

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



Giovanni Polverino<sup>a,\*</sup>, Tommaso Ruberto<sup>b</sup>, Georg Staaks<sup>a</sup>, Thomas Mehner<sup>a</sup>

<sup>a</sup> Department of Biology and Ecology of Fishes, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany

<sup>b</sup> Department of Ecophysiology and Aquaculture, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany

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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.

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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, Dieneske, & 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](mailto:polverino@igb-berlin.de) (G. Polverino).

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,  $+40^{\circ}17'20.44''$ ,  $+17^{\circ}49'24.30''$ ; 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  $23 \pm 0.5^{\circ}\text{C}$ , 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  $23^{\circ}\text{C}$ . 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

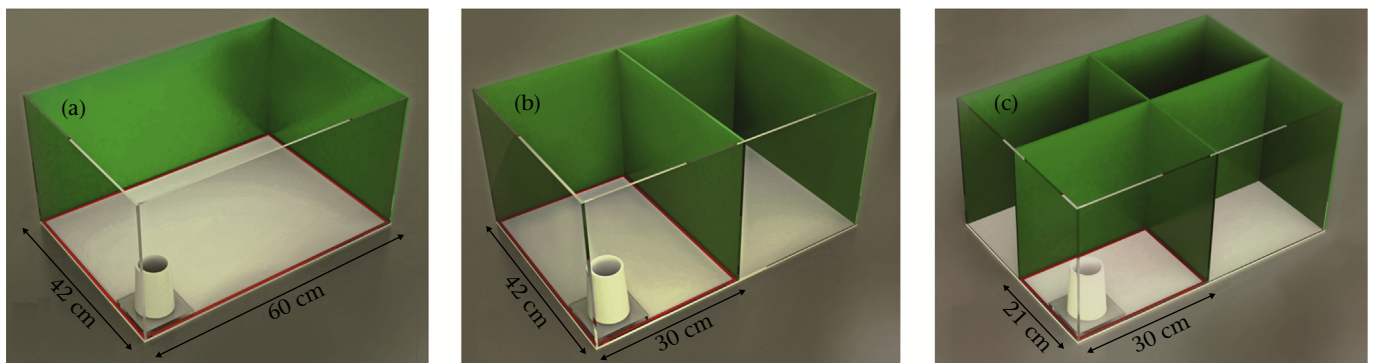
experimental climate chamber and equipped as described above for the 10-litre tanks utilized for collecting juveniles. On the following 2 days, each fish was tested again twice, once per day, as described above but in test tanks that differed in size to that used on the first day. In other words, each 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<sup>2</sup>/s<sup>2</sup>) 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; Dingemans & Dochtermann, 2013) between each pair of the six behavioural traits by using bivariate linear mixed-effects models (LMMs), as suggested by Dingemans 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 (Dingemans & 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 (Dingemans & 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 1500 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

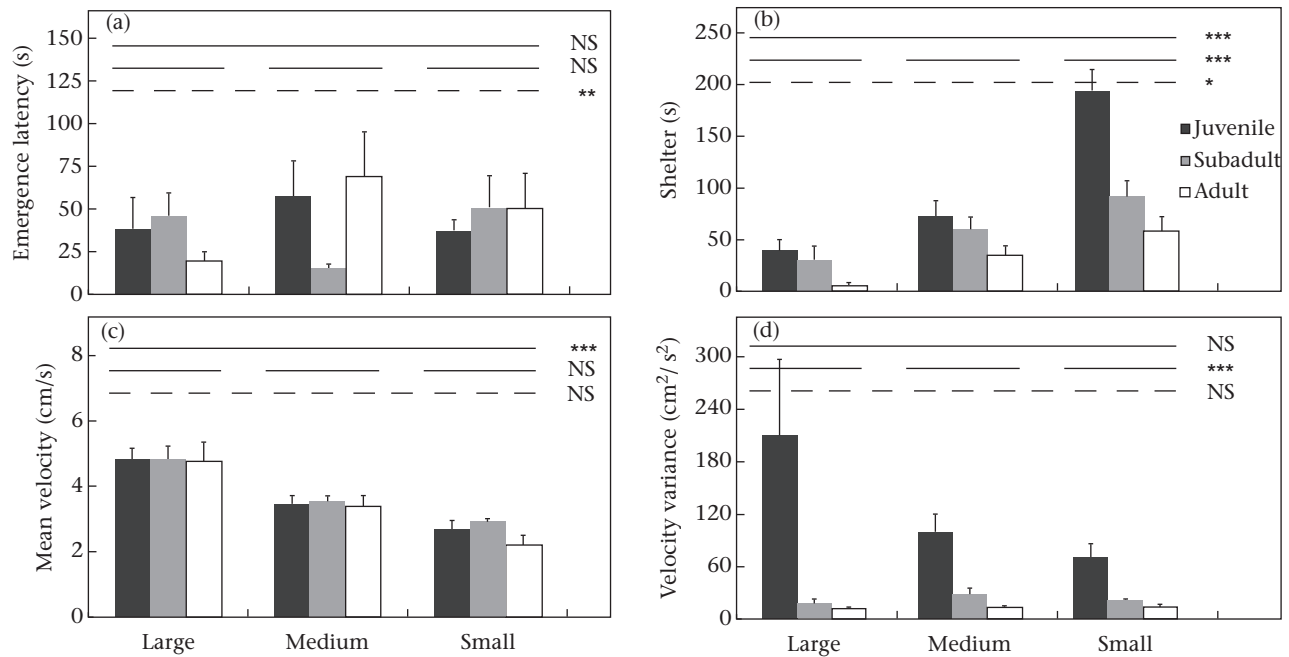
For each behavioural trait, we tested whether individuals from a given ontogenetic stage maintained their rank order across test tanks that varied in size. We observed an inconsistent individual rank order (i.e. nonsignificant effects of the random intercepts) for all four behavioural traits for at least 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<sup>2</sup>/s<sup>2</sup>, 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

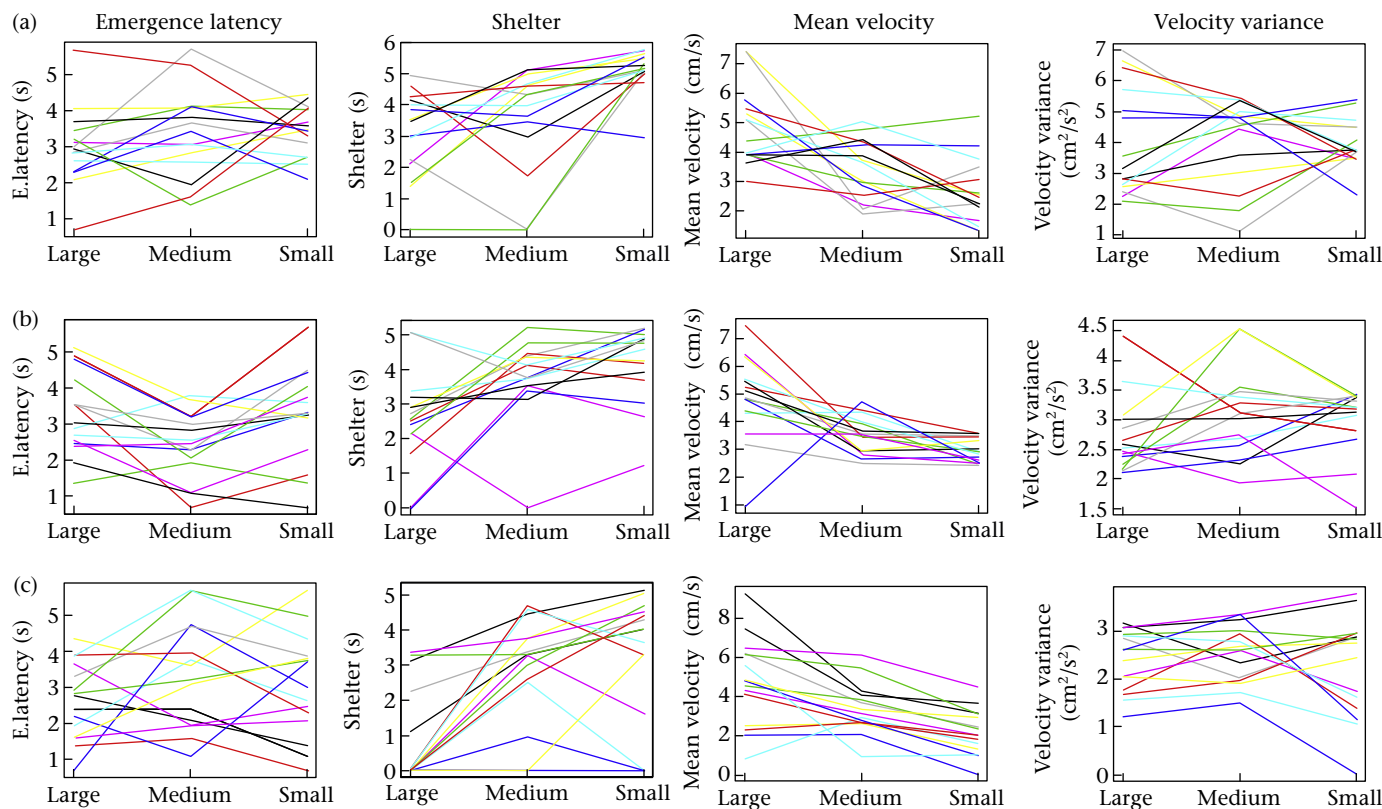
Behavioural trait	O.stage	AIC Full model Null model	$P$	$V_{\text{within}} \pm \text{SEM}$	$V_{\text{among}} \pm \text{SEM}$	$R$ (95% CI)
Emergence latency	Juvenile	136.24	0.06	0.74 $\pm$ 0.13	0.32 $\pm$ 0.08	0.28 (0.04, 0.55)
	Subadult	137.72				
		133.05	<0.01	0.51 $\pm$ 0.11	0.75 $\pm$ 0.13	0.58 (0.31,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

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 ( $V_{\text{within}}$ ), intercepts ( $V_{\text{among}}$ ) 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.

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