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Does short-term salinization of freshwater alter the behaviour of the Iberian barbel (*Luciobarbus bocagei*, Steindachner 1864)?

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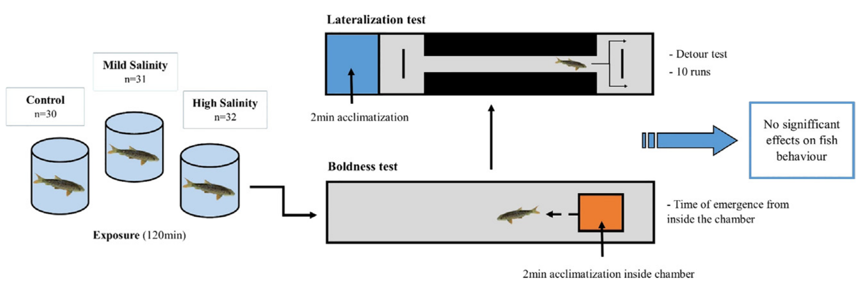
HIGHLIGHTS

- We evaluate the effects of salinity on freshwater fish behaviour.
- Fish seem to become bolder and shyer with higher levels of salinity.
- Fish behaviour was not significantly affected by the salinity stress.
- With higher salinities fish seem to become more lateralized.
- Population lateralization left trend was maintained throughout treatments.

GRAPHICAL ABSTRACT



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ABSTRACT

Stream salinization is a great environmental hazard being aggravated by anthropogenic disturbances. Harmful conditions, as increasing salinity in freshwater systems, may negatively affect river fish fauna and possibly influence fish behaviour, such as boldness and/or cerebral lateralization. Salinity has been proven to affect behavioural expression, despite the tolerance of some species. It is thus relevant to study these behaviours, as the salinity exposure effects could represent greater environmental consequences. The impact of salinity stress was evaluated by exposing Iberian barbels, *Luciobarbus bocagei* (Steindachner, 1864) (Cypriniformes, Cyprinidae), to three levels of salinity (0.9, 9 and 19 mS/cm, using NaCl) and by conducting boldness and lateralization experiments, regarding population trends. Results show that, with increased salinity, fish diverged to the extremes of the shy-bold gradient, the population was slightly lateralized to the left, and seemed to become more lateralized with increasing salinity. However, there were no statistical differences between the treatments. Fish living in a Mediterranean climate are especially resilient to various stressors, which may confer them additional tolerance, and in this case, acute punctual exposure to increased salinity may not be detrimental for behaviour maintenance. We encourage the expansion of the research to different freshwater fish species that would help to recognise salinity thresholds and use them to implement effective conservation measures and appropriate ecological restoration actions for these sensible systems.

1. Introduction

The intensification of anthropogenic pressures is leading to freshwater ecosystems deterioration endangering many aquatic species (Malmqvist and Rundle, 2002; Santos et al., 2015; Valle et al., 2015). Stream salinization is a pressing environmental issue, especially in semi-arid and arid regions (Dugan et al., 2017; Williams, 2001), as

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outcomes will be intensified by climate changes (Mantyka-Pringle et al., 2014), and these global variations will interact with increasing salinities, inducing physiochemical stress in freshwater ecosystems, by increasing ionic concentrations (Kaushal et al., 2018; Olson, 2015). One of the main activities leading to salinization, i.e. secondary salinization, is field irrigation in agriculture, (Cañedo-Argüelles et al., 2013, 2015; Lerotholi et al., 2004). Excess salts can be leached out and end up in rivers and streams. Other significant sources include the use of road salts as de-icing agents, industrial discharges or mining (Cañedo-Argüelles et al., 2013; Lerotholi et al., 2004). Most of these are point-source pressures that produce an acute, but temporary stress. Anthropogenic salinization may cause great loss of freshwater biodiversity, (Herbert et al., 2015; Kefford et al., 2016), through the acidification of streams (Lofgren, 2001) and translocation of toxic metals (Norrström and Bergstedt, 2001). At an organism level, it affects metabolism and osmoregulation, threatening long-term growth, reproduction, and ultimately the viability of the species (Hasan et al., 2017; Nielsen et al., 2003; Rind et al., 2017), which translates into consequences on the structure, functioning and sustainability of the aquatic system (Kaushal et al., 2005) through influences on competition and predation (Braukmann and Böhme, 2011; Millán et al., 2011). Hence, elevated salinity concentrations could have lethal effects on several species, possibly causing extinction (e.g. carps, Koç, 2008), thus being a selective pressure. Freshwater fish species can tolerate salinities between 7 and 13 g/L (14 and 26 mS/cm), however when salinity exceeds this range, metabolism is compromised and osmoregulatory mechanisms fail (Bacher and Garnham, 1992; James et al., 2003; Pistole et al., 2008; Rind et al., 2017), which will impact development of fish (Boeuf and Payan, 2001), as more energy is spent on osmoregulation (Bradley, 2009; Griffith, 2017; Hintz and Relyea, 2017).

Studies have shown that behavioural responses of fish can also be altered by sub-lethal levels of salinity, such as overall activity, evasive responses, and ineffective responses to possible risk (e.g. fathead minnows, Hoover et al., 2013). The shy-bold continuum is considered to be a fundamental gradient of behavioural variation and a strong driving force of evolution (Frost et al., 2007), and disturbances, such as increasing salinity, on freshwater systems and fish populations could result in a cascade of behavioural and ecological consequences. This gradient describes the willingness of individuals to take risks in novel contexts (Magnhagen and Bunnefeld, 2009; Riesch et al., 2009; Wilson et al., 1993). Shy individuals retreat in unfamiliar situations, and bold individuals are more curious about the surrounding environment. Individual variation in boldness affects how animals react to the physical environment, forage, reproduce and interact with prey, predators and conspecifics (Réale et al., 2000), therefore, it has repercussions for population structure (Coleman and Wilson, 1998). Concomitantly, cerebral lateralization in fish has also been proven to be affected by environmental factors, such as, elevated temperature and CO_2 (Domenici et al., 2014), and hypoxic conditions (Lucon-Xiccato et al., 2014), leading to the belief that increasing salinity might also have similar effects on salinity intolerant fish species. Interspecific variation in both strength and direction of laterality in fish, at a population level, along with boldness, may determine survival in many natural scenarios such as feeding and predator interactions (Brown et al., 2007; Yasugi and Hori, 2012), and plays a role on coordination among individuals living in social groups (Bisazza et al., 2000; Lucon-Xiccato et al., 2014).

In conclusion, fish experiencing potentially harmful conditions, such as high levels of salinity, respond to stress by redirecting energy towards re-establishing homeostasis, and therefore, behaviour can be affected (Gül et al., 2004; Hoover et al., 2013; Pottinger et al., 2002). Consequently, our main aim was to determine if salinity, as a stressor, can alter boldness behaviour and cerebral lateralization of fish. Increasing knowledge on the effects of salinization on these behaviours is important as the approaches are non-invasive and the behaviours easy to test and indicators for other behaviours.

2. Materials and methods

2.1. Species selection

The Iberian barbel, *Luciobarbus bocagei* (Steindachner, 1864) (Cypriniformes), is a freshwater fish from the Cyprinidae family. It is endemic to the Iberian Peninsula, and occurs in a wide range of lotic and lentic habitats and in almost all the river basins of northern and central Portugal from Lima to Sado drainages (Lobón-Cerviá and Fernández-Delgado, 1984). It has stable populations and is considered a non-threatened species by the IUCN Red List. Juveniles (9–13 cm Total Length, TL) were selected for this study as they may be more affected by salinity stress due to their higher surface/volume ratio. An eco-morphologic approach was followed, and the Iberian barbel was considered as representative of bottom-oriented, medium-size potamodromous cyprinids (Branco et al., 2013). This kind of approach has been considered suitable to study systems where several similar species exist, and when exhaustively studying all species present is logistically challenging (Boeuf and Payan, 2001). This species has the added advantage of being abundant in several systems which allows all fish to be captured in the same river stretch minimizing the probability of multi population capture and reducing the time taken for capture and concomitantly capture stress is maintained to a minimum. All of these contribute for a reduced experimental bias.

2.2. Fish capture and holding

Fish were captured from the Lizandro River (N 38°54.040′, W 9°21.711′, altitude = 33 m a.s.l.) in Carvalhal county (Mafra, Portugal) during day-time in late-summer (September and October 2017). It is a small (29 km long) coastal Mediterranean river, draining a 374 km2 catchment, whose uppermost reaches are forested, but otherwise alternates between small urban areas and remnant agricultural and open-space lands. Capture site selection was based on habitat representativeness, i.e. taking into account the presence of well-defined riffle sections, alternated with deep pools (mean depth = 0.8 m) where the species often occurs (Santos et al., 2018), and avoiding the lowermost brackish portion of the river. Water quality parameters were fairly constant throughout the sampling period (electrical conductivity: 740 ± 50.3 μS/cm; pH = 7.5 ± 0.5; water temperature: 18.8 ± 0.6 °C). Capture was achieved by using standard electrofishing protocols closely following the CEN standards (CEN, 2003). During the experiments, fish were maintained in tanks with freshwater at the School of Agriculture (University of Lisbon). Maintenance tanks (approx. 800 L) were kept under natural light and temperature, and with mechanical and biological filtration (Fluvial FX4) with a turnover of 1700 L/h. Fish were acclimated for at least 48 h and feeding stopped 24 h before the experiments took place. Fish were used only once and were never maintained for >5 days before they were returned alive to the same location from where they were captured. In total 93 barbels were used (TL: 11.34 ± 1.33 cm, mean ± SD).

2.3. Fish experiments

Fish experiments took place in a mesocosms system composed by 2 zinc channels (400 cm length × 40 cm width × 20 cm depth) (Fig. 1). Channels were fed by an in situ natural spring (pH = 8.06; Conductivity = 0.87 mS/cm; DO = 9 mg/L) and conducted to 3000 L central reservoir and evenly delivered to the channels. Each individual was tested for boldness, and then transported to the other channel to test for lateralization. Salinity levels of the mesocosms were maintained the same as the treatment being tested and water depth was maintained constant throughout treatments and replicates (10.06 ± 0.21 cm boldness and 7.49 ± 0.42 cm lateralization channel, mean ± SD).