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Is personality of young fish consistent through different behavioural tests?

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ABSTRACT

Most studies carried out on personality recognized that personality is defined by behavioural traits consistent through time and/or contexts. In fish, most studies on personality were performed either on juveniles (aged between 6 months and 1 year) or adults, but very few focused on the early life stages. The main goal of this study is to characterize behavioural syndromes and to highlight the existence of a personality in young juvenile pikeperch, a species with a strong economic value. To study the consistency of behavioural responses of juvenile (50 and 64 days post-hatch) pikeperch *Sander lucioperca* (n = 41, total length = 5.8 ± 1.0 cm and mass = 1.6 ± 0.7 g), we performed three tests per fish in one day: exploration (cross-maze), dyadic and restraint test. In the cross-maze test, exploratory fish were more active and bolder. In the dyadic test, fish with the highest number of contacts, showed also more approaches, orientations and avoidance behaviours. In the restraint test, bolder fish were more active and tried to escape more often. Consequently, the investigation of the different behavioural responses of each fish highlighted behavioural syndromes in this species. Furthermore, for the first time, we showed, with a cross-context analysis, that young juvenile pikeperch, responded in the same way to exploration and dyadic test but their responses were opposite in the restraint test. Our results opened new opportunities for testing individual personality in very young fish that may help solving some aquaculture problems, such as intra-cohort cannibalism.

1. Introduction

Animal behaviour studies have allowed establishing that individuals exhibit consistent behavioural differences (Koolhaas et al., 1999; Bell et al., 2009; Killen et al., 2016), which are described by different terms, among which personality or behavioural syndromes. Yet, these two terms are not equivalent. Personality is defined as behavioural individual differences stable over time and/or in varying environmental contexts (Réale et al., 2007). Behavioural syndrome is a suite of correlated behaviours reflecting between- individual consistency in behaviour across multiple (two or more) situations (Sih et al., 2004). A population or species can exhibit a behavioural syndrome (Sih et al., 2004).

Different personality traits are recognized in animals in relation to diverse behavioural responses of individuals within the same population and the performances of these traits followed a continuum along the personality axis. Five traits have been identified: boldness-shyness, exploration-avoidance, activity, aggressiveness, and social tolerance (Sih et al., 2004; Réale et al., 2007; Cote et al., 2008). The boldness-shyness trait is a measure of the individual's reaction to a situation perceived as dangerous (Réale et al., 2007). Several tests exist to test boldness-shyness among which the open-field test, maze-test or novel object test (Adriaenssens and Johnsson, 2013; Castanheira et al., 2013; Ferrari et al., 2014). In fish, several kinds of measures have been used to assess boldness-shyness (Toms et al., 2010). Behavioural variables include the latency to approach a novel object (Pasquet et al., 2015), or to explore a novel environment (Fraser et al., 2001), the time to emerge from a shelter (Ferrari et al., 2014), or to respond to a frightening stimulus (Millot et al., 2009). The exploration-avoidance trait includes behaviours that involve the individual willingness to investigate novel environments, food items or objects in the absence of predators or other frightening stimulus (Conrad et al., 2011). In fish, these behaviours could be analysed by the number of explored zone in a maze (Ferrari, 2014), as well as by the latency to explore a novel space in an open-field test and by the travelled distances in an open area (Adrianssens et al., 2012; Ferrari et al., 2014). The activity trait is measured by the number or the frequency of movement in a familiar environment or by the time spent swimming in a given area (Pasquet et al., 2015; Colchen et al., 2016). The aggression is often measured by either the defence of a territory or by food resource competition (Conrad et al., 2011). For this trait, another approach is the assessment of 'competitive abilities' (Huntingford et al., 2010) rather than a direct

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measure of aggression, such as the number of attacks or bites. To study aggressiveness, the mirror test is classically used (Höjesjö et al., 2011; Adrianssens et al., 2012) or direct confrontations are realized in a dyadic test (Sundström, 2004). The sociability or social tolerance trait refers to an individual's reaction towards conspecifics, which does not correspond to an aggressive interaction. In fish, sociability is classically measured by avoidance or attraction to conspecifics (Conrad et al., 2011).

In fish, most studies on personality were performed either on juveniles (between 6 months and 1 year age) or adults (Dahlbom et al., 2011). In contrast, very few studies focused on the early life stages (larval and the first juvenile's stages) (Sundström, 2004; Pasquet et al., 2015; Tudorache et al., 2015). The small size as well as the difficulty to manipulate these first developmental stages could explain why there are so few studies focusing on personality. Nevertheless, understanding personality of the early life stages is an essential question to improve our knowledge on these stages and may contribute to solve one of the main bottlenecks in the rearing of new species, which is the intra-cohort larval cannibalism (Conrad et al., 2011; Baras, 2012; Teletchea and Fontaine, 2014), as observed in pikeperch *Sander lucioperca* (Kestemont et al., 2007).

Pikeperch has a strong economic potential for inland aquaculture diversification in Europe (Wang et al., 2009). To our knowledge, personality in larvae and juveniles of pikeperch has never be performed. To establish a personality profile, it is necessary that individuals respond consistently to different behavioural tests. To be sure that a behavioural test allows us to show personality, it is necessary to realize cross-context validations of behavioural measures (Réale et al., 2007). In our study, we aim at exploring the relationship between the fish behavioural responses, and for that we used three among the most common behavioural tests (see Conrad et al., 2011 for review). For a determined fish, the three tests (novel environment, social and net restraint) were all performed within the same day. The main goals of this study are: (i) to characterize behavioural syndromes between behaviours in each test, (ii) to highlight the existence of a personality and (iii) to realize a cross-context analysis of behavioural syndromes between tests in young pikeperch juveniles.

2. Material and methods

2.1. Fish and experimental conditions

Eggs were obtained from stripping of two domesticated females reared in a fish farm (Asialor, Pierrevillers 57, France) on January 26th, 2015. After hatching (January 27th, 2015), larvae were raised at URAFPA (http://www.urafpa.fr/, France) in a RAS (Recirculating Aquatic System). Fish were hand fed commercial food (first with nauplii of *Artemia* (550–600 µm, Catvis, Hertogenbosch, The Netherlands) from 4 dph to 18 dph, followed by Larviva PROWEAN 100, 300, 500, 700 µm (BIOMAR®, France) until 35 dph and INICIOplus 0.8 mm (BIOMAR®, France) according to the protocol used by Schram and Philipsen (2003). Fish were fed seven times per day (between 8:30 to 17:30). At 44 dph (stage 2, Ott et al., 2012), 41 fish were randomly sampled (total length = 5.8 ± 1.0 cm and mass = 1.6 ± 0.7 g). After anaesthesia (Tricaine methanesulfonate (MS 222): 80 mg L^{-1}), fish were tagged with Visible Implant Elastomers (VIE tag, Northwest Marine Technologies, USA; Bell and Sih, 2007). We used two different colours (blue and pink) and tags were injected in the operculum and/or in flanks of each fish on the right or the left side. Then, fish were distributed in three 700 L tanks (13–14 fish per tank) without enrichment. Throughout the entire experiment, artificial lighting followed a 12 h light/12 h darkness cycle with light on from 08:00 to 20:00 and a flow rate of 100L h^{-1}. Water temperature was maintained at 20 ± 0.3 °C. The physico-chemical properties of the water were monitored twice per week (dissolved oxygen: 7.66 ± 0.35 mg L^{-1}; ammonia 0.16 ± 0.09 mg L^{-1}; nitrite: 0.02 ± 0.02 mg L^{-1}). Tanks were cleaned every day in the morning.

2.2. Characterization of behaviours

All behavioural tests were carried out on juveniles (N = 41) aged between 50 and 64 dph (stage 2, Ott et al., 2012). For each 700 L tank, fish were caught, identified and isolated in a bucket, one hour before the first test. Then each fish was submitted to the three tests during the same day: cross-maze test, dyadic test and net restraint test. Exploration and restraint test were performed randomly and dyadic test was always performed the last one. All tests were executed in a dedicated room separate from the rearing tanks. The water in the test devices was changed between each fish. Tests were video recorded (Sony HDR-CX550VE). All variables in the three tests were measured with continuous focal observations considering all the recording period (30 min) except for the swimming activity that was recorded during three time periods of two minutes. Behavioural data were obtained with the Observer XT software (Noldus, The Netherlands, version 10.0). After the tests, all fish were euthanized by over-anesthetizing them in MS 222 bath (240 mg L^{-1}).

2.2.1. Behaviours in the cross-maze test

Swimming activity, exploration and boldness were evaluated using a cross-maze test. The cross-maze (16 cm × 5 cm with 2.5 cm of water) was divided into five zones (Fig. 1), and placed on a translucent table with a light below. Fish were tested one by one. Each fish was placed in the acclimatization zone (7 cm × 5 cm) separated from the maze by a vertical divider. After a five-minute acclimatization period, the divider was opened and fish behaviour was video recorded for 30 min. If a fish did not go out of the acclimatization zone (AZ) during the 30 min' period, a latency of 1800s was attributed. For exploration and boldness, three variables were analysed: the individual latency to emerge from the acclimatization zone *LE* (in s), the time spent outside the acclimatization zone *TOAZ* (in s) and the total number of visited zones *TNVZ* (for definitions see Table 1). They were analysed over the whole 30 min period. Swimming activity *SA* (for definition see Table 1) was calculated over three periods of time: from the 5th to the 8th minute, from the 15th to the 18th minute and from the 25th to the 28th minute (adapted from Pasquet et al., 2015).

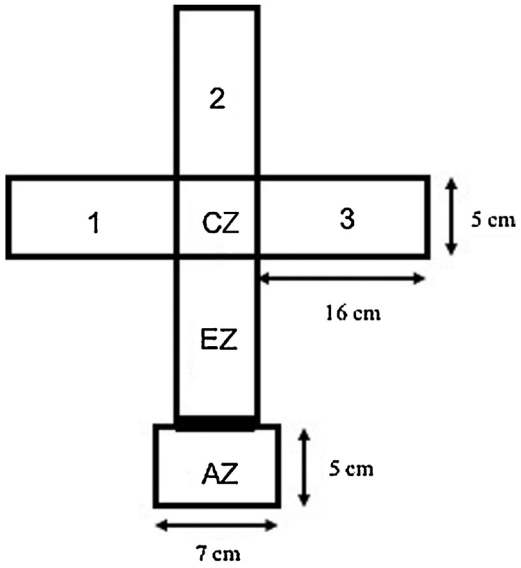

Fig. 1. Scheme of the device used for cross-maze tests. Maze was divided in six zones: acclimatization zone (AZ), entry zone (EZ), central zone (CZ) and three others zones (1,2,3). Between AZ and EZ, there was a removable divider.