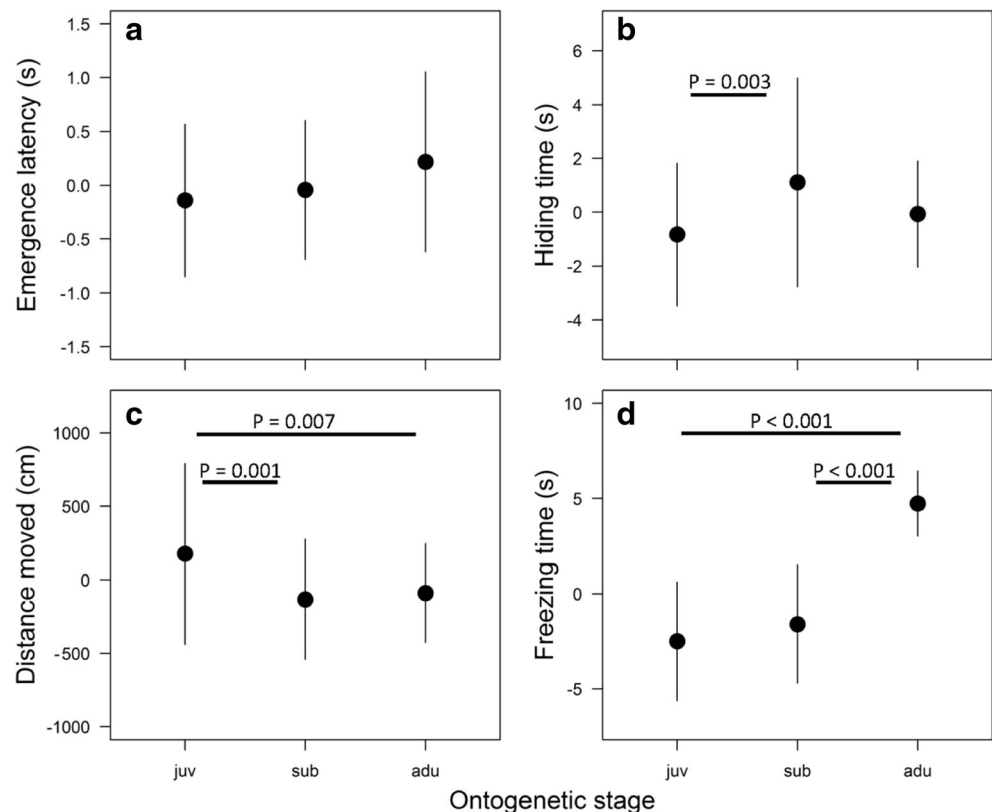
obtain the posterior distribution. Then, a linear regression was fitted to the variance scores randomly sampled from the posterior distribution at each stage using ontogenetic time (in weeks) as a predictor variable, since we assumed a linear change in behavior over ontogeny. However, a categorical variable 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 criteria (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 , $g/(mm)^3 \times 100,000$), 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

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

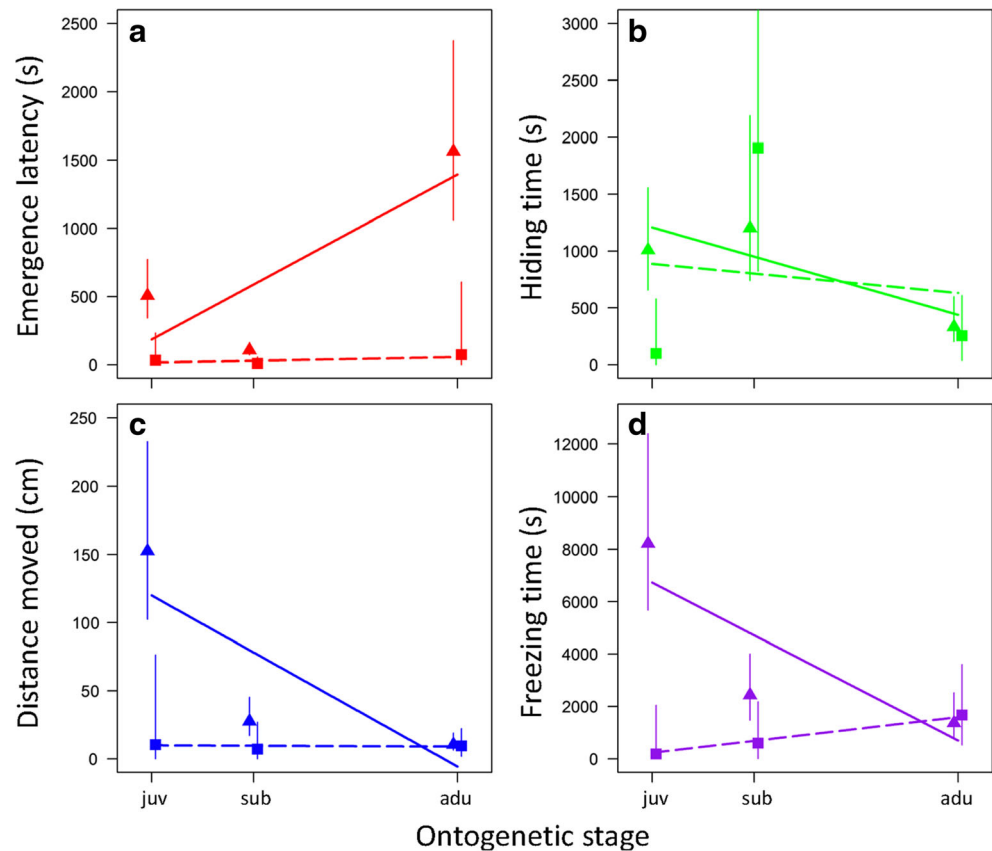
Variance components	P_{within}	P_{among}
Emergence latency	<0.001	0.69
Hiding time	<0.001	0.29
Distance moved	<0.001	0.94
Freezing time	<0.001	0.12

“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

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)).

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.

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Compliance with ethical standards

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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.

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