Aquatic Toxicology 170 (2016) 323–329

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Adult vertebrate behavioural aquatic toxicology: Reliability and validity

Matthew O. Parker

School of Health Sciences and Social Work, University of Portsmouth, Portsmouth, UK

ARTICLE INFO

Article history:
Received 26 June 2015
Received in revised form 27 August 2015
Accepted 1 September 2015
Available online 2 September 2015

Keywords:
Toxicology
Aquatic vertebrates
Anxiety
Behaviour
Reliability
Validity

ABSTRACT

Current advances in the ability to assay adult aquatic vertebrate behaviour are potentially very useful to aquatic toxicologists wishing to characterise the effects of pollutants on behaviour, cognition or neurodevelopment. This review considers two specific challenges faced by researchers wishing to exploit these technologies: maximising reliability and validity. It will suggest two behavioural procedures, with the potential for automation and high-throughput implementation, which can be used to measure social cohesion and anxiety, two areas of interest in behavioural aquatic toxicology. In addition, the review will make recommendations about how these procedures (and others) could be carried out to maximise reliability and validity.

1. Introduction

There have been a number of recent technological and intellectual advances in understanding the behaviour of aquatic vertebrates. This raises some exciting possibilities for research in aquatic toxicology, particularly in terms of the critical need to link behaviour to physiological and biochemical processes (Sloman and McNeil, 2012). First, it increases the potential for understanding how environmental pollutants in aquatic ecosystems may affect subtle behaviours of aquatic populations (and the wider connotations of this). Second, there may be increased potential for expanding translational toxicology (Kalueff et al., 2014a; Mattes and Walker, 2009). For example, there is potential to replace classical mammalian models, or indeed, refine existing aquatic models, in particular in the light of increasing potential for non-invasive physiological measurement in aquatic vertebrates (Scott et al., 2008). Third, it presents the opportunity for increasing throughput in an age of 'big data'; thus, increasing the potential for scientific breakthrough. However, extensive experience with rodent models, coupled with increasing evidence from laboratory-based aquatic vertebrate research, has demonstrated that there are a number of challenges that accompany the developmental of large-scale laboratory animal behavioural testing. The goal of this review is to consider what are the main challenges faced by researchers when implementing behavioural tests. Finally, the review will suggest behavioural tests that could be used to overcome these challenges.

2. What are the main challenges faced by researchers when implementing behavioural tests?

When attempting to carry out work of a translational nature (i.e., work that uses observaions in model systems to inform human condition; Mattes and Walker, 2009), a challenge faced by all involved in preclinical research is confirming validity to ensure the usefulness of data derived from the model (Nestler and Hyman, 2010). There are three types of validity associated with translational models (as first discussed by Willner, 1997): (i) face validity; the phenomenological and subjective similarity between the model and the intended translational target (e.g. superficial similarities between the effects of anxiolytic drugs on marine vertebrates/invertebrates and humans; Brodin et al., 2013; Guler and Ford, 2010; Olsén et al., 2014); (ii) construct validity; a solid theoretical basis for the model (e.g. endocrine disruptors such as bisphenol A have developmental effects in mammals and marine vertebrates/invertebrates through mimicking the sex hormone oestradiol; Howdeshell et al., 1999; Kang et al., 2007); and (iii) predictive validity; the ability of the model make accurate predictions (e.g. if an animal was exposed to an endocrine disrupting compound during early development, and this increases stress reactivity in later life, is this rescued by anxiolytic drugs?). A fourth type of validity, ecological validity, describes the degree to which

E-mail address: matthew.parker@port.ac.uk

http://dx.doi.org/10.1016/j.aquatox.2015.09.001
0166-445X/

work carried out within a laboratory can be generalised outside to the 'real world', and this is discussed in detail below.

Other major challenges faced in behavioural aquatic toxicology involve maximising the critical components of good experimental design: (1) within- and between-laboratory reproducibility of experiments to ensure reliability; and (2) high external validity (e.g. ecological validity) to ensure biological relevance of the findings/observations/measurements. Generally, the most significant challenge is to reach equilibrium, with high reliability often leading to low external validity, and highly reliable experiments often being low in generalisability to situations outside of the laboratory (Carter et al., 2013). These considerations are particularly important during the increasing momentum towards high-throughput testing. Below, each of these challenges will be considered in turn with respect to aquatic toxicology.

High within- and between-laboratory reproducibility of experiments is essential in ecotoxicology research, not only in terms of ensuring good science and reducing redundant duplication of experiments, but also to ensure that reliable advice is given with respect to risk-assessment to user-groups (Klimisch et al., 1997). In order to ensure high levels of reproducibility, the assumed intelligence in laboratory animal science was to reduce within-animal variation, referred to as environmental standardisation (Paylor, 2009). However, some have argued that instead of increasing reliability, standardised environments reduce individual variability; thus, increasing the risk of subtle extraneous factors affecting the dependent variable (Richter et al., 2009). Direct evidence for this came from Richter et al. (2011), who carried out a multi-laboratory standardisation vs. heterogenisation procedure, examining strain × environment interactions in a series of commonly used behavioural procedures in mice (including open field test and elevated maze). They demonstrated that systematic heterogenisation (i.e. increasing variation between cages/tanks/group allocation) increased both within- and between-laboratory reliability. This view is not undisputed; however, with some laboratory animal scientists arguing that rigorous standardisation remains essential (Jonker et al., 2013; Josef van der Staay et al., 2010; Örink and Rehbinder, 2000).

As it is clear from this debate, the argument surrounding standardisation is not one for which there is a straightforward answer. In aquatic vertebrates, work with zebrafish has demonstrated that environmental enrichment increases exploration (Collymore et al., 2015), but reduces locomotor behaviour, and increases neuronal proliferation and whole-body cortisol (von Krogh et al., 2010). von Krogh et al. (2010) kept fish in social isolation, which theirs, and other studies, have shown reduced whole-body cortisol. For example, we found keeping fish either in isolation or in pairs for two-weeks prior to testing removes potential ceiling effects observed in group housed fish during the novel tank diving test (Parker et al., 2012); effects that may mask the efficacy of some environmental toxins to affect subtle behaviours. However, while chronic social isolation reduces cortisol, acute isolation reliably increases cortisol (Kalueff et al., 2014a). It is clear in zebrafish that changes in the environment can have severe effects on behaviour and physiology masking, or in some cases reversing, the effects of treatments.

Aquatic vertebrates have a number of personality/individual differences factors that must be accounted for in experimental design. Much of the work in this area hags concentrated on social hierarchies. For example, Galhardo et al. (2012) observed notable differences in the exploration of novel objects between male and female cichlids (*Oreochromis mossambicus)*, and that these effects were strongly mediated by social context (e.g. social isolation, unfamiliar social groups). In addition, social plasticity has been observed in a number of teleost species (Matessi et al., 2010; Taborsky and Oliveira, 2012), and represents an important function both for survival and evolutionary fitness (Oliveira, 2012).

Furthermore, choosing an testing environment and assay that best mimics conditions experienced in the relevant ecosystem is critical in behavioural aquatic toxicology in order to ensure the external validity (generalisability to subjects outside of the study sample) of the findings (Moore and Robinson, 2004). This relates also to ensuring ecologically relevant concentrations of putative contaminants are used (Brodin et al., 2013), but a detailed discussion of this is beyond the scope of this review (see Carvalho et al., 1995). In terms of behavioural testing, during animal experimentation, the subject is removed from its social group (e.g., shoal), taken

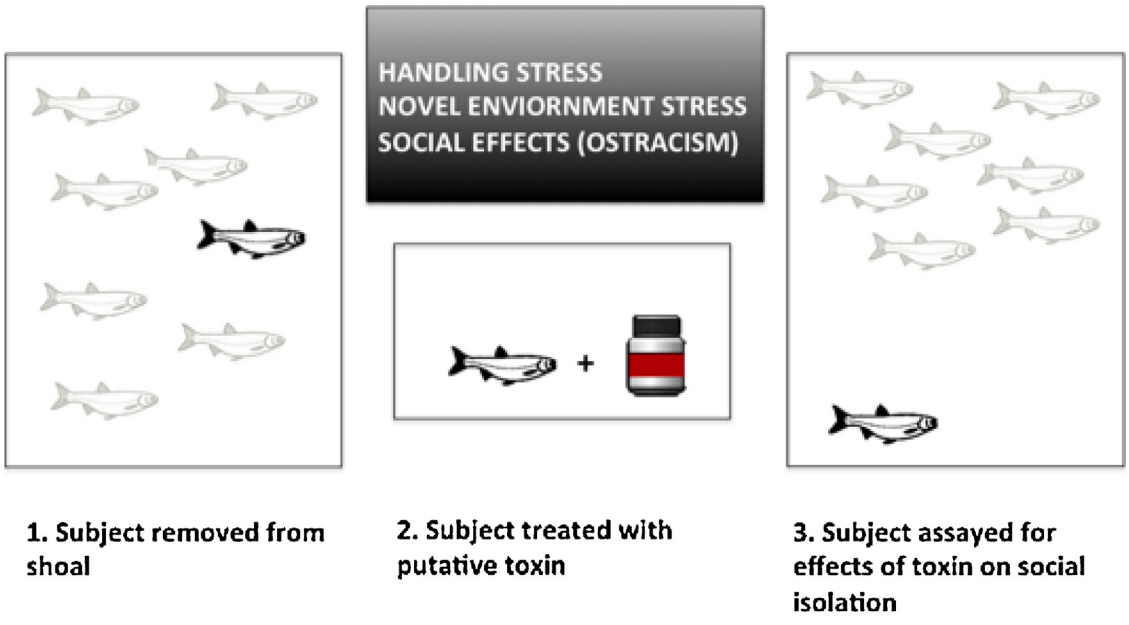

Fig. 1. Model of putative environmental confounds affecting behavioural effects of a toxin on social behaviour.