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

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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.

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,

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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.