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Does environmental enrichment promote recovery from stress in rainbow trout?

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ABSTRACT

The EU Directive on animal experimentation suggests that all protected animals should have enrichment to improve welfare yet relatively little research has been conducted on the impact of enrichment in fish. Studies employing enrichment in zebrafish have been contradictory and all fish species should be provided with species-specific enrichments relevant to their ecology. Salmonids are important experimental models in studies within aquaculture, toxicology and natural ecosystems. This study therefore sought to establish whether an enriched environment in an experimental aquarium may promote improved welfare in rainbow trout (*Oncorhynchus mykiss*) by enhancing their recovery from invasive procedures. Trout were held individually in either barren (no tank ornamentation) or enriched (gravel, plants and an area of cover) conditions. Recovery rates after a noxious stimulus and a standard stressor were investigated by monitoring behaviour, opercular beat rate and plasma cortisol concentrations. Fish were randomly assigned to one of four treatment groups: Control (undisturbed), Sham (handled but not manipulated), Stress (air emersion) and Pain (subcutaneous injection of acetic acid). The results suggest that for rainbow trout environmental enrichment appears to promote recovery and ameliorate adverse effects following a stressor. However, recovery rate did not differ between environments in the pain treatment groups. Thus environmental enrichment may not be an important factor when the fish is responding to a painful stimulus. These results have important implications for the husbandry and welfare of captive rainbow trout and possibly other salmonids and suggest that enriched environments may be preferable to barren environments in experimental studies.

1. Introduction

There is a plethora of evidence supporting the benefits of enrichment for animals held in captivity, particularly mammals (Simpson and Kelly, 2011; Singhal et al., 2014). However, evidence for the benefits or otherwise of enriched environments for fish is lacking. Fish are a widely exploited research model, second only to mice in numbers used (UK Home Office, 2013), but more importantly they also constitute a major source of protein with an estimated two million tonnes of farmed fish being produced across Europe annually (FEAP, 2014). Globally, aquaculture is a growing industry and this growth is inevitably accompanied by concerns about the welfare of intensively-farmed fish employed in research aimed at resolving production problems.

Natural environments are more heterogeneous than those found in captivity and this disparity may result in stress or impaired cognitive function among captive animals with obvious implications for welfare (e.g. Kellison et al., 2000; Brown and Day, 2002; Huntingford, 2004; Sundstrom et al., 2004; Araki et al., 2008). For animals kept in captivity the EU directive on the protection of animals used for scientific purposes (EU Directive, 2010) recommends, although does not enforce, that captive-held fish should be kept in enriched conditions. However, despite this recommendation, relatively little is known about the benefits of enrichment for captive fish and what is required to maintain a high standard of welfare. In captivity for example the habitat often remains non-enriched (from this point onward referred to as barren) with no heterogeneity, for ease of cleaning, removal and transfer of fish, reduction of the spread of disease etc. Whilst there are many different def-

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initions of environmental enrichment, the same general concept applies: increasing environmental complexity within an animal's surroundings that is in some way beneficial to not only maintaining but also improving general animal welfare. Enrichment can be further categorised into areas that target the different aspects of an animal's life; social, diet, cognitive, sensory, and physical (Näslund and Johnsson, 2014).

The results of research on environmental enrichment in fish are contradictory and highlight the extensive variation between and even within species. As reviewed in Näslund and Johnsson (2014), there are several studies demonstrating the positive effects of environmental enrichment on welfare across many fish species. Compared with captive-held fish in barren environments, those provided with some form of enrichment have been found to have increased brain development (Marchetti and Nevitt, 2003; Kihslinger and Nevitt, 2006; von Krogh et al., 2010; Salvanes et al., 2013), reduced impact from stressors (Braithwaite and Salvanes, 2005; Naslund et al., 2013; Batzina et al., 2014), improved foraging ability (Brown et al., 2003; Strand et al., 2010; Rodewald et al., 2011), improved post-release survival (D'Anna et al., 2012) and positive effects on growth (Leon, 1975; Hansen and Moller, 1985; Batzina et al., 2014). This general increase in neural plasticity results in the development of behaviourally flexible fish that are better at coping with a variety of situations. Although it must be noted that there are also studies demonstrating negative and neutral associations of environmental enrichment (See Näslund and Johnsson, 2014).

As a consequence of the diversity in natural histories exhibited in fish, as well as the wide range of both physiological and behavioural traits, it is likely that the ideal enrichment will have to be judged on a species by species and possibly even on a life stage basis. Here we examine the rainbow trout (*Oncorhynchus mykiss*), a commercially important salmonid with more than 380,000 t (FEAP, 2014) being produced annually through aquaculture in Europe and as such this species is also widely used in scientific research (Thorgaard et al., 2002) but there remains a paucity of information on enrichment in captivity in this species.

If a lack of environmental enrichment affects fish behaviour, physiology or welfare then this would undoubtedly be a confounding factor when interpreting experimental results and might lead to erroneous conclusions from experiments (Williams et al., 2009; Killen et al., 2013). In laboratory rodent studies, enrichment can improve the health and welfare of the test subjects but may also reduce individual variation such that the data sets are more robust and scientifically valid (Singhal et al., 2014). The present study was conducted to investigate the effects of simple environmental enrichment on (i) the recovery of rainbow trout from stressors and invasive procedures, and (ii) the degree of inter-individual variability among the data collected.

2. Methods

2.1. Fish husbandry

Experiments were conducted with approval from the Home Office, U.K. (licence no.PPL 40/3435) and the University of Liverpool's Ethics Committee. Juvenile rainbow trout, *O. mykiss* (average weight 92.48 ± 2.72 g; $n = 64$), were obtained from a commercial supplier and maintained in stock tanks ($2 \times 2 \times 0.5$ m) in a semi-recirculating system at 11 ± 1 °C, with constant aeration and a 14:10 h light:dark cycle. The trout were allowed at least two weeks in the stock tanks to recover from the stress of transport. Fish were fed commercial trout diet (Skretting, Northwich, U.K.) at 1% body weight per day. For experiments, fish were caught at random and transferred individually to separate glass aquaria ($90 \times 50 \times 45$ cm) with either barren (air stone only) or enriched (air stone, gravel, plastic plant and an overhead area of cover) conditions that were screened from visual disturbance. Tanks were provided with filtered water and aeration by a semi-closed recirculation system; light, temperature and feeding regimes were identical to those of the stock tank. Rainbow trout are a naturally territorial species and form dominance hierarchies where subdominants and subordinates are chronically stressed due to low social status (Gilmour et al., 2005; Sneddon et al., 2011). Therefore, this species is less stressed when held individually where they are allowed to form a "territory" within their holding tank without the stress of social subordination or territorial disputes (e.g. Frost et al., 2007; Thomson et al., 2011; 2012; Frost et al., 2013) thus we tested fish individually to ensure social stress was not a confounding factor and behaviour and physiological responses were consistent over the experimental period and any responses were due to the treatments imposed.

2.2. Experimental procedure

Fish (enriched $n = 32$; barren $n = 32$) remained in individual tanks for at least two weeks until acclimatised and were allowed at least seven days after resumption of feeding. To avoid any biasing, fish were randomly assigned to one of four treatment groups: Control where the fish were left undisturbed; Sham where fish were anaesthetised in benzocaine (Sigma–Aldrich Co., UK) dosed water ($0.033\ \mathrm{g\,l^{-1}}$; Mettam et al., 2012) but no invasive procedure undertaken; Pain where a subcutaneous injection of 1% acetic acid was administered into the frontal lips (0.5 ml in each) during anaesthesia; and stress where fish were subject to 1 min of air emersion by holding the fish in a net (Pickering and Pottinger, 1989). All fish were tested at the same time each day to account for diel variations, and treatments were conducted out of view of other subjects. Trout and other fish are commonly exposed to stressors and invasive treatments causing tissue damage that may give rise to pain in laboratory studies (e.g. invasive tagging (Weigel et al., 2014), vaccination (Bjorge et al., 2011) and exposure to necrotic diseases (Fredriksen et al., 2013) and low pH chemicals (Mettam et al., 2012)) thus it is vital that we understand if enrichment can enhance the resilience and recovery from experimentally induced stress and pain to refine experimental protocols. We, therefore used a standard pain test that has been validated and does not cause lasting harm. Previous research has shown subcutaneous injection of acetic acid activates nociceptors in fish (Sneddon et al., 2003a; Ashley et al., 2009) and fish do indeed recover at approximately 3 h with behaviour and physiology returning to normal (Sneddon, 2003; Sneddon et al., 2003a,b; Reilly et al., 2008a). This allows us to measure recovery from a painful stimulus over a relatively short period of time and prevents longer-term pain or lasting harm to the fish. In this study benzocaine was used over other anaesthetics because it has analgesic properties, has been used in several other studies investigating behaviour and opercular beat rate (OBR) (Sneddon, 2003; Reilly et al., 2008a; Ashley et al., 2009; Mettam et al., 2011), and recovery is reported to be around 10 min post exposure (Gilderhus and Marking, 1987; Gilderhus, 1989) thus making it appropriate to use for investigating short term responses from stressors. However, benzocaine, a commonly used anaesthestic has been reported to be aversive to zebrafish (Readman et al., 2013) thus the anaesthetic procedure (handling, confinement in the anaesthetic vessel and exposure to a potentially aversive chemical for approximately 10 min) and is known to be stressful in rainbow trout (Review in Sneddon, 2012). We kept sample sizes to a minimum for statistical analysis and used trout reared for commercial production rather than using a wild species. We believe the benefits of understanding whether enrichment improves recovery in a