

# **Revisiting the foundations of animal personality**

**Samuel O'Neill**

**Masters of Research Thesis**

Department of Biological Sciences  
Macquarie University

January 2017



## **Declaration**

I wish to acknowledge the following assistance in the research detailed in this report:

1. Associate Professors Jane Williamson and Culum Brown for assistance with experimental design, statistical analysis, and manuscript preparation.
2. Louise Tosetto, for performing the repeatability analyses and assisting with the methods and results in chapter two.

All other research described in this report is my own original work.

A handwritten signature in dark ink, appearing to read 'Sam O'Neill', with a long, sweeping underline.

Samuel J O'Neill

10<sup>th</sup> January 2017

## **NOTE TO EXAMINERS**

This thesis is written in the form of two journal articles for the journal *Animal Behaviour*, and represents one year (nine months and ten days) of research. This thesis has been formatted per guidelines from the journal *Animal Behaviour*, except where overridden by the requirements of Macquarie University for thesis submission.

Because this thesis includes two chapters, I have included an overarching general abstract.

## TABLE OF CONTENTS

<b>Acknowledgements</b> .....	4
<b>General Abstract</b> .....	5
<b>Chapter One: Developing trait theory in animal personality: Lessons from psychology and phylogenetics</b> .....	6
Title .....	6
Abstract .....	7
Introduction .....	19
Trait Theory: A Brief History .....	11
Theoretical Problems in Animal Personality .....	16
Unvalidated Measures .....	17
Competing Theories .....	18
Developing a Statistical Model of Personality .....	22
Conclusion .....	28
References .....	30
 <b>The effects of acclimatisation on behavioural repeatability in two commonly assayed behaviours of the guppy <i>Poecilia reticulata</i></b> .....	34
Title .....	34
Abstract .....	35
Introduction .....	37
Methods .....	42
Results .....	49
Discussion .....	55
Conclusion .....	59
References .....	61

## **ACKNOWLEDGEMENTS**

If it takes a village to raise a child, it makes sense that it takes much more to raise a scientist.

I will forever appreciate everyone who has helped me on the journey so far.

I thank my two supervisors, Jane Williamson and Culum Brown for their support, feedback, and for enduring my thousands of questions over the last year. I'm glad you never stopped answering them, because I learnt so much from both of you.

I thank my friends and family, the Marine Ecology Group and the Behaviour, Ecology and Evolution of Fishes Laboratory.

Thanks to my two volunteers, Zac Warham, for designing and programming the 'Fish Finder' application, and Belinda Showler for her assistance with data collection and image analysis used in chapter two.

I owe a lot to the wonderful academics who took the time to answer emails, meet me for lengthy discussions, or look over drafts. Thanks to Simon Boag, Sergey Bugaev, Alecia Carter, Sasha Dall, Michael Gillings, and Kate Lynch for their feedback, support and suggestions for chapter one. Thanks to Kate Lynch and Louise Tosetto for their feedback, support and suggestions for chapter two.

I owe the most to Sally Dupont, my partner in crime, for her constant support throughout this entire thesis.

## GENERAL ABSTRACT

Animal personality research is incredibly important, but at the same time faces constant criticisms over its methodological and theoretical foundations. These foundational problems undermine the progress of the field, its reputation, and its impact, and need to be addressed so that previous research can be understood clearly, and that future research can progress in a grounded and efficient way. This thesis addresses two concerns in the animal personality field: 1) a lack of developed theory of personality traits in animals, and 2) a lack of research informing basic methodological decisions.

Chapter one develops a clear pathway for developing and testing trait theory in animals. An underdeveloped trait theory is the ultimate cause for most of the problems raised in recent reviews of the field. Learning from approaches used in human psychology and in phylogenetics, I describe a bottom up approach to develop the structure of animal personality in a species. This structure will both determine the appropriate number of personality traits and trait categories/dimensions, and determine which behaviours reflect each trait.

Chapter two investigates the effect of acclimatisation on behaviour in two different populations of the guppy, *Poecilia reticulata*. I found that longer acclimatisation leads to more repeatable behaviour in an activity assay, and recommend that activity be measured in a familiar environment. For an emergence assay, individuals must be moved to an unfamiliar apparatus and cannot be acclimatised for long periods due to the nature of the starter box. Furthermore, acclimatisation periods are limited due to unideal starting conditions for the animal. I found that although intermediate acclimatisation time led to the highest behavioural repeatability, the emergence assay produced highly repeatable behaviour overall regardless of acclimatisation period. This suggests that the emergence assay generally generates robust behavioural responses. I recommend that researchers investigate acclimatisation times within their study population using their chosen behavioural assays prior to commencing research.

Although further research is required to answer the criticisms of animal personality research, these chapters are each a step towards addressing two of the foundational issues in the field.

## **Chapter One**

# **Developing trait theory in animal personality: Lessons from psychology and phylogenetics**

**Samuel J. O'Neill\*, Jane E. Williamson and Culum Brown**

*Department of Biological Sciences, Macquarie University, Sydney, Australia*

\*Correspondence: S. J. O'Neill, Department of Biological Sciences, Macquarie University, North Ryde, NSW 2109, Australia.

E-mail address: [samuel.oneill@students.mq.edu.au](mailto:samuel.oneill@students.mq.edu.au) (S. J. O'Neill)

## HIGHLIGHTS

- Trait theory is undeveloped in animal personality, leading to incoherencies in the field
- Current attempts to solve this have been helpful, but may not be sufficient in many cases
- We suggest using a bottom up approach to generate the structure of animal personality
- This would address both theoretical and methodological problems in the field
- This will allow comparisons of personality across taxa

We review the problem concerning the definitions and classification of personality traits, and the development of trait theory, in contemporary animal behaviour research. There is theoretical disagreement about how animal behaviours should be sorted into traits, and therefore over how to interpret behavioural observations in even common assays. This has led to a contradictory animal personality literature. There is a growing movement to test the validity of behavioural assays using a few behaviours at a time. This is not optimal, however, as many traits are theorised to be linked by behavioural syndromes or overarching trait categories and therefore will correlate to some degree. Moreover, the level of correlation between behaviours suggesting a trait, or suggesting behaviours under two traits under a trait category has not been defined. We propose that the solution to this problem is the proper development of trait theory, which has been used and developed successfully in human psychology but has been neglected and largely taken for granted by animal behaviour researchers. We outline four steps to developing trait theory in a species using statistics: 1) conceptualisation of relevant behaviours, 2) data collection and analysis, 3) comparison of the statistical model to *a priori* expectations, and 4) repeating the model across populations. This will allow the bottom-up generation of personality traits that will each consist of statistically clustered behaviours, and therefore not suffer from the current *impasse* reached



by current theoretical only attempts. Furthermore, multiple levels can be specified prior to analysis so behaviours can be clustered into traits, and traits can be clustered into trait categories without having to judge relatedness on a case by case basis.

## **KEYWORDS**

Philosophy, personality structure, psychology, phylogenetics, animals, factor analysis, statistics

The study of animal personality is complex, but critical for understanding animal behaviour and the ecological interactions between animals. A simple idea lies at the core of personality research: Individuals within species show consistent differences in behaviour over time and within contexts (Réale, Reader, Sol, McDougall, & Dingemanse, 2007). Animal personality research has focused on understanding the variations that exist between the behaviour of individuals in a population, and the cause of these variations. Similar types of behaviours are generally considered to group together, these being manifestations of the same underlying causes, or personality traits. Overall, consistent behavioural differences between individuals have been found to be manifestations of an interacting and underlying combination of physiology, genetics, development, cognition, and experience (Brown & Braithwaite, 2004; Careau, Thomas, Humphries, & Réale, 2008; Carere & Locurto, 2011; Dingemanse et al., 2009; Sommer-Trembo, Zimmer, Jourdan, Bierbach, & Plath, 2016; Van Oers, De Jong, Van Noordwijk, Kempenaers, & Drent, 2005).

Personality has profound impacts on the life-time fitness of an individual. For example, personality can affect an individual's growth, survival and social status (Mittelbach, Ballew, Kjolvik, & Fraser, 2014). It can also influence group dynamics, mate selection, predator-prey interactions, dispersal and migration, and habitat use (Mittelbach et al., 2014; Smith & Blumstein, 2008; Toms, Echevarria, & Jouandot, 2010). Animal personality research therefore integrates proximate mechanisms into ecological and evolutionary studies by accounting for the effects that environment, physiology, and genetics have on an individual's behaviour. Furthermore, animal personality research must also be accounted for when using animals in non-personality laboratory and field-based experiments (Careau et al., 2008; Carere & Locurto, 2011). Despite the clear value of personality research, the study of personality in

animals is underdeveloped and problems, both methodologically and conceptually, appear throughout the literature. Although researchers have touched on some key issues in the past (Biro & Stamps, 2008; S. Budaev, 1998; Réale et al., 2007), and others have directly addressed the problems, both in the field and theoretically (Biro, 2012; Burns, 2008; Carter, Feeney, Marshall, Cowlshaw, & Heinsohn, 2013; Toms et al., 2010), it is clear that current literature does not fully reflect the critical insights of these authors. Furthermore, there is still substantial progress to be made to eliminate these issues.

As we will argue below, because questions about personality can only be answered by studying behaviour, we need to develop our understanding of personality structure to properly research personality traits, and to solve the conceptual problems of animal personality. To do this there are two, non-mutually exclusive, pathways forward: (1) theoretically driven and (2) statistically driven approaches. Previous attempts in animal personality research have primarily been theoretical (Réale et al., 2007), or have used statistics to investigate the validity of behavioural measures as indicators of a personality trait by examining a few traits at a time (Beckmann & Biro, 2013; Perals, Griffin, Bartomeus, & Sol, 2017). We argue that this trait-by-trait approach is unsatisfactory. Although human psychology research has had its own issues, there are still many lessons that animal personality can draw from. Here we focus on the use of statistics in psychology, and factor analysis in particular, which has been used to generate the largely successful five-factor model of personality structure in humans.

Clearly, contemporary and future attempts to address conceptual problems in animal personality can also benefit from the strengths of both theory and robust statistical analysis. In the field of animal behaviour, analyses such as factor analysis have been raised as a

potential tool for clarifying some conceptual issues in animal personality (Carter et al., 2013; Toms & Echevarria, 2014), and have been used to examine personality at the broad level of dimensions (S. Budaev, 1998). However, we believe that statistical analyses such as factor analysis could play a far more substantial role than identified so far in the literature and could be used to investigate the structure of personality at the species level.

The goal of this review is to both synthesise and build on the understanding of trait theory in animal personality, by:

1. Briefly describing the history and criticisms of trait theory in psychology, to show that the development of trait theory was difficult, and that the development of trait theory in animal personality is no trivial exercise.
2. Using literature from one research area to demonstrate conceptual problems that occur in the personality literature, largely because of our undeveloped trait theory
3. Outlining why previous attempts to rectify these problems have been unsuccessful or insufficient; and
4. Outlining a methodology for developing a theoretical and statistical model to fully investigate and develop a more comprehensive understanding of personality structure.

## **TRAIT THEORY: A BRIEF HISTORY**

The use of personality traits in animal personality research stems from the dominance of trait theory in contemporary human psychology, however, the development of trait theory in psychology took considerable effort. In psychology, personality traits are defined as the enduring patterns of thoughts, feelings, and behaviours that drive behaviour, and differ between individuals (Roberts & Mroczek, 2008). The five-factor model is the most dominant

version of trait theory (although Eysenck's three factor model is also popular), and is currently the dominant theory for personality in humans more generally (Judge et al., 2013). The five-factor model of personality describes five overarching personality trait categories consisting of agreeableness, conscientiousness, extraversion, neuroticism, and openness to experience (McCrae & Costa, 1987). This model was developed on the back of the lexical hypothesis, which posits that important personality characteristics will become embedded in common language as the language evolves (Cattell, 1957; McCrae & Costa, 1987). Therefore, by sampling language, researchers were able to generate a list of important personality descriptors. This list was simplified from 4504 personality descriptors down to 171 by removing synonyms (Cattell, 1957), and this shorter list was used in questionnaires to gather peer ratings covering these areas of personality (Digman, 1990). Separate efforts to analyse the questionnaire data produced differing opinions on how many factors best described the data (Eysenck, 1991), but a general consensus ensured that the five factor model became the main theory of personality. Despite its popularity, however, there are multiple critiques concerning the both five factor model and use of trait theory in psychology today.

There are many criticisms of trait theory and the five-factor model of personality. While it is impossible to do them justice here, the primary criticisms of trait theory were summarised by Block (2010). The most relevant criticisms for animal researchers concern 1) the atheoretical nature of the generation of the five factors using a statistics-focused approach, 2) the use of factor analysis as the sole method of conceptualising personality given the subjectivity of the analysis, and 3) claims that there are personality aspects that are not covered by the five-factor model.

Some criticisms are not as relevant when considering the development of trait theory in animal personality. For instance, one major criticism concerns using analysis of lay person questionnaires as the sole basis for developing an understanding of human psychology (Block, 1995, 2010). While valid, this criticism does not transfer as readily to a behaviourally-focused conceptualisation in the animal personality field. Regardless, animal personality researchers should be aware that there are multiple criticisms of trait theory beyond those covered here, and understand that developing a deep understanding of personality should not be taken lightly.

Although at one stage it might have been a deliberate and careful decision by animal researchers to utilise trait theory, contemporary researchers appear to take trait theory as a given. It has been noted in the past that animal personality has come to rely heavily on the terminology and constructs generated by trait theory in psychology (Gosling & John, 1999). While some researchers have tried to directly apply the human five factor model of personality to animals (e.g., S. Budaev, 2000; Frost, Winrow-Giffen, Ashley, & Sneddon, 2007; Nettle, 2006), this approach has been problematic because 1) these traits are generally measured in humans using self-reporting, a method unavailable in animal research, and 2) the reasoning behind using the lexical hypothesis to generate these traits in humans may not be valid when applied animal species (see Uher and Asendorpf 2008 for discussion of these problems and others). There is little justification in thinking that an human psychology will have developed an adequate, systematic list of personality descriptors concerning other animal species, particularly species that we have rarely observed and interacted with (Uher & Asendorpf, 2008). As a result, most contemporary research does not try use the human personality models.

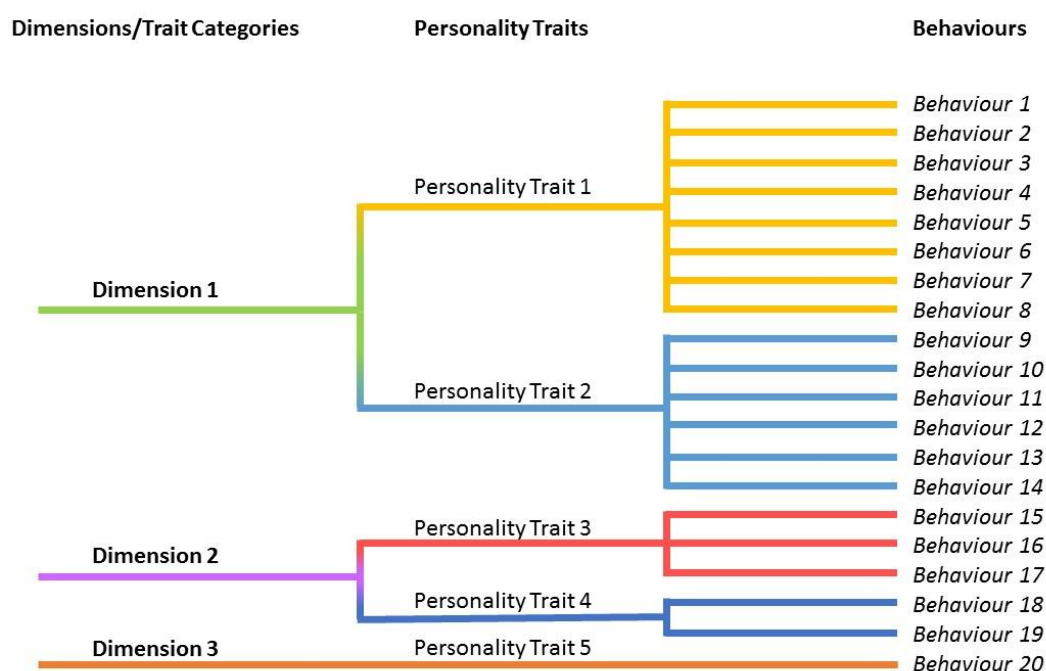
It is not surprising that animal personality research adopted a trait based approach, however this has not been without issue. Conceivably, within animal species there are thousands of different behaviours that vary consistently over time and contexts. To some extent, this diversity is reflected in the literature, with studies investigating behavioural traits ranging from risk taking, escape behaviour, food neophobia, to sociability (Dzieweczynski, Campbell, & Kane, 2016; Fischer, Ghalambor, & Hoke, 2016; Gosling, 2001; Jolles, Manica, & Boogert, 2016; Laubu, Dechaume-Moncharmont, Motreuil, & Schweitzer, 2016). However, many of these behaviours are correlated and therefore might be appropriately grouped into overarching personality traits. Instead of identifying an individual's location on these thousands of behavioural axes, it is simpler to locate an individual on a smaller number of personality trait axes. For instance, an individual that scores highly for consistently risky behaviour in the face of a predator as well as risky behaviour in the face of a dominant conspecific could be described more simply as scoring highly on one personality trait axis concerning risk taking in a more general context. Not only is trait theory a simplifier, but understanding these groupings also allowed researchers to predict broad behaviour using the results of only one or two assays.

The dominant terminology used in animal personality is that of the five trait categories proposed by Réale et al. (2007), although Réale et al. themselves brought together traits that were already being studied in the literature. The five trait categories defined were activity, aggressiveness, boldness-shyness, exploration-avoidance, and sociability, each defined by the ecological context of the behaviours being measured. For each personality trait, individuals exist on a continuum between two extremes (Toms et al., 2010) and observed behaviour is

the primary indicator of where an individual places on that continuum (Biro & Stamps, 2008; Réale et al., 2007; Uher & Asendorpf, 2008).

There are three levels of interest in animal personality studied under trait theory: behaviour, traits, and dimensions (groups, or categories of personality traits). The way that animal behaviours are organised into their representative traits, and these traits into dimensions (see Figure 1 for an example), is what we will refer to as ‘personality structure’ throughout this paper. While the five trait categories outlined by Réale et al. (2007) are more popular and have been more successful than using the human-specific five factor model, there has been little empirical effort to investigate the validity or suitability of these five theoretical trait categories or to further develop the theoretical understanding of personality. For instance, are these trait categories accurately describing the personality landscape of animals, and are there key traits that are not included under these models? It might be the case that only certain behavioural axes that seem related correlate well. Even more fundamentally, when animal researchers describe traits, are they describing patterns of behaviour or are they describing the causes of these patterns of behaviour? Are individuals sociable because they engage with others, or do they engage with others because they are sociable? This lack of theoretical development has led to some major problems in personality research, and interpreting the literature as a whole more generally.





**Figure 1.** A hypothetical personality structure for one species. Using a multilevel statistical analysis such as a factor analysis, behaviours can group into personality traits and one or more personality traits can form overarching dimensions based on the level of correlation of the different behaviours. In this example, behaviours 1-8 correlate highly enough to be considered grouped under personality trait one, but also correlate more weakly with behaviours 9-14 so that they are all considered to be linked under one broad dimension. However, behaviour 20 does not correlate with any other behaviour and therefore forms its own trait and dimension.

## UNVALIDATED MEASURES

Having multiple behavioural measures for each personality trait is necessary for diligent personality research, but is problematic if these assays have not been validated prior to their use. To establish the validity of behavioural measures as a measure of a personality trait, it is important to compare results between trait measures (Burns, 2008; Carter et al., 2013). If one individual does not rank similarly on the two assays, it suggests that one or both assays are not measuring the intended trait in the study species. In animal behaviour, establishing multiple valid behavioural measures for a personality trait is important because not all assays will make sense for all species. This is especially the case when species come from broadly different environmental niches. For instance, the climbing assay used to investigate boldness in European glass eels (*Anguilla Anguilla*) (Podgorniak, Blanchet, De Oliveira, Daverat, &

Pierron, 2016), would obviously not be suitable for species such as the Atlantic cod (*Gadus morhua*), which have no need to climb obstacles during a migration.

The problem in the literature is that the number of assays used in animal personality research has expanded dramatically without any underlying framework of validity. For instance, in early 2016 there were 16 different types of boldness assay used over nine species within 15 studies in fish behaviour alone (Table 1). Although some studies used more than one behavioural measure, it is not currently clear that studies are investigating the personality traits they claim to be studying without any single, holistic investigation of all these behavioural measures. For instance, is activity in the home tank, novel object exploration and also time spent in a risky habitat best categorised by the one trait, boldness, as suggested by some authors (Härkönen, Hyvärinen, Niemelä, & Vainikka, 2015; Vrtělová, Ferrari, Manek, & Chivers, 2016), rather than a combination of activity, curiosity/exploration and boldness? Ideally, theory would guide researchers to which behaviours to use. However, in animal personality research this approach has been problematic.

## **THEORETICAL PROBLEMS IN ANIMAL PERSONALITY**

Theoretically-focused approaches to structuring personality have been valuable, but have not successfully permeated the animal personality field. Réale et al. (2007) proposed the five trait categories listed above as mutually exclusive; for instance, boldness is risk taking in the absence of novelty, while exploration deals with all situations of novelty, and so on for each trait category. However, there is a lack of consensus between competing theories of personality and therefore a lack of consensus in assigning behaviours to a trait of interest (Carter et al., 2013). For instance, some authors argue that novelty is an integral part of the boldness definition, while others argue that behaviours in the context of novelty are not

boldness at all (Coleman & Wilson, 1998; Réale et al., 2007; Toms et al., 2010). This has led to a confused literature with, for example, boldness assays often emphasising novelty (Dzieweczynski et al., 2016; Forsatkar, Nematollahi, & Brown, 2016; Vainikka, Tammela, & Hyvärinen, 2016), and exploration assays often emphasising risk taking (Kalb, Lindström, Sprenger, Anthes, & Heubel, 2016; Řežucha & Reichard, 2016).

Despite the efforts of researchers to develop theoretical guidelines for animal personality, many researchers appear to design their experiments outside of the defined theoretical paradigms, often using their own or other's "common sense" definitions or methods. Furthermore, there is currently no convention for researchers to outline which theoretical paradigm, if any, a study is operating under, making researchers' conclusions about personality traits difficult to put into a broader context. This lack of both theoretical consensus and rigour has divided researchers, and led to two main problems in the literature (Carter et al., 2013). Firstly, there is no clear theoretical guidance for which behaviours are valid measures of a given personality trait of interest. Secondly, single behavioural measures are often used to investigate different personality traits in different studies.

## **COMPETING THEORIES**

The use of a single behavioural measure for multiple personality traits is perhaps the biggest problem in animal personality research today. For example, forced open field assays, where an animal is released into a large open area with no shelter, are commonly used to measure boldness (Benhaïm, Ferrari, Chatain, & Bégout, 2016; Brown, Burgess, & Braithwaite, 2007; Dzieweczynski et al., 2016), exploration (De Serrano, Fong, & Rodd, 2016; Fischer et al., 2016) and activity (Fischer et al., 2016), with each study emphasising different trait interpretations for the same behaviour. Likewise, emergence tests, which are generally thought of as

measuring boldness (Brown & Braithwaite, 2004; Brown et al., 2007), have also been used to measure exploration (Kalb et al., 2016; Řežucha & Reichard, 2016). It does not appear that researchers are in active disagreement. Rather, researchers tend to reference prior papers and common sense theory to support their use of a specific assay for that particular trait, and therefore different research ‘cultures’ develop. The point here is not to criticize specific authors, but rather to outline the problem that emerges when attempting to review the literature and combine individual papers to generate a broader understanding of personality through, for example, meta-analyses. However, when there are competing theories what is the best way to move forward?

In some cases, it is likely that assays inherently measure more than one trait. For example, both emergence assays and open-field assays doubtlessly involve aspects of risk taking, exploration, and possibly activity personality traits. Unfortunately, this realisation does not seem to be the answer to the problem above, as each the debate shifts to what trait is represented most in the results of each assay. The mix of traits measured for a species in each assay needs to be understood, so that conclusions can rightly reflect the broad mix of personalities tested and so that we can identify optimal behavioural measures that are more specific to the trait of interest<sup>1</sup>.

Although the literature contains many conclusions that personalities affect, are affected by, or relate to some other factor, we cannot be certain that different authors’ findings apply to the same personality trait. For example, the following three empirical findings concerning boldness in fish are published:

---

<sup>1</sup> Such as testing predator response to a familiar predator in a familiar environment to reduce the impact of curiosity on the result.

1. Boldness does not affect fry growth rate or survival (Höjesjö et al., 2011)
2. Bold fish make faster decisions (Croft et al., 2009)
3. Bold fish have weaker social networks than shy fish (Mamuneas, Spence, Manica, & King, 2015)

It appears that here we have built some understanding of how boldness may affect the ecology of a fish. However, the methods for these studies vary significantly. Höjesjö et al. (2011) used novel object/novel prey assays as a measure for boldness, Croft et al. (2009) used response to a novel predator model, and Mamuneas et al. (2015) used the time spent in the open (not in cover). Therefore, applying the theoretical framework developed by Réale et al. (2007) excluding novelty from the boldness definition, only the third finding clearly concerns boldness in fish. Under that paradigm, we would conceivably restate these findings thus:

1. Exploration does not affect fry growth rate or survival (Höjesjö et al., 2011)
2. More exploratory fish make faster decisions (Croft et al., 2009)
3. Bold fish have weaker social networks than shy fish (Mamuneas et al., 2015)

To investigate current literature on personality one must foremost refer to the methods of a paper to place the results into one's own theoretical framework. Sufficient understanding of the behaviour and ecology of the species in question is also required. To have to reinterpret a significant proportion of animal personality papers is inefficient and problematic. This reinterpretation must also be done with the understanding that any given framework in animal personality is underdeveloped and risks being overturned in favour of a competing theory. This issue needs to be resolved as soon as possible, since the volume of research that needs to be evaluated under any future framework grows steadily each year.

This problem compounds if one tries to investigate personality at higher levels. For example, if we wish to investigate personality dimensions – the correlation of different personality traits to each other (e.g. boldness and activity) – we need to have a very clear structure in the lower levels of classification to begin with. The challenge of trying to interpret personality studies across species is already difficult (Gosling & John, 1999), and inconsistencies caused by a lack of understanding of the structure of personality only enhance this difficulty.

**Table 1:** A select list of measures of personality in fishes used by authors in 2016.

Target Trait	Behavioural Assay	Study	Species
Boldness	Emergence	Rey et al. (2016)	<i>Cyprinus carpio</i> (common carp)
		Vainikka et al. (2016)	<i>Perca fluviatilis</i> (Eurasian perch)
	Forced open field	Dzieweczynski et al. (2016)	<i>Betta splendens</i> (Siamese fighting fish)
		Forsatkar et al. (2016)	<i>Betta splendens</i> (Siamese fighting fish)
	Feeding under predation	Kim (2016)	<i>Gasterosteus aculeatus</i> (three-spined stickleback)
		Stein, Trapp, and Bell (2016)	<i>Gasterosteus aculeatus</i> (three-spined stickleback)
	Predator/threat response in home tank	Sommer-Trembo et al. (2016)	<i>Poecilia reticulata</i> (Trinidadian guppies)
		Vrtělová et al. (2016)	<i>Cyprinus carpio</i> (Common Carp)
	Time spent in open versus cover/shelter	Jolles et al. (2016)	<i>Gasterosteus aculeatus</i> (three-spined stickleback)
		Vrtělová et al. (2016)	<i>Cyprinus carpio</i> (Common Carp)
	Time to emerge from burrow	Kalb et al. (2016)	<i>Pomatoschistus microps</i> (Common Goby)
	Novel object exploration	Forsatkar et al. (2016)	<i>Betta splendens</i> (Siamese fighting fish)
	Novel environment assay	Dzieweczynski et al. (2016)	<i>Betta splendens</i> (Siamese fighting fish)
	Unforced open field	Benhaïm et al. (2016)	<i>Dicentrarchus labrax</i> (European sea bass)
	Time spent in risky habitat	Härkönen et al. (2015)	<i>Perca fluviatilis</i> (Eurasian perch)
	Feeding behaviour / latency to eat	Kalb et al. (2016)	<i>Pomatoschistus microps</i> (Common Goby)
	Forced open field assay under predation	Řežucha and Reichard (2016)	<i>Poecilia wingei</i> (Endler's guppies)
	Activity under predation	Vainikka et al. (2016)	<i>Perca fluviatilis</i> (Eurasian Perch)
	Novel Environment exploration under predation	Vainikka et al. (2016)	<i>Perca fluviatilis</i> (Eurasian Perch)
	Latency to Explore	Ólafsdóttir and Magellan (2016)	

<i>Exploration</i>	Forced open field under predation	Řežucha and Reichard (2016)	<i>Poecilia wingei</i> (Endler's guppies)
	Emergence	Kalb et al. (2016) Řežucha and Reichard (2016)	<i>Pomatoschistus microps</i> (Common Goby) <i>Poecilia wingei</i> (Endler's guppies)
<i>Activity</i>	Forced-open field	De Serrano et al. (2016) Fischer et al. (2016)	<i>Poexilia reticulata</i> (Trinidadian guppies) <i>Poecilia reticulata</i> (Trinidadian guppies)
	Climbing behaviour	Podgorniak et al. (2016)	<i>Anguilla anguilla</i> (European eel)
	Activity in home tank	De Serrano et al. (2016) Kalb et al. (2016)	<i>Poexilia reticulata</i> (Trinidadian guppies) <i>Pomatoschistus microps</i> (Common Goby)
<i>Aggressiveness</i>	Forced-open field	Vrtělová et al. (2016) Fischer et al. (2016)	<i>Cyprinus carpio</i> (Common Carp) <i>Poecilia reticulata</i> (Trinidadian guppies)
	Mirror task	Forsatkar et al. (2016) Jolles et al. (2016) Hesse, Bakker, Baldauf, and Thünken (2016)	<i>Betta splendens</i> (Siamese fighting fish) <i>Gasterosteus aculeatus</i> (three-spined stickleback) <i>Pelvicachromis taeniatus</i> (striped kribensis)
	Male-male contest	Fischer et al. (2016) Laubu et al. (2016)	<i>Poecilia reticulata</i> (Trinidadian guppies)
<i>Sociability</i>	Time spent near conspecifics	Dzieweczynski et al. (2016) Jolles et al. (2016)	<i>Amatitlania siquia</i> (Convict cichlid) <i>Betta splendens</i> (Siamese fighting fish) <i>Gasterosteus aculeatus</i> (three-spined stickleback)
	Shoaling assay	Kim (2016)	<i>Gasterosteus aculeatus</i> (three-spined stickleback)

## DEVELOPING A STATISTICAL MODEL OF PERSONALITY

One alternative to the theoretical-centred approach outlined above is to generate a statistical model that helps describe the structure of animal personalities. It is worth mentioning early on that this approach assumes that the behaviours of any given personality trait should correlate. While this condition has been excluded by some key theories (Réale et al. 2007), there are two advantages to this assumption:

1. It allows for the validity testing that many argue is necessary for conducting rigorous personality research in animal behaviour (Beckman & Biro, 2013; Burns, 2008; Carter et al., 2013; Peral, Griffin, Bartomeus, & Sol, 2017);
2. The attempt of understanding what, why, and how these patterns of correlating behaviours develop is arguably what gives value to animal personality research.

The five trait categories suggested by Réale et al. (2007) were only a starting point for animal personality structure, and lack of assay validation in animal personality has been raised multiple times in the literature (Beckmann & Biro, 2013; Carter et al., 2013; Toms & Echevarria, 2014; Toms et al., 2010). As a result, more often than before researchers are using multiple behavioural measures of a personality trait of interest and we now know more about the relationship between behaviours.

Unfortunately, this research has limited applications for determining the structure of animal personality because of two main problems. Firstly, animal behaviour papers cover a broad range of taxa, and it should not be expected that specific relationships are represented across all species. Secondly, it is not entirely clear what level of correlation we should expect between measures of a single trait, measures of unrelated traits, and measures of different traits connected under a broader dimension or trait category. This is because the strength of these relationships are relative. A similar problem is faced when constructing a phylogenetic tree and designating structures such as species, genus and family. In both cases, the degree to which we decide a group of species or behaviours belong to a genus or personality trait will depend on the data more generally. In the same way that researchers should not declare two species as belonging to a single genus without comparing them to a wide sample of species, researchers should not examine a small sample of behaviours and declare that they belong



to the same trait without knowing the broader context. This is supported statistically; for example, the assigned eigenvalues used to determine the number of factors present by a factor analysis are dependent on the variables used in the factor analysis and thus depend on the dataset. If key variables are not entered, then the eigenvalues and therefore the factors constructed might be misleading. How are we to know what level of correlation to expect between behaviours of different traits, of the same trait, between behaviours of two different traits that are aligned under a trait category? These are empirical questions for which we will not know the answer without investigation. What then is the best way to move forward?

The work required to develop this personality structure describing the relationship between behaviour, traits and dimensions, is daunting but necessary. A well-developed structure will allow simplification of the field, which in turn should lead to clearer and more precise experimental design, and a more coherent literature. By grouping correlated behaviours into traits, and traits under broader dimensions, a developed structure will inform diverse but appropriate behavioural measures with which to investigate personality traits. Similar to behavioural syndrome research, the structure could reflect relationships between traits and thus direct research into underlying mechanisms of behaviour. There are four apparent steps in determining the personality structure of a species:

1. *Conceptualisation*: Based on our understanding of the species or even population of interest, many relevant behavioural measures reflecting as many personality traits as possible should be identified. Key questions to consider include: what do we know about this species and in which ways would we expect individuals of the species to have developed alternate strategies, or wide ranges of behaviour? This is an important step that has been lacking across many areas of animal personality research (Dall &

Griffith, 2014). The same behaviour can represent entirely different contexts for different species, for instance moving into a sheltered area means very different things for a fish with open water predators and a fish with predators ambushing from such shelters. Furthermore, missing key behaviours will most likely skew the result of any analysis, which are highly dependent on the input data, so special thought should be given towards identifying behaviours that may be highly representative of a trait<sup>2</sup> (Zuckerman, 1992).

2. *Data Collection and Analysis:* These measures should be tested in a single model species or population measuring as broad a range of behaviours possible. A statistical analysis such as a factor-analysis could then be used to produce the least number of robust traits required to sufficiently describe the variation present.
3. *Reflection:* The list of traits should be critically analysed to determine if any traits are not represented and why. A trait might not be represented because it was not truly independent from another trait, however, care needs to be taken as there is a level of arbitrariness and subjectivity to factor analysis (S. V. Budaev, 2010). Importantly, statistical analysis can miss smaller independent factors. Such might be the case with traits like activity, where there are relatively fewer measures compared to the more “complex” traits such as boldness.
4. *Repetition:* These criticisms should feed back into the model and the process repeated in the same population to check consistency. The process should then be repeated

---

<sup>2</sup> Currently, it appears that ‘activity’, i.e. the amount an individual moves around in familiar environments (not exploration), is a behaviour that strongly represents the personality trait of activity, with few other measures such as fin movements in fish also being used.

across different populations, as populations often show personality differences (Bell, 2005; Dingemanse et al., 2007).

In some cases, this process is already being used in the literature, and has shown greater success than other methods (Uher & Asendorpf, 2008). The process should be repeated in the same species to determine how reliable it is, and also repeated across taxa. It would be naïve to assume that personality structure will carry across exactly between species and different structures for different clades are likely to emerge. Researching different species would also allow researchers to more effectively investigate big picture questions, such as the role of environment versus the role of phylogeny in determining personality. How much structure differs between closer- and lesser-related species is, once again, an empirical question that we cannot comprehensively answer without undertaking this research. Simply using meta-analysis on the current data on animal personality would likely not be feasible, due to the difficulties outlined above.

The need to look for correlations to establish the validity of assays and even the structure of personality that has been outlined in the past; however the necessity of undertaking this task and the consequences of having not already done so have been largely understated. As mentioned above, statistical analysis was the primary method used to develop the five-factor model in psychology. Are the criticisms that were raised against psychology equally applicable to this model just outlined? Firstly, is the model outlined a purely theoretical model? No. While this model draws on the ideas and terminology that surround personality structure from trait theory, there is substantially more emphasis on finding the cause of personality, and the methods of developing trait theory have been far removed from the much argued-against lexical approach used in psychology. Likewise, there is ample opportunity, and need,

for this to be informed by the currently developed theory of animal personality. Because of the emphasis on physiological, genetic, developmental and external causes, it could be said that the animal trait theory also aligns with biopsychological, evolutionary, and perhaps ‘whole trait theory’ understandings of personality, which emphasise the need to understand but also explain personality (Fleeson & Jayawickreme, 2015). Regardless, it is clear that both theory and causal explanations, do and will play a part in determining personality structure.

The second criticism is that statistical analysis is not an ideal method for determining personality structure. This criticism primarily arises because of the multiple subjective decisions pervading statistical analysis which prevent it being the “objective” structure generator that some proponents claim. Data are often restructured before factor analysis, which can lead to conclusions that would not have been found otherwise (Block, 1995). Likewise, there are other researcher decisions, such as stopping rules and rotational preferences that heavily influence the number of factors found by analyses such as factor analysis (Block, 2010). Multiple trait dimensions have been generated using factor analysis in the psychology literature in this way. Apart from the five factors, other examples include three, 12 and 16 factor theories developed from the results from different questionnaires (Eysenck, 1991; Shedler & Westen, 2014). For reasons such as these, critics have warned that analyses like factor analysis should not be “treated as a mechanical truth generator” (Meehl 1992, cited by Block 1995). Given that factor analysis can be forced to search for greater or fewer factors, critics say that the analysis must be supported by theory, rather than replace or drive theory. As mentioned earlier, animal behaviour researchers face many of the same problems as comparative psychologists; that is, how sure can we be when applying knowledge about one species or population to another? Can results from one factor analysis be easily

applied to another group of organisms? Once again, it is clear that our theoretical understanding of personality and the species must support the choices made in the analysis, but this does not detract from the value of statistical analysis.

Thirdly, traits have been criticised as statistical generalizations, not always corresponding to an individual's behaviour (Block, 1995). The accusation is that trait theory washes out individual behaviour, ignoring odd moments, or conditional behaviours, such as a sudden outburst of aggression, or compassion in a certain circumstance that might otherwise be a window into that individual's personality (McAdams, 1994). The individual who is highly aggressive when cornered is treated the same as the individual who shows the same number of random bouts of aggression. A response to these criticisms is difficult. We might expect that, at least to some extent, an individual's aggression to defend their young will reflect an amplification of their base aggression. Alternatively, it may be that aggression to defend one's young has nothing to do with normal aggression levels, and should therefore not be grouped together regardless. However, as discussed below, statistical analysis can miss these small, nuanced factors. It might then be the case, as some have concluded in human personality research, that trait theory is a necessary first step, but is not sufficient, in fully describing the whole personality of an individual (McAdams, 1994; McAdams & Pals, 2006). Regardless, trait theory and personality structure need to be developed in animal personality research to move it forward.

Lastly, while not a criticism of the use of statistics to help generate personality structure, it should be noted that statistical analysis will not generate labels for each factor, and naming factors has an interpretive nature that cannot be avoided. Personality traits in general can carry connotations of motivation or intention. For instance, if we say an individual is bold

because of their risk-taking behaviour then it might be taken to imply that their risk-taking is happening on the proximate causal level, both calculated and conscious. However, is an individual who leaves shelter quickly actually leaving early because of a risk calculation, or are there other equally likely explanations? For instance, perhaps some individuals have a stronger desire to seek food more than others. Helpfully, factors generated by factor analysis should require more specific explanatory behaviour compared to single behaviours such as in this case, which should rule out all but a few possible explanations. Regardless, care should be taken when naming the factors and thus personality traits used in animal behaviour.

While trait theory and factor analysis may have their problems, and should not be portrayed as a universal solution for determining personality structure, we believe it is a necessary step towards clearing up the confusion surrounding personality structure and theory. Animal behaviour researchers are all using some version of trait theory; however, it seems that most do this with little understanding of the work that was required to establish its sister theory in human psychology. Although the field of animal personality is relatively young, it should be of some concern that multiple competing theories of personality have been developed in human psychology while only a small portion of animal behaviour papers have been devoted to developing theory at all. It would be highly valuable for the field to better establish the trait theory, if only to find its limitations and replace it later.

## **CONCLUSION**

Carter et al. (2013) recommended that behavioural ecologists consider three aspects when designing animal personality experiments. These were (1) carefully consider assay design, (2) develop and use multiple assays for each trait of interest, and (3) validate these assays using different measures of validity. As of 2016, there appears to have been some progress, with

more studies including multiple behavioural measures to more reliably measure a trait category. We believe that the lack of developed structure in animal personality theory is currently resulting in a confused literature. Consequently, despite many personality traits being identified and used throughout the literature, it is clear that researchers are not all talking about the same thing. Animal personality research has utilised a wide range of behaviours to infer conclusions about personality traits, however, this has been done in the absence of careful validation. Likewise, different researchers use single behaviours to infer different, supposedly independent, personality traits, the worst case being the overlap between boldness and exploration research. Clearly, conclusions about animal personality cannot currently be taken at face value in this context.

The use of statistics in developing a structural framework has been successful in psychology, although it is not without its challenges. Regardless, more thought and research needs to be put into the fundamentals of the field; what are the key personality traits, what behaviours belong to which personality traits, and what is the relationship between personality traits? Furthermore, how generalizable are these findings across populations, species and taxa? Regardless of the answer, a large proportion of scientific papers on animal personality may need to be reinterpreted. Although the solution proposed here requires a large amount of work, this work is nothing compared to the amount of time that will be wasted reinterpreting past and future works on animal personality if we do not rapidly adopt a consistent framework for studying animal personality.

## **ACKNOWLEDGEMENT**

We would like to thank Simon Boag, Sergey Budaev, Alecia Carter, Sasha Dall and Michael Gillings for their critical insights and feedback surrounding the ideas discussed in this review.

This work was partially completed under a Research Training Pathway scholarship from Macquarie University.



## REFERENCES

- Balcombe, J. P., Barnard, N. D., & Sandusky, C. (2004). Laboratory routines cause animal stress. *Journal of the American Association for Laboratory Animal Science*, 43(6), 42-51.
- Beckmann, C., & Biro, P. A. (2013). On the Validity of a Single (Boldness) Assay in Personality Research. *Ethology*, 119(11), 937-947. doi: 10.1111/eth.12137
- Bell, A. (2005). Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*). *Journal of evolutionary biology*, 18(2), 464-473.
- Benhaïm, D., Ferrari, S., Chatain, B., & Bégout, M.-L. (2016). The shy prefer familiar congeners. *Behavioural Processes*. doi: <http://dx.doi.org/10.1016/j.beproc.2016.03.008>
- Biro, P. A. (2012). Do rapid assays predict repeatability in labile (behavioural) traits? *Animal Behaviour*, 83(5), 1295-1300.
- Biro, P. A., & Stamps, J. A. (2008). Are animal personality traits linked to life-history productivity? *Trends in Ecology & Evolution*, 23(7), 361-368.
- Block, J. (1995). A contrarian view of the five-factor approach to personality description. *Psychological bulletin*, 117(2), 187.
- Block, J. (2010). The five-factor framing of personality and beyond: Some ruminations. *Psychological Inquiry*, 21(1), 2-25.
- Brown, C., & Braithwaite, V. A. (2004). Size matters: a test of boldness in eight populations of the poeciliid *Brachyraphis episcopi*. *Animal Behaviour*, 68(6), 1325-1329.
- Brown, C., Burgess, F., & Braithwaite, V. A. (2007). Heritable and Experiential Effects on Boldness in a Tropical Poeciliid. *Behavioral Ecology and Sociobiology*, 62(2), 237-243.
- Budaev, S. (1998). How many dimensions are needed to describe temperament in animals: A factor reanalysis of two data sets. *International Journal of Comparative Psychology*, 11, 17-29.
- Budaev, S. (2000). The dimensions of personality in humans and other animals: A comparative and evolutionary perspective.
- Budaev, S. V. (2010). Using Principal Components and Factor Analysis in Animal Behaviour Research: Caveats and Guidelines. [Article]. *Ethology*, 116(5), 472-480. doi: 10.1111/j.1439-0310.2010.01758.x
- Burns, J. G. (2008). The validity of three tests of temperament in guppies (*Poecilia reticulata*). *Journal of Comparative Psychology*, 122(4), 344.
- Careau, V., Thomas, D., Humphries, M., & Réale, D. (2008). Energy metabolism and animal personality. *Oikos*, 117(5), 641-653.
- Carere, C., & Locurto, C. (2011). Interaction between animal personality and animal cognition. *Curr. Zool*, 57(4), 491-498.
- Carter, A. J., Feeney, W. E., Marshall, H. H., Cowlshaw, G., & Heinsohn, R. (2013). Animal personality: what are behavioural ecologists measuring? *Biological Reviews Of The Cambridge Philosophical Society*, 88(2), 465-475. doi: 10.1111/brv.12007
- Cattell, R. B. (1957). Personality and motivation structure and measurement.
- Coleman, K., & Wilson, D. S. (1998). Shyness and boldness in pumpkinseed sunfish: individual differences are context-specific. *Animal Behaviour*, 56(4), 927-936. doi: <http://dx.doi.org/10.1006/anbe.1998.0852>
- Croft, D. P., Krause, J., Darden, S. K., Ramnarine, I. W., Faria, J. J., & James, R. (2009). Behavioural trait assortment in a social network: patterns and implications. *Behavioral Ecology and Sociobiology*, 63(10), 1495-1503.
- Dall, S. R., & Griffith, S. C. (2014). An empiricist guide to animal personality variation in ecology and evolution. *Frontiers in Ecology and Evolution*, 2, 3.
- De Serrano, A. R., Fong, C., & Rodd, F. H. (2016). Effects of methylphenidate on responses to novelty in a teleost fish (*Poecilia reticulata*). *Behavioural Brain Research*, 302, 53-59. doi: <http://dx.doi.org/10.1016/j.bbr.2016.01.021>

- Digman, J. M. (1990). Personality structure: Emergence of the five-factor model. *Annual review of psychology*, 41(1), 417-440.
- Dingemanse, N. J., Van der Plas, F., Wright, J., Réale, D., Schrama, M., Roff, D. A., . . . Barber, I. (2009). Individual experience and evolutionary history of predation affect expression of heritable variation in fish personality and morphology. *Proceedings of the Royal Society of London B: Biological Sciences*. doi: 10.1098/rspb.2008.1555
- Dingemanse, N. J., Wright, J., Kazem, A. J., Thomas, D. K., Hickling, R., & Dawnay, N. (2007). Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. *Journal of Animal Ecology*, 76(6), 1128-1138.
- Dziewieczynski, T. L., Campbell, B. A., & Kane, J. L. (2016). Dose-dependent fluoxetine effects on boldness in male Siamese fighting fish. *Journal of Experimental Biology*, 219(6), 797-804. doi: 10.1242/jeb.132761
- Eysenck, H. J. (1991). Dimensions of personality: 16, 5 or 3?—Criteria for a taxonomic paradigm. *Personality and individual differences*, 12(8), 773-790.
- Fischer, E. K., Ghalambor, C. K., & Hoke, K. L. (2016). Plasticity and evolution in correlated suites of traits. *Journal of Evolutionary Biology*, n/a-n/a. doi: 10.1111/jeb.12839
- Fleeson, W., & Jayawickreme, E. (2015). Whole Trait Theory. *Journal of Research in Personality*, 56, 82-92. doi: <http://dx.doi.org/10.1016/j.jrp.2014.10.009>
- Forsatkar, M. N., Nematollahi, M. A., & Brown, C. (2016). The toxicological effect of Ruta graveolens extract in Siamese fighting fish: a behavioral and histopathological approach. *Ecotoxicology*, 1-11. doi: 10.1007/s10646-016-1639-5
- Frost, A. J., Winrow-Giffen, A., Ashley, P. J., & Sneddon, L. U. (2007). Plasticity in animal personality traits: does prior experience alter the degree of boldness? *Proceedings of the Royal Society of London B: Biological Sciences*, 274(1608), 333-339.
- Gosling, S. D. (2001). From mice to men: what can we learn about personality from animal research? *Psychological bulletin*, 127(1), 45.
- Gosling, S. D., & John, O. P. (1999). Personality dimensions in nonhuman animals a cross-species review. *Current directions in psychological science*, 8(3), 69-75.
- Härkönen, L., Hyvärinen, P., Niemelä, P. T., & Vainikka, A. (2015). Behavioural variation in Eurasian perch populations with respect to relative catchability. *acta ethologica*, 19(1), 21-31. doi: 10.1007/s10211-015-0219-7
- Hesse, S., Bakker, T. C. M., Baldauf, S. A., & Thünken, T. (2016). Impact of social environment on inter- and intrasexual selection in a cichlid fish with mutual mate choice. *Animal Behaviour*, 111, 85-92. doi: <http://dx.doi.org/10.1016/j.anbehav.2015.10.004>
- Höjesjö, J., Adriaenssens, B., Bohlin, T., Jönsson, C., Hellström, I., & Johnsson, J. I. (2011). Behavioural syndromes in juvenile brown trout (*Salmo trutta*); life history, family variation and performance in the wild. *Behavioral Ecology and Sociobiology*, 65(9), 1801-1810.
- Jolles, J., Manica, A., & Boogert, N. (2016). Food intake rates of inactive fish are positively linked to boldness in three-spined sticklebacks *Gasterosteus aculeatus*. *Journal of fish biology*.
- Kalb, N., Lindström, K., Sprenger, D., Anthes, N., & Heubel, K. U. (2016). Male personality and female spawning consistency in a goby with exclusive male care. *Behavioral Ecology and Sociobiology*, 1-11. doi: 10.1007/s00265-016-2089-z
- Kim, S.-Y. (2016). Fixed behavioural plasticity in response to predation risk in the three-spined stickleback. *Animal Behaviour*, 112, 147-152. doi: <http://dx.doi.org/10.1016/j.anbehav.2015.12.004>
- Laubu, C., Dechaume-Moncharmont, F.-X., Motreuil, S., & Schweitzer, C. (2016). Mismatched partners that achieve postpairing behavioral similarity improve their reproductive success. *Science Advances*, 2(3). doi: 10.1126/sciadv.1501013
- Mamuneas, D., Spence, A. J., Manica, A., & King, A. J. (2015). Bolder stickleback fish make faster decisions, but they are not less accurate. *Behavioral Ecology*, 26(1), 91-96. doi: 10.1093/beheco/aru160

- McAdams, D. P. (1994). A psychology of the stranger. *Psychological Inquiry*, 5(2), 145-148.
- McAdams, D. P., & Pals, J. L. (2006). A new Big Five: fundamental principles for an integrative science of personality. *American Psychologist*, 61(3), 204.
- McCrae, R. R., & Costa, P. T. (1987). Validation of the five-factor model of personality across instruments and observers. *Journal of personality and social psychology*, 52(1), 81.
- Mittelbach, G. G., Ballew, N. G., Kjølvik, M. K., & Fraser, D. (2014). Fish behavioral types and their ecological consequences. *Canadian Journal of Fisheries and Aquatic Sciences*, 71(6), 927-944.
- Nettle, D. (2006). The evolution of personality variation in humans and other animals. *American Psychologist*, 61(6), 622.
- Ólafsdóttir, G. Á., & Magellan, K. (2016). Interactions between boldness, foraging performance and behavioural plasticity across social contexts. *Behavioral Ecology and Sociobiology*, 70(11), 1879-1889. doi: 10.1007/s00265-016-2193-0
- Perals, D., Griffin, A. S., Bartomeus, I., & Sol, D. (2017). Revisiting the open-field test: what does it really tell us about animal personality? *Animal Behaviour*, 123, 69-79. doi: <http://dx.doi.org/10.1016/j.anbehav.2016.10.006>
- Podgorniak, T., Blanchet, S., De Oliveira, E., Daverat, F., & Pierron, F. (2016). To boldly climb: behavioural and cognitive differences in migrating European glass eels. *Royal Society Open Science*, 3(1). doi: 10.1098/rsos.150665
- Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews*, 82(2), 291-318. doi: 10.1111/j.1469-185X.2007.00010.x
- Rey, S., Ribas, L., Morera Capdevila, D., Callol, A., Huntingford, F. A., Pilarczyk, M., . . . MacKenzie, S. (2016). Differential responses to environmental challenge by common carp *Cyprinus carpio* highlight the importance of coping style in integrative physiology. *Journal of Fish Biology*, 88(3), 1056-1069. doi: 10.1111/jfb.12877
- Řežucha, R., & Reichard, M. (2016). The Association Between Personality Traits, Morphological Traits and Alternative Mating Behaviour in Male Endler's Guppies, *Poecilia wingei*. *Ethology*, n/a-n/a. doi: 10.1111/eth.12491
- Roberts, B. W., & Mroczek, D. (2008). Personality Trait Change in Adulthood. *Current Directions in Psychological Science*, 17(1), 31-35. doi: 10.1111/j.1467-8721.2008.00543.x
- Shedler, J., & Westen, D. (2014). Dimensions of personality pathology: an alternative to the five-factor model. *American Journal of Psychiatry*.
- Smith, B. R., & Blumstein, D. T. (2008). Fitness consequences of personality: a meta-analysis. *Behavioral Ecology*, 19(2), 448-455.
- Sommer-Trembo, C., Zimmer, C., Jourdan, J., Bierbach, D., & Plath, M. (2016). Predator experience homogenizes consistent individual differences in predator avoidance.
- Stein, L. R., Trapp, R. M., & Bell, A. M. (2016). Do reproduction and parenting influence personality traits? Insights from threespine stickleback. *Animal Behaviour*, 112, 247-254. doi: <http://dx.doi.org/10.1016/j.anbehav.2015.12.002>
- Toms, C. N., & Echevarria, D. J. (2014). Back to basics: searching for a comprehensive framework for exploring individual differences in zebrafish (*Danio rerio*) behavior. *Zebrafish*, 11(4), 325-340.
- Toms, C. N., Echevarria, D. J., & Jouandot, D. J. (2010). A methodological review of personality-related studies in fish: focus on the shy-bold axis of behavior. *International Journal of Comparative Psychology*, 23(1).
- Uher, J., & Asendorpf, J. B. (2008). Personality assessment in the Great Apes: Comparing ecologically valid behavior measures, behavior ratings, and adjective ratings. *Journal of Research in Personality*, 42(4), 821-838.
- Vainikka, A., Tammela, I., & Hyvärinen, P. (2016). Does boldness explain vulnerability to angling in Eurasian perch *Perca fluviatilis*? *Current Zoology*. doi: 10.1093/cz/zow003

- Van Oers, K., De Jong, G., Van Noordwijk, A. J., Kempenaers, B., & Drent, P. J. (2005). Contribution of genetics to the study of animal personalities: a review of case studies. *Behaviour*, 142(9-10), 1185-1206.
- Vrtělová, J., Ferrari, M. C. O., Manek, A. K., & Chivers, D. P. (2016). Consistent long-term behavioural traits are linked to morphological defences in common carp (). *Behaviour*, 153(1), 15-30. doi: <http://dx.doi.org/10.1163/1568539X-00003320>
- Zuckerman, M. (1992). What is a basic factor and which factors are basic? Turtles all the way down. *Personality and Individual Differences*, 13(6), 675-681. doi: [http://dx.doi.org/10.1016/0191-8869\(92\)90238-K](http://dx.doi.org/10.1016/0191-8869(92)90238-K)

## Chapter Two

# **The effects of acclimatisation on behavioural repeatability in two commonly assayed behaviours of the guppy *Poecilia reticulata***

**Samuel J. O'Neill <sup>a\*</sup>, Jane E. Williamson <sup>a</sup>, Louise Tosetto <sup>a</sup>, Culum Brown <sup>a</sup>**

*<sup>a</sup> Department of Biological Sciences, Macquarie University, Sydney, Australia*

\*Correspondence: S. J. O'Neill, Department of Biological Sciences, Macquarie University, North Ryde, NSW 2109, Australia.

E-mail address: samuel.oneill@students.mq.edu.au (S. J. O'Neill)

## HIGHLIGHTS

- We investigated the effects of acclimatisation time on behavioural repeatability
- Activity became more repeatable over time as the fish acclimatised
- Intermediate acclimatisation led to the most repeatable emergence behaviour
- The feral and domestic sourced population showed substantially different repeatability

The way animal personality experiments are designed can have far reaching effects on results and data interpretation, however, these designs are rarely evaluated. Understanding the effects of experimental design on both behaviour and behavioural repeatability in a population – the primary measure used to identify personality – is crucial to understanding if current methods are optimal. The importance of acclimatisation is widely accepted by animal researchers, yet the length of acclimatisation times used in personality studies vary dramatically. We investigated the effects of different acclimatisation times on the repeatability of activity and emergence behaviour in the guppy, *Poecilia reticulata*. Two divergent populations (one feral-sourced and one domesticated) were assessed to understand the universality of the behaviour. We found that activity levels from individuals exposed to short acclimatisation times were erratic, not repeatable and not indicative of future behaviour, while acclimatisation of one day or more led to activity that was considerably more repeatable. Fish exposed to 10-minutes of acclimatisation in the emergence assay showed high repeatability, and were more repeatable than fish with two, five and 30-minute acclimatisation periods. However, all except for one treatment generated moderate to high repeatability, suggesting that the emergence assay largely generates robust behavioural responses. Although one population (domestic) was more active and emerged

faster than the other population (feral-sourced), the influence of acclimatisation time on repeatability was consistent across both populations. These results suggest that the ideal acclimatisation time for a model species is not always obvious. In cases where acclimatisation conditions may become stressful over time the benefits of longer acclimatisation may be nullified or even reversed. Additionally, some behavioural assays may not need long acclimatisation due to the context of the behaviour. We recommend that researchers investigate acclimatisation times within their study population using their chosen behavioural assays prior to commencing research.

#### **KEYWORDS**

Methodology, activity, emergence, personality, fish, rest, behavioural plasticity, acclimation.

Despite its great impact, there is still significant progress to be made in the field of animal personality. Critically, animal personality research has shown that behaviour is not infinitely flexible as once thought (Sih, Bell, Johnson, & Ziemba, 2004), and is constrained by broader characteristics such as personality dimensions and behavioural syndromes (Budaev, 1998; Sih & Del Giudice, 2012). Animal personality research has also shifted attention from a focus on mean population behaviour to a focus on individuals and the variation between individuals (Clark & Ehlinger, 1987). Overall, research has revealed that animal personality has far reaching effects on all manner of behaviours, and is providing greater explanatory power to animal behaviour research (Roche, Careau, & Binning, 2016). Despite a rapid growth in the number of studies conducted, animal personality research is hampered by its underdeveloped theoretical and methodological foundations. Several reviews have identified problems, including a lack of method validation, non-repeated sampling of behaviour, questionable behavioural measures, problematic study design, and contradictory definitions of key terms (Biro, 2012; Budaev & Brown, 2011; Carter, Feeney, Marshall, Cowlshaw, & Heinsohn, 2013; Dall & Griffith, 2014; Toms & Echevarria, 2014). Although important methodological guidelines and considerations accompanied these criticisms, overall the development of methodology has been limited.

Measuring and demonstrating the repeatability of behaviour is essential to the methodology of personality research. Animal personality is commonly defined as: “individual behavioural differences that are consistent over time and/or across situations” (page 294 Réale, Reader, Sol, McDougall, & Dingemanse, 2007). Thus, to study personality, each individual’s behaviour must be measured multiple times (although many studies measure behaviour only once to infer personality; see Table 1 for examples)(Biro & Stamps, 2008; Carter et al., 2013). Measures of repeatability are considered the best way to experimentally quantify consistent



differences in individual behaviour (Bell, Hankison, & Laskowski, 2009). In personality, repeatability is defined as the proportion of behavioural variation that can be attributed to between-subject variation (Nakagawa & Schielzeth, 2010). Simply, a higher repeatability score characterises more consistent behaviour in an individual over time. Thus, a relatively highly active animal at time T will also be relatively highly active at time T + 1. An increase in repeatability is generated by a wide and consistent gap between individuals' behaviour. If researchers cannot show repeatability of a behaviour, then the behavioural variation cannot be defined as personality.

Many factors can influence the repeatability of personality in a population. When looking across taxa, there is variation in the overall repeatability of different behaviours (Bell et al., 2009). Additionally, there can be variation in the repeatability of a single behaviour between different populations of the same species (Brown, Jones, & Braithwaite, 2005; Lynch, Jean, & Kemp, Forthcoming). The consistency of behaviour, measured by repeatability, can vary due to the context of the behaviour, enclosure size (Polverino, Ruberto, Staaks, & Mehner, 2016), temperature (Biro, Beckmann, & Stamps, 2010), feeding regime (Lichtenstein et al., 2016), salinity (O'Mara & Wong, 2016) and acclimatisation time given prior to behavioural observation (Biro, 2012). Although many factors can be controlled experimentally, research methodology is still in its infancy.

Acclimatisation time, also referred to as acclimation or rest and recovery time, plays an important role in studying behaviour by allowing animals to recover from short-term stress caused by relocating animals to a new testing environment. Handling stress can easily mask or interfere with the expression of natural behaviour (Balcombe, Barnard, & Sandusky, 2004), and therefore potentially personality. Although researchers try to keep stress consistent

across replicate individuals, stress can be increased or decreased by factors outside of the experimenters' control. For example, an individual may be easy to catch in one instance, and harder to catch on another causing stress to vary between trials and therefore reducing the consistency of behaviour across trials. Likewise, there are questions surrounding the effect that personality differences in curiosity or exploration may have when investigating other personality traits in novel test apparatus (Carter et al., 2013). Theoretically, individuals will become more familiar with an enclosure over time and the need to explore will decrease, reducing behaviours that otherwise might be used to indicate commonly investigated traits like activity and boldness. If an individual was highly exploratory, but otherwise relatively inactive it might take some time after introduction for activity levels to truly represent activity levels in a familiar environment. Theoretically, longer acclimatisation times will allow for these confounding effects to decline, and thus produce a more repeatable behaviour for the trait of interest. Thus, there is good reason to study the potential impacts of variation in acclimatisation time on the repeatability of personality traits.

The time given for individuals to acclimatise varies widely in the personality literature. For a single behaviour, methods vary from starting immediately after the animal is added to the test arena to allowing minutes, hours, or days for the animal to acclimatise (Table 1). For some assays, such as the activity assay, there are few researcher costs associated with using longer acclimatisation periods other than increasing experimental time. Furthermore, there are compelling reasons to believe that assays like the activity assay should only be conducted in familiar environments to avoid the impact of novelty and the handling stress associated with rapid assays (Biro, 2012; Budaev & Brown, 2011). However, there are some potential downsides to longer acclimatisation times. Using longer acclimatisation periods requires more time and may lead to a smaller sample size and less experimental power if researchers

are time-limited. Prolonged acclimatisation times may lead to a change in non-targeted behaviours, such as a reduction in subject motivation to perform the targeted task. Furthermore, many of the typical designs of behavioural assays may not be suitable for long term acclimatisation. In behavioural assays, such as the emergence assay and open field assay the novelty of the area is integral to their validity. In most cases, individuals are removed from the home tank and often start in small starter-box chambers, where long-term acclimatisation could impact on the physiology of the individual, such as by increasing stress and reversing the intended effect of acclimatisation (Balcombe, Barnard, & Sandusky, 2004; Strange, Schreck, & Ewing, 1978). Despite the problems with using low acclimatisation time, the emergence assay has been found to lead to high repeatability with limited acclimatisation periods (Chapman et al., 2011), suggesting that this assay is relatively robust compared with others. Despite the importance of acclimatisation time the effect of different acclimatisation times is poorly understood particularly in the context of animal personality assays.

**Table 1.** Acclimatisation time given before the first measurement of activity, number of times activity was measured and the time between these trials in personality research using fish. Fish were chosen because they are a model species for personality research and are our area of research.

Study	Acclimatisation Time	Times tested	Time between trials
Kekäläinen, Lai, Vainikka, Sirkka, and Kortet (2014)	2 minutes	1	N/A
Cote, Fogarty, Weinersmith, Brodin, and Sih (2010)	10 minutes	2	3 weeks
Cote, Fogarty, Tymen, Sih, and Brodin (2013)	10 minutes	1	N/A
Jones and Godin (2010)	1 hour	1	N/A
Dingemanse et al. (2009)	2 hours	2	2 hours
Millot et al. (2014)	2 hours	1	N/A
Brown and Irving (2013)	1 day	2	2 days
Colléter and Brown (2011)	1 day	1	N/A
Irving and Brown (2013)	1 day	2	2 days
Biro et al. (2010)	1.5 days	15	1 day
Biro (2012)	3 Days	11	5 hours / 17 hours
Øverli, Kotzian, and Winberg (2002)	7 Days	1	N/A
Vallon et al. (2016)	9 days	15	24 hours

To date only two papers have investigated the effects of acclimatisation on animal behaviour and behavioural repeatability, and specifically examined the effects of acclimatisation and habituation on activity and predator response in fish. Although not a main feature of the study, Burns (2008) found that a small increase of acclimatisation time from two minutes to five minutes significantly increased the reliability of behaviour in the guppy *Poecilia reticulata*, although repeatability was not directly calculated. Biro (2012) established wild-captured juvenile marine fish (*Pomacentrus wardi*) in home tanks and examined behaviour over seven days as fish acclimatised to captivity. While responses of individuals to simulated-predator attacks were not repeatable over the first two days, response times after two days were highly repeatable. Activity levels in individuals showed low repeatability regardless of acclimatisation and habituation. As a result, Biro (2012) advised against rapid assaying of behaviour. However, lab-reared animals experiencing handling or isolation during their development can experience lower stress reactions to similar events later in life (Caldji, Francis, Sharma, Plotsky, & Meaney, 2000), and Biro (2012) highlighted that further research is needed to corroborate these findings, and to determine if divergent populations of fish, such as lab reared populations, have different acclimatisation patterns.

This study assessed the effects of different acclimatisation times on two different behavioural measures commonly used in animal personality research, using two populations of the guppy *Poecilia reticulata*. *P. reticulata* is routinely used as a study model in behavioural experiments (E.g. Burns, 2008; Harris, Ramnarine, Smith, & Pettersson, 2010; Irving & Brown, 2013). Population differences (e.g., maternal effects) and ecological contexts (e.g., predation levels) can lead to different patterns of behaviour within a population (Biro & Stamps, 2008; Dingemanse et al., 2009; Lynch et al., Forthcoming), so two distinct populations, one feral-sourced and one domesticated, were used to assess the universality of acclimation effects.

Activity measures are primarily used to investigate the activity personality trait (Colléter & Brown, 2011; Cote et al., 2013; Dingemanse et al., 2009), and allowed a direct comparison to the findings of Biro (2012). Emergence assays are commonly used to infer boldness personality (Brown & Braithwaite, 2004; Caroline & Reeb, 2006; Chapman et al., 2011; Cote et al., 2013), although they have also been used to investigate exploration (Kalb, Lindström, Sprenger, Anthes, & Heubel, 2016; Řežucha & Reichard, 2016). Due to the problematic nature of extending the starting conditions of the emergence assay, we hypothesised that:

1. longer acclimatisation time will lead to the highest repeatability of activity behaviour of individuals in both populations. However;
2. moderate (5-10 minute) acclimatisation periods will lead to the higher repeatability of emergence behaviour in individuals in both populations.

## **METHODS**

### *Study Subjects*

Two separate populations of guppies (*Poecilia reticulata*) were used for our observational experiments, taken from breeding populations maintained in a laboratory setting at Macquarie University, Sydney, Australia. One population was originally sourced from a feral guppy population living in a drainage ditch in Darwin, Australia (see Trompf & Brown 2014 for details), with the current population likely being well past the 10<sup>th</sup> generation lab reared. The second population consisted of domestic guppies obtained locally from aquarium stores from 2014 to 2016, with most individuals being at least third generation lab reared. Because both populations had been housed in the lab for some time, we do not make conclusions concerning the origin of these populations (i.e., feral versus domesticated guppies) other than they differ in their provenance. However, we will refer to these two populations as ‘feral’ and

‘domesticated’ guppies for brevity. Both populations were maintained across several group tanks within the laboratory. Fish were routinely moved between tanks to maintain similar densities and social conditions.

#### *Activity Test Procedure*

Test arenas consisted of nine 15 L (38 x 20 x 20 cm) glass tanks supplied with a gravel substrate and the sides lined with opaque partitions to obscure vision between individuals. Tanks were filled with aged freshwater to a height of 16 cm. The room was maintained at 27°C, and illuminated on a 12-hour photoperiod (lights on at 0700 hours).

Twenty-two fish from each population, with equal numbers of both sexes and of various sizes (larger than 15mm to avoid problems finding them in photographs), were taken from their group tanks and placed individually in small 4.5 L (20 x 15x 15 cm) tanks to become accustomed to isolation and avoid variation in capture stress. Because of the time intensive nature of this design compared to the emergence experiment, 44 fish were used in total. Fish were given 72 h in isolation before being moved into the larger 15 L (38 x 20 x 20 cm) experimental tanks; one day longer than the 48 hours required for fish effectively adjust to isolation (Jolles, Taylor and Manica 2016). The behaviour of each individual was recorded for blocks of five minutes, with recording commencing at two minutes, 10 minutes, 60 minutes, 120 minutes, 24 hours, 48 hours, 96 hours, and seven days after their introduction into the tank. Fish were fed a commercial flake food each morning during both isolation and observation periods. To record activity, a GoPro™ camera was positioned centrally above the tank to mitigate lens distortion and set to take a photo of the tank every 10 seconds. Pictures were analysed using the custom software, ‘Fish Finder’, which allowed us to generate an x-y

coordinate for the fish in each photo and therefore estimate the distance each fish travelled during the five-minute observation period.

As data were longitudinal in nature, linear mixed models (LMM) were used to assess variables likely to significantly impact on fish activity. All models were built using the lme function in the nlme package in R Studio (R Core Team, 2013). Activity data met normality assumptions. The full model (all main effects and interactions) was compared with a main effects model, with fish identity included in both models as a random effect, and maximum likelihood used to estimate model parameters. The two models were compared using a likelihood ratio test and a significant effect of interactions ( $P = 0.012$ ) was observed. Interaction models, fit with restricted maximum likelihood (REML), were then compared using AIC values to ascertain the final model (see appendix for models and relevant AIC values). The final model included time, population, size and sex as fixed effects with interactions between all variables. Individual fish were included as the random effect:

$$\text{activity} \sim \text{time} + \text{population} + \text{sex} + \text{size} + \text{time} * \text{population} * \text{sex} * \text{size} + (1 | \text{Fish ID})$$

Models to assess activity levels in domestic and feral populations separately were also created with time, sex and size as fixed effects and with fish ID as random effects in models for both populations.

To assess repeatability, data were subset into 28 different time pairings to generate a repeatability ‘heat map’, with results set out as per Figure 1. This allowed us to assess repeatability across acclimatisation periods and determine not only when repeatability first occurs between two observations, but also if (and when) there is an acclimatisation ‘break point’ after which behaviour will be reliably repeatable across future observations. With so many comparisons the risk of type one error, a false positive in testing for statistical

significance, is considerable, so only results with  $p < 0.001$  were considered significant. Additionally, because we only investigated repeatability on this level to establish temporal trends the impact of any single type one error was low.

	2 Minutes	10 Minutes	60 Minutes	120 Minutes	One Day	Two Days	Four Days	Seven Days
2 Minutes		1	2	3	4	5	6	7
10 Minutes			8	9	10	11	12	13
60 Minutes				14	15	16	17	18
120 Minutes					19	20	21	22
One Day						23	24	25
Two Days							26	27
Four Days								28
Seven Days								

**Figure 1.** Map showing the order of the 28 different time pairings for the assessment of activity repeatability.

Repeatability was estimated for each population independently for each of the 28 time pairings using the within – and between – variance components. LMM with REML was used in the package rptR (R Core Team, 2013), with individual fish identity specified as the grouping factor. Confidence intervals and standard errors for both repeatability ( $R$ ) and adjusted repeatability ( $R_A$ ) were calculated from parametric bootstraps that created the distributions of likelihood ratios (1000 times) (Nakagawa & Schielzeth, 2010). Statistical significance of the repeatability was tested by likelihood ratio tests, comparing the log-likelihood of models with and without the grouping effect of individual fish.



A visual assessment of the heat maps for each population identified a break point in repeatability after one day of acclimatisation in both populations (see Tables 3 and 4 in results). This was similar to the results of Biro (2012), who found a break in the consistency of predator response after two days of acclimatisation, after which repeatability was high. Repeatability was then examined using the same methodology as above for the full data set (all time periods) compared to a truncated data set that only included observations taken after one day of acclimatisation (time periods: one, two, four and seven days).

#### *Emergence Assay Procedure*

Two hundred guppies, *P. reticulata*, 100 from each population, were used in the emergence acclimatisation assays. Guppies of various sex and age (we excluded those under 9mm to avoid escape from the starter box) were randomly chosen from stock tanks and placed individually into 4.5 L (20x15x15 cm) glass fish tanks. Individuals of different size and sex were intentionally included to capture the true variability within each population. Fish were block randomised to produce groups of 25 fish from each population for four treatment groups: fish undergoing 2, 5, 10, and 30-minute acclimatisation periods. Fish were given 72 hours to adjust to isolation in their smaller tanks to reduce variation in capture stress and improve repeatability, as per the findings of Biro (2012) and Jolles, Aaron Taylor, and Manica (2016). Fish were fed flake food in the morning each day, including the mornings on days where trials occurred, which has been shown to potentially increase repeatability of behaviour in some contexts (Lichtenstein et al., 2016). Fish were then assayed individually with an emergence apparatus.

The setup for the emergence apparatus was as follows (see appendix for illustration). A large 26 L (45 x 24 x 24 cm) tank was lined with gravel and filled to 16cm height with water. All sides

of the tank were covered with an opaque lining to block the fish viewing the researcher. An opaque grey acrylic chamber (10 x 10 x 17 cm tall) was placed at one end of the tank with an opaque lid on top and a 4 x 4 cm opening facing the open area. A sliding door covered the opening at the start of each assay, and could be removed remotely by a pulley system. Small slits along each corner of the chamber allowed some light to penetrate inside once covered.

To begin a trial, a fish was placed in the chamber, the top opening covered, and a timer started. After the individual's allotted acclimatisation time (two, five, 10 or 30 minutes) had passed the door was lifted, and the time taken for the fish to fully emerge from the chamber was recorded. Once emerged, a few minutes was given before recapturing the fish to minimise the fish forming an association between emerging and capture stress that might impact the repeat assay. Individuals were then returned to their individual tanks. Individuals were given approximately 96 hours before being tested again using the same methods to establish an overall stable repeatability. The time between repeated trials tends to vary in the methodological literature from 24 hours to a week or more (e.g. Beckman & Biro, 2013; Biro, 2012; Burns, 2008), with more studies using a shorter rather than longer period. We therefore chose 96 hours (four days) as a healthy median. After the second assay, individuals were captured, measured and then released back into their relevant group tanks. Water in the individual home tanks was changed during the trial. Water in the experimental tank was aerated and partially changed (30 % of the volume) between trials to minimize the risk of accumulating chemical cues, and the experimental tank was washed out and refilled completely between trial days.

To assess whether emergence times differed across populations and acclimatisation periods, and that any differences were repeatable across both populations and acclimatisation

periods, we analysed data in line with their longitudinal nature (repeated measures over time) using LMM. Emergence times were log transformed to meet normality assumptions and fish size was mean-centred by sex and population (range -1 to +1, with 0 as the mean). It was necessary to generate a relative size measure because size differed between populations and sexes (Brown & Braithwaite 2004). The full model (with all main effects and possible interactions) was first compared with a main effects model, both models including fish identity as the random effect and both fit using the maximum likelihood method. A likelihood ratio test was performed using the ANOVA function in R package, nlme. No significant effect of the full model with interactions ( $P = 0.366$ ) was observed. The main effects model included acclimatisation time as the principal variable of interest with the variables; trial number, fish population, sex, size and housing tank also included as fixed effects. To account for variability between individuals, fish identity was included as a random effect. No effect of sex ( $t = 1.16_{192}$ ,  $P = 0.246$ ), fish size ( $t = 0.435_{192}$ ,  $P = 0.664$ ) or holding tank ( $t = 1.24_{192}$ ,  $P = 0.217$ ) on the emergence times of fish was observed, and these variables were thus excluded from the final model. Models were fitted with REML and ranked based on AICs with the best fitting models having the lowest AIC values (Gutzwiller, 2007) (see appendix for summary of AIC model scores). We compared models with and without the inclusion of a random slope (fish identity / tank) to determine its effect on model strength. The final model included trial and fish population as fixed effects with individual fish included as the random effect:

$$emergence \sim acclimatisation + trial + population + (1|Fish\ ID)$$

To assess the repeatability of emergence at different acclimatisation times we first obtained agreement repeatability ( $R$ ) of component scores. We included the time-related effects of multiple trials to ensure validity of the repeatability score (Biro & Stamps 2015, Jolles et al.

2016b). Agreement repeatability for each population was estimated independently for each acclimatisation period using the within – and between – variance components with LMM. REML estimation was used in the package rptR, with individual fish identity specified as a random effect. We then assessed adjusted repeatability ( $R_A$ ) at each acclimatisation period based on the final mixed-effects model above; trial was included as a fixed factor and individual fish as the grouping factor. Confidence intervals and standard errors for repeatability estimates were calculated from parametric bootstraps that created the distributions of likelihood ratios (1000 times). Statistical significance of the repeatability was tested by likelihood ratio tests comparing the log-likelihood of models with and without the grouping factor of individual fish.

#### *Ethical Note*

All experimental methods conformed to the standards set by the Macquarie University Animal Ethics Committee (ARA 2013/024). Fish were checked daily to ensure their health, and ensure optimal environmental and housing conditions (e.g. temperature, lighting, filtration). At the completion of the study fish were moved back into stock breeding tanks.

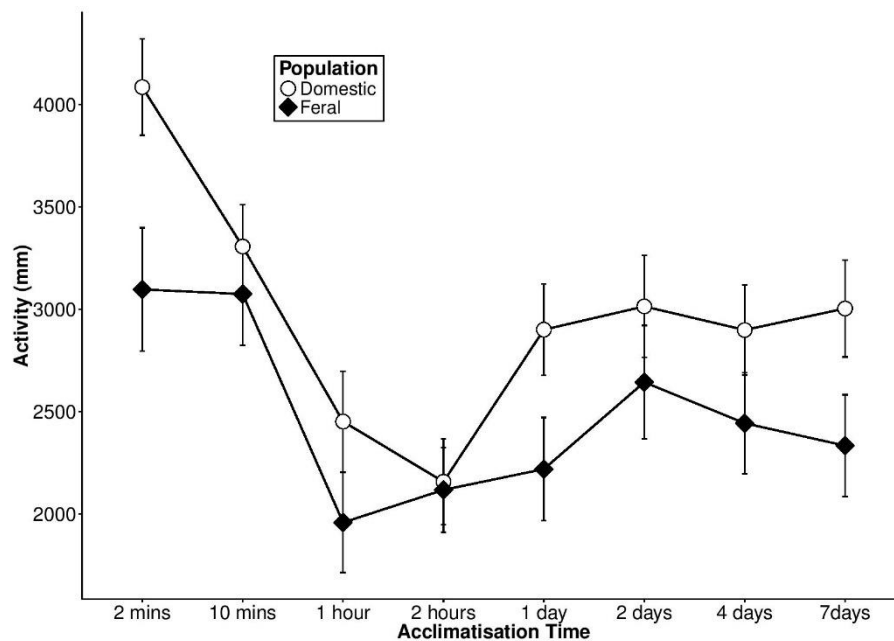
## **RESULTS**

### *Activity*

There was a complex interaction between time, size and sex ( $F_{37} = 3.440$ ,  $P = 0.002$ ), however, there was no obvious pattern in this interaction and its effect on fish activity. No other interactions were significant.

Fish from the feral population were significantly less active than the domestic population across the seven days ( $t_{37} = -2.629$ ,  $P = 0.012$ ). There was a significant effect of acclimatisation

time on the activity of fish. In the domestic population, individuals were most active at two minutes of acclimatisation, after which activity declined and was significantly less at all other acclimatisation periods ( $p < 0.05$ ). In the feral population, individuals were also most active at two minutes of acclimatisation. Although there was no significant difference between activity between two and ten minutes ( $t_{154} = -0.077$ ,  $P = 0.939$ ) and two minutes and two days ( $t_{154} = -1.553$ ,  $p = 0.123$ ), activity was significantly lower at all other acclimatisation times ( $p < 0.05$ ) (Figure 2).



**Figure 2.** Mean activity ( $\pm$  SE) for feral and domestic populations over 7 days of acclimatisation.

In both populations, we visually determined a break point in repeatability at one day of acclimatisation (Figures 3, 4). As expected, repeatability was often higher for observations taken in close temporal proximity. This was particularly true across the first four observations which all occurred over a ~two-hour period, although this effect was far more noticeable in the behaviour of the feral population (Figure 4).

	2 Minutes	10 Minutes	60 Minutes	120 Minutes	1 Day	2 Days	4 Days	7 Days
2 Minutes		0.265 (0.18)	0.000 (0.13)	0.000 (0.13)	0.069 (0.14)	0.225 (0.18)	0.337 (0.18)	0.335 (0.18)
10 Minutes			0.154 (0.16)	0.000 (0.12)	0.059 (0.14)	0.151 (0.16)	0.022 (0.13)	0.000 (0.12)
60 Minutes				0.710 (0.13)	0.475 (0.18)	0.332 (0.18)	0.040 (0.14)	0.307 (0.19)
120 Minutes					0.243 (0.18)	0.164 (0.16)	0.000 (0.12)	0.132 (0.16)
1 Day						0.733 (0.11)	0.344 (0.18)	0.509 (0.17)
2 Days							0.108 (0.16)	0.331 (0.18)
3 Days								0.402 (0.17)
7 Days								

**Figure 3.** Heat map showing repeatability estimates for activity comparisons in domestic guppies, with confidence intervals in parentheses, across all acclimatisation subsets for domestic fish. All repeatability estimates were highly significant ( $P < 0.001$ ). Intensity of red represents increasing repeatability in intervals of 0.10.

	2 Minutes	10 Minutes	60 Minutes	120 Minutes	1 Day	2 Days	4 Days	7 Days
2 Minutes		0.476 (0.16)	0.370 (0.18)	0.435 (0.17)	0.196 (0.17)	0.164 (0.16)	0.576 (0.15)	0.050 (0.13)
10 Minutes			0.504 (0.16)	0.503 (0.16)	0.361 (0.17)	0.148 (0.16)	0.258 (0.17)	0.000 (0.12)
60 Minutes				0.560 (0.15)	0.232 (0.17)	0.210 (0.17)	0.398 (0.17)	0.167 (0.16)
120 Minutes					0.398 (0.17)	0.170 (0.16)	0.400 (0.17)	0.041 (0.13)
1 Day						0.576 (0.15)	0.577 (0.14)	0.416 (0.17)
2 Days							0.530 (0.15)	0.680 (0.12)
3 Days								0.632 (0.13)
7 Days								

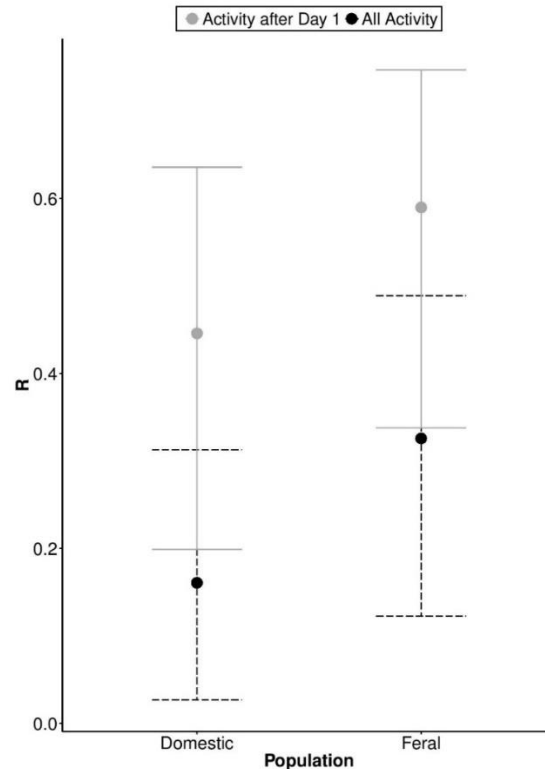
**Figure 4.** Heat map showing repeatability estimates for activity comparisons in feral guppies, with confidence intervals in parentheses, across all acclimatisation subsets for domestic fish. All repeatability estimates were highly significant ( $P < 0.001$ ). Intensity of red represents increasing repeatability in intervals of 0.10.

We analysed behavioural repeatability for both populations using 1) activity across the entire experiment, and 2) using only activity observed only after one day of acclimatisation. Adjusted repeatability ( $R_A$ ) results are provided in Table 2. The repeatability assessment was statistically significant for in all cases ( $p < 0.001$ ), however, the repeatability statistic itself was

considerably higher in both populations when using activity observed after one day of acclimatisation ( $R = 0.161$  compared to  $R = 0.446$  in the domestic population, and  $R = 0.326$  compared to  $R = 0.590$  in the feral population). Overall, the adjusted repeatability of the domestic population's activity was lower than that of the feral population (Figure 5).

**Table 2.** Repeatability estimates of activity for both the domestic and feral populations calculated both using all observations and using only observations made after one day of acclimatisation. Adjusted repeatability ( $R_A$ ), standard errors (SE), variance among individuals ( $Var_{among}$ ), variance within individuals ( $Var_{within}$ ), the 95% confidence interval (CI) and associated P value of each estimate is given. All tests were significant.

Treatment	Observations	$R_A$ (SE)	$Var_{among}$	$Var_{within}$	CI	P-value
Domestic	All observations	0.161 (0.073)	228860	1423744	[0.022, 0.300]	<0.001
	Observations after one day of acclimatisation	0.446 (0.118)	548832	1229462	[0.199, 0.636]	<0.001
Feral	All observations	0.326 (0.091)	539839	1656622	[0.142, 0.487]	<0.001
	Observations after one day of acclimatisation	0.590 (0.106)	941814	1591356	[0.338, 0.747]	<0.001



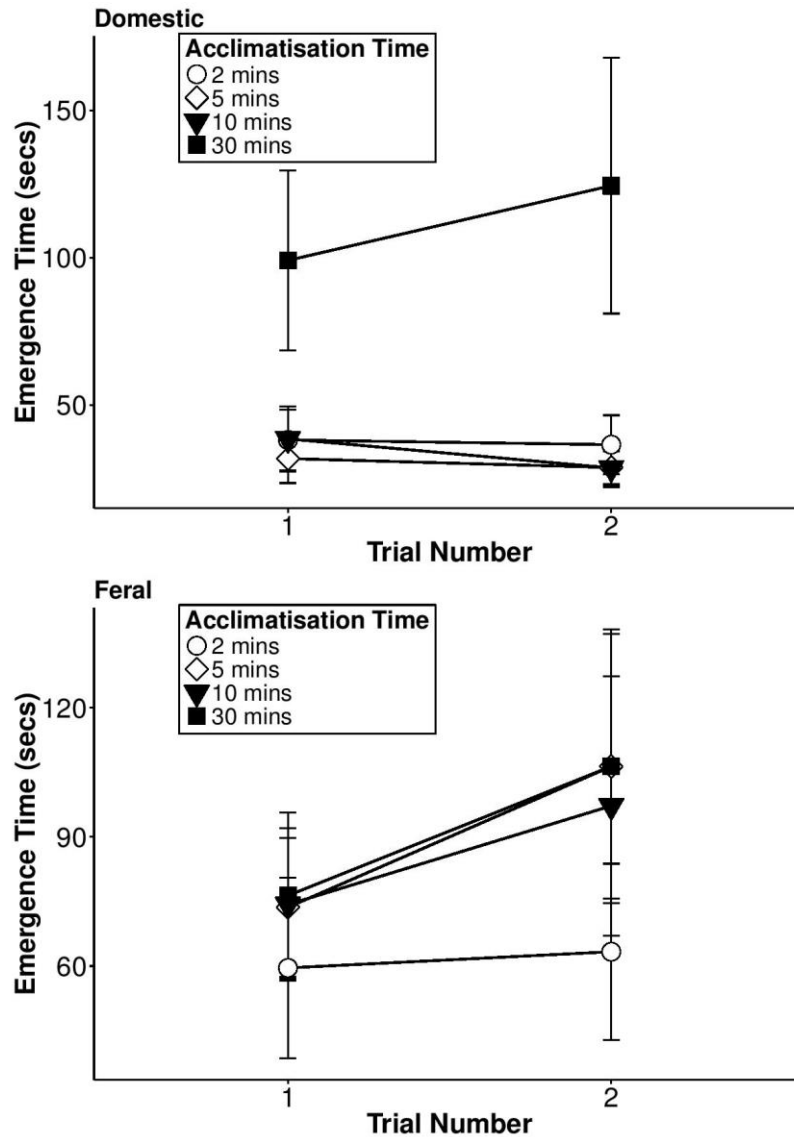
**Figure 5.** Comparison of the repeatability point estimates and 95% confidence intervals for the activity levels of domestic and feral populations. Values of all activity (black) compared to repeatability of activity after one day of acclimatisation (grey).

## *Emergence*

The final model demonstrated a significant effect of population on emergence times with fish from the feral population taking longer on average to emerge from the chamber than those in the domestic population ( $29 \pm 14$  seconds,  $t_{195} = 3.690$ ,  $P = 0.003$ ). No significant difference was observed in overall emergence time between trial one and two ( $t_{199} = 0.551$ ,  $P = 0.585$ ). Fish in the five and 10-minute acclimatisation treatments both took, on average,  $11 \pm 20$  seconds longer than those in the two-minute acclimatisation treatment, but these differences were not significant ( $t_{195} = 0.908$ ,  $P = 0.365$  &  $t_{195} = 1.113$ ,  $P = 0.267$  respectively). Fish acclimatised for 30 minutes prior to being tested took significantly longer to emerge than fish acclimatised for two minutes ( $51 \pm 21$  seconds,  $t_{195} = 2.396$ ,  $P = 0.018$ ).

Analyses of the fish populations were also undertaken separately. In domestic fish, there was no significant difference in emergence times for fish acclimated at five ( $t_{94} = 0.142$ ,  $P = 0.888$ ) and ten minutes ( $t_{94} = 0.430$ ,  $P = 0.668$ ) when compared with the two-minute acclimation treatment. However, fish exposed to the thirty-minute acclimatisation treatment took  $74 (\pm 25)$  seconds longer to emerge than those in the two-minute treatment ( $t_{94} = 2.355$ ,  $P = 0.021$ ). For those fish from the feral population, there was no difference in emergence times for acclimation periods of five ( $t_{94} = 1.436$ ,  $P = 0.154$ ), ten ( $t_{94} = 1.147$ ,  $P = 0.254$ ) or 30 ( $t_{94} = 1.112$ ,  $P = 0.269$ ) when compared to two minutes (Figure 6).





**Figure 6.** Mean ( $\pm$ SE) emergence times for domestic fish (top box) and feral fish (bottom box) at the four different acclimatisation time periods.

Adjusted repeatability ( $R_A$ ) results are provided in Table 3 (see appendix for a side by side comparison of repeatability and adjusted repeatability results). The repeatability assessment was statistically significant for all acclimatisation periods ( $P < 0.05$ ) except the 30-minute feral treatment ( $P = 0.06$ ), however, the repeatability statistic itself ranged from 0.44 in the 30-minute domestic treatment, to 0.74 in the 10-minute feral treatment. Repeatability for emergence scores was highest in both populations of fish in the 10-minute acclimatisation treatment.

**Table 3.** Repeatability estimates across trials for emergence times for individuals from the domestic and feral populations, and for both populations combined. Acclimatisation time (Accl.), adjusted repeatability (RA), standard errors (SE), variance among individuals (Var<sub>among</sub>), Variance within individuals (Var<sub>within</sub>), the 95% confidence interval (CI) and associated P value of each estimate is given. Significant repeatability estimates are shown in bold.

Treatment	Accl.	RA (SE)	Var <sub>among</sub>	Var <sub>within</sub>	CI	P-value
Domestic	2	0.444 (0.161)	0.667	1.501	[0.077, 0.698]	<b>0.002</b>
	5	0.562 (0.147)	0.654	1.163	[0.211, 0.778]	<b>0.005</b>
	10	0.603 (0.140)	0.624	1.035	[0.271, 0.802]	<b>0.003</b>
	30	0.506 (0.151)	1.246	2.463	[0.152, 0.743]	<b>0.008</b>
Feral	2	0.607(0.127)	0.803	1.322	[0.295, 0.801]	<b>0.002</b>
	5	0.494(0.159)	0.704	1.424	[0.135, 0.749]	<b>0.016</b>
	10	0.742 (0.096)	1.057	1.424	[0.498, 0.875]	<b>&lt;0.001</b>
	30	0.399 (0.163)	0.682	1.756	[0.035, 0.653]	0.060

## DISCUSSION

Our results demonstrate that acclimatisation time can impact on the repeatability of behaviour in two distinct populations of guppies. As predicted, longer acclimatisation led to higher behavioural repeatability for activity in guppies. Activity was considerably more repeatable when data prior to one day of acclimatisation was excluded. This trend was present in both populations but clearest in the feral-sourced population. While complete acclimatisation often takes long periods of time, this finding is in line with substantial recovery to stress over a one day acclimatisation period previously demonstrated (e.g. Atwell et al., 2012; Wedemeyer, 1972). Biro (2012) documented a similar phenomenon, but found a break point after two days of acclimatisation when bringing wild fish into the lab rather than the one day in our results. Stress responses are proportional to the stress event itself (Barton & Iwama, 1991), and our methods were able to minimise stressors because all components of the experiment occurred in the lab environment, allowing capture, handling, and the

matching of water conditions to be relatively easy. Given this, and that lab reared animals can experience lower stress responses to familiarised stressors (Caldji et al., 2000), it is not surprising that we found a shorter necessary acclimatisation time and higher overall repeatability than that in the results of wild-captured fish found by Biro (2012). A future study comparing replicate populations of lab-reared and wild-captured individuals of the same species would elucidate this further. Interestingly, activity behaviour in this study showed substantially higher repeatability than the average activity repeatability in the literature (Bell et al., 2009), where activity was found to be one of the least repeatable behaviours. We propose that the average activity repeatability in the literature may be low because of the insufficient acclimatisation times used in many personality studies. These findings show the need for behavioural researchers to test activity in familiar environments, as rapid assays of activity in new environments will not reliably detect activity personality because of the likely trade-offs with other traits such as exploration.

The two populations differed marginally in both activity levels and emergence time, with the domesticated population emerging faster and being more active than the feral-sourced population. Reports of population differences in personality are common in the literature (e.g., Brown et al., 2005; Dingemanse et al., 2007; Bell & Sih, 2007), but recent research investigating personality differences between domestic and feral guppies have found mixed results, ranging from significant differences (Lynch, Jean & Kemp, In press) to few differences at all (Swaney, Cabrera-Álvarez, & Reader, 2015). Given that only one population of each was represented in this study and that the feral-sourced population was many generations removed from the wild, we cannot draw general conclusions regarding the possible ecological drivers of this variation other than to say there is a heritable component.

Although the intermediate acclimatisation time in the emergence assay led to the highest behavioural repeatability, almost all acclimatisation treatments showed substantial repeatability. We hypothesised that individuals stress levels would decline as fish acclimatised, but then increase due to negative impacts of confinement. This is because the benefits of longer acclimatisation times for assays with suboptimal starting conditions – such as confined starter boxes – likely peak and then decline if an individual is left for too long. The effect of these secondary stressors over 30 minutes of acclimatisation are complex as individuals react differently depending on prior stress experience (Pitman, Ottenweller, & Natelson, 1990), and we therefore predicted that repeatability would be highest at intermediate stages of acclimatisation. While intermediate acclimatisation did produce the highest behavioural repeatability for both populations, the differences between acclimatisation treatments were low. The high repeatability across most treatments suggests that the emergence assay generates robust behaviour that is less affected by the uncontrolled minor stressors than the activity assay in our study populations. It has been proposed that emergence behaviour is necessarily linked to stress because individuals must choose when to exit the chamber into an unfamiliar and potentially risky environment (Brown, Burgess, & Braithwaite, 2007). The necessity of some stress in this assay may therefore explain why this assay may do well with lower acclimatisation time compared to the activity assay. Given that wild caught animals will undergo higher levels of stress, and that environmental factors will interact with populations differently, we recommend that experimental design is considered on a population basis. These results demonstrate the need to investigate acclimatisation times for population or species in pilot studies prior to behavioural research.

Animal behaviour and personality researchers should be mindful of individual variation and population differences. The personality of animals can be investigated at multiple levels: the

individual, the population, the species, and broader taxonomic categories (e.g. fish). However, repeatability estimates are essential in personality research but are generated at the population level, and it is therefore crucial that researchers consider how likely their repeatability estimates indicate trends relevant to other populations, and other levels of interest. The effects of acclimatisation on emergence and activity behaviour were similar in two genetically distinct populations of guppy in this study, despite significant behavioural difference between the populations. We are therefore confident that these findings are rigorous for lab reared guppies.

It is important to keep sight of what repeatability means for animal personality research. Repeatability represents the proportion of variance that is due to differences between individuals rather than within individuals or, essentially, the proportion of variation of animal behaviour likely due to personality. Generally, differences in repeatability have been interpreted as actual differences between populations or between behaviours (Bell et al., 2009), however, recent research shows that repeatability can be increased or decreased in a behaviour of a single population (Biro, 2012; Biro et al., 2010; Lichtenstein et al., 2016; O'Mara & Wong, 2016; Polverino et al., 2016). In this study, it was an interaction of both an increase in individual consistency and an increase in variation between individuals that contributed to higher repeatability. In the activity assay, where individuals had a significant time to acclimatise, the variation between individuals approximately doubled while individuals also became more consistent. In the emergence assay, where individuals did not have much time to acclimatise, the interaction was less clear. Although these components of repeatability are not always reported, our results demonstrate how both these aspects of behavioural repeatability can vary, and may potentially be influenced by experimental design.

This study demonstrates that experimental design can strongly impact the findings of animal behaviour research. Personality researchers assume that, at least to some degree, experimental measurements of individual behaviour reflect how those individuals or populations would behave outside experimental conditions (Biro, 2012). Thus, experimental research can investigate relationships between aspects of personality and other factors (such as whether activity levels are decreased by previous exposure to a predator), or investigate relationships between two or more aspects of personality (such as correlations between activity and exploratory tendencies). However, methodological research is discovering that there are a range of experimental factors that act differently across individuals within a study and therefore muddy the expression of consistent individual differences in behaviour, personality, under experimental conditions. Variations in individual responses to habituation (Bell & Peeke, 2012; Biro, 2012), changes in social conditions (Jolles et al., 2016; Wilson, Coleman, Clark, & Biederman, 1993), enclosure size (Polverino et al., 2016), and acclimatisation (Biro, 2012) can all act in this way unless experiments are designed carefully. Researchers should consider all aspects of experimental design, and minimise the confounding variables most concerning for their research question.

In animal behaviour and personality research, there are still many questions surrounding proper methodology. Is the current methodology actually measuring the behaviour or personality of interest? Are there experimental decisions affecting animal behaviour in a way that alters our understanding of animal behaviour and personality? For acclimatisation, the picture is becoming clearer. Pilot studies on a population or species are a first preference for assessing optimal acclimatisation times. Failing this, in experiments where an animal will be in 'home tank'-like conditions, longer acclimatisation will likely lead to higher repeatability. For experiments that employ problematic starting conditions, which may vary between

populations and species but could include factors like social isolation or small enclosures, longer acclimatisation may be better only to a point, after which further acclimatisation time loses benefit and can reduce behavioural repeatability. Regardless, rapid assays of behaviour are rarely an ideal methodology, and researchers are encouraged to assess the costs and benefits of their species and experimental system in relation to their hypotheses.

## **CONCLUSION**

Careful design of behavioural experiments and methodologies is essential to our understanding of animal personality. We have shown that acclimatisation time affects behavioural repeatability, the primary indicator of animal personality and that these effects are similar in two discrete populations of guppies. For many assays, longer acclimatisation periods will lead to best results. However, in some methodologies, such as the emergence assay in this study, the best acclimatisation time may be restively short, and may vary between populations and species. Overall, more consideration is needed prior to experimentation in personality research to ensure that the data will robustly reflect a response to any given hypothesis. We strongly suggest that, where possible, researchers assess methodologies with pilot studies prior to the actual experiments to determine ideal acclimatisation periods.

## REFERENCES

- Atwell, J. W., Cardoso, G. C., Whittaker, D. J., Campbell-Nelson, S., Robertson, K. W., & a, E. D. (2012). Boldness behavior and stress physiology in a novel urban environment suggest rapid correlated evolutionary adaptation. *Behavioral Ecology*, 23(5), 960-969. doi: 10.1093/beheco/ars059
- Barton, B. A., & Iwama, G. K. (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases*, 1, 3-26. doi: [http://dx.doi.org/10.1016/0959-8030\(91\)90019-G](http://dx.doi.org/10.1016/0959-8030(91)90019-G)
- Bell, A. M., Hankison, S. J., & Laskowski, K. L. (2009). The repeatability of behaviour: a meta-analysis. *Animal behaviour*, 77(4), 771-783.
- Bell, A. M., & Peeke, H. V. S. (2012). Individual variation in habituation: behaviour over time toward different stimuli in threespine sticklebacks (*Gasterosteus aculeatus*). *Behaviour*, 149(13-14), 1339-1365. doi: 10.1163/1568539X-00003019
- Bell, A. M., & Sih, A. (2007). Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). *Ecology Letters*, 10(9), 828-834.
- Biro, P. A. (2012). Do rapid assays predict repeatability in labile (behavioural) traits? *Animal Behaviour*, 83(5), 1295-1300.
- Biro, P. A., Beckmann, C., & Stamps, J. A. (2010). Small within-day increases in temperature affects boldness and alters personality in coral reef fish. *Proceedings of the Royal Society of London B: Biological Sciences*, 277(1678), 71-77.
- Biro, P. A., & Stamps, J. A. (2008). Are animal personality traits linked to life-history productivity? *Trends in Ecology & Evolution*, 23(7), 361-368.
- Brown, C., & Braithwaite, V. A. (2004). Size matters: a test of boldness in eight populations of the poeciliid *Brachyrhaphis episcopi*. *Animal Behaviour*, 68(6), 1325-1329.
- Brown, C., Burgess, F., & Braithwaite, V. A. (2007). Heritable and experiential effects on boldness in a tropical poeciliid. *Behavioral Ecology and Sociobiology*, 62(2), 237-243. doi: 10.1007/s00265-007-0458-3
- Brown, C., & Irving, E. (2013). Individual personality traits influence group exploration in a feral guppy population. *Behavioral Ecology*(25), 95-101.
- Brown, C., Jones, F., & Braithwaite, V. (2005). In situ examination of boldness–shyness traits in the tropical poeciliid, *Brachyrhaphis episcopi*. *Animal Behaviour*, 70(5), 1003-1009.
- Budaev, S. (1998). How many dimensions are needed to describe temperament in animals: A factor reanalysis of two data sets. *International Journal of Comparative Psychology*, 11, 17-29.
- Budaev, S., & Brown, C. (2011). Personality traits and behaviour. *Fish Cognition and Behavior. Fish and Aquatic Resources Series*. Oxford, UK: Wiley-Blackwell.
- Burns, J. G. (2008). The validity of three tests of temperament in guppies (*Poecilia reticulata*). *Journal of Comparative Psychology*, 122(4), 344-356. doi: 10.1037/0735-7036.122.4.344
- Caldji, C., Francis, D., Sharma, S., Plotsky, P. M., & Meaney, M. J. (2000). The Effects of Early Rearing Environment on the Development of GABAA and Central Benzodiazepine Receptor Levels and Novelty-Induced Fearfulness in the Rat. *Neuropsychopharmacology*, 22(3), 219-229.
- Caroline, L., & Reeb, S. G. (2006). Individual Leadership and Boldness in Shoals of Golden Shiners (*Notemigonus crysoleucas*). *Behaviour*, 143(10), 1263-1280.
- Carter, A. J., Feeney, W. E., Marshall, H. H., Cowlshaw, G., & Heinsohn, R. (2013). Animal personality: what are behavioural ecologists measuring? *Biological Reviews Of The Cambridge Philosophical Society*, 88(2), 465-475. doi: 10.1111/brv.12007
- Chapman, B. B., Hulthén, K., Blomqvist, D. R., Hansson, L. A., Nilsson, J. Å., Brodersen, J., . . . Brönmark, C. (2011). To boldly go: individual differences in boldness influence migratory tendency. *Ecology Letters*, 14(9), 871-876.
- Clark, A. B., & Ehlinger, T. J. (1987). Pattern and adaptation in individual behavioral differences *Perspectives in ethology* (pp. 1-47): Springer.



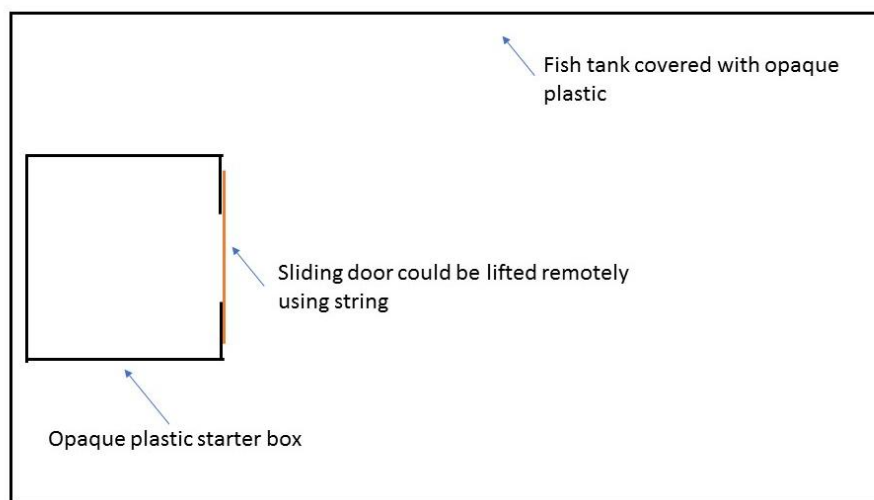
- Colléter, M., & Brown, C. (2011). Personality traits predict hierarchy rank in male rainbowfish social groups. *Animal Behaviour*, 81(6), 1231-1237.
- Cote, J., Fogarty, S., Tymen, B., Sih, A., & Brodin, T. (2013). *Personality-dependent dispersal cancelled under predation risk*. Paper presented at the Proc. R. Soc. B.
- Cote, J., Fogarty, S., Weinersmith, K., Brodin, T., & Sih, A. (2010). Personality traits and dispersal tendency in the invasive mosquitofish (*Gambusia affinis*). *Proceedings of the Royal Society of London B: Biological Sciences*, rspb20092128.
- Dall, S. R., & Griffith, S. C. (2014). An empiricist guide to animal personality variation in ecology and evolution. *Frontiers in Ecology and Evolution*, 2, 3. doi: 10.3389/fevo.2014.00003
- Dingemanse, N. J., Van der Plas, F., Wright, J., Réale, D., Schrama, M., Roff, D. A., . . . Barber, I. (2009). Individual experience and evolutionary history of predation affect expression of heritable variation in fish personality and morphology. *Proceedings of the Royal Society of London B: Biological Sciences*, 276, 1285-1293. doi: 10.1098/rspb.2008.1555
- Dingemanse, N. J., Wright, J., Kazem, A. J., Thomas, D. K., Hickling, R., & Dawnay, N. (2007). Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. *Journal of Animal Ecology*, 76(6), 1128-1138.
- Gutzwiller, K. J. R., Samuel K. (2007). Using Statistical Models to Study Temporal Dynamics of Animal—Landscape Relations. In J. A. Bissonette & I. Storch (Eds.), *Temporal Dimensions of Landscape Ecology: Wildlife Responses to Variable Resources* (pp. 93-118). Boston, MA: Springer US.
- Harris, S., Ramnarine, I. W., Smith, H. G., & Pettersson, L. B. (2010). Picking personalities apart: estimating the influence of predation, sex and body size on boldness in the guppy *Poecilia reticulata*. *Oikos*, 119(11), 1711-1718. doi: 10.1111/j.1600-0706.2010.18028.x
- Irving, E., & Brown, C. (2013). Examining the link between personality and laterality in a feral guppy *Poecilia reticulata* population. *Journal of fish biology*, 83(2), 311-325.
- Jolles, J. W., Aaron Taylor, B., & Manica, A. (2016). Recent social conditions affect boldness repeatability in individual sticklebacks. *Animal Behaviour*, 112, 139-145. doi: <http://dx.doi.org/10.1016/j.anbehav.2015.12.010>
- Jones, K. A., & Godin, J.-G. J. (2010). Are fast explorers slow reactors? Linking personality type and anti-predator behaviour. *Proceedings of the Royal Society of London B: Biological Sciences*, 277(1681), 625-632.
- Kalb, N., Lindström, K., Sprenger, D., Anthes, N., & Heubel, K. U. (2016). Male personality and female spawning consistency in a goby with exclusive male care. *Behavioral Ecology and Sociobiology*, 70(5), 683-693. doi: 10.1007/s00265-016-2089-z
- Kekäläinen, J., Lai, Y.-T., Vainikka, A., Sirkka, I., & Kortet, R. (2014). Do brain parasites alter host personality?—Experimental study in minnows. *Behavioral ecology and sociobiology*, 68(2), 197-204.
- Lichtenstein, J. L. L., DiRienzo, N., Knutson, K., Kuo, C., Zhao, K. C., Brittingham, H. A., . . . Pruitt, J. N. (2016). Prolonged food restriction decreases body condition and reduces repeatability in personality traits in web-building spiders. *Behavioral Ecology and Sociobiology*, 70(11), 1793-1803. doi: 10.1007/s00265-016-2184-1
- Lynch, K. E., Jean, S., & Kemp, D. J. (Forthcoming). Boldness differences in feral and domestic guppies (*Poecilia reticulata*).
- Millot, S., Péan, S., Labbé, L., Kerneis, T., Quillet, E., Dupont-Nivet, M., & Bégout, M.-L. (2014). Assessment of genetic variability of fish personality traits using rainbow trout isogenic lines. *Behavior genetics*, 44(4), 383-393.
- Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biological Reviews*, 85(4), 935-956.
- O'Mara, K. M., & Wong, M. Y. L. (2016). Body size mediated effects of multiple abiotic stressors on the growth and social behaviour of an estuarine fish, Australian Bass (*Macquaria*

- novemaculeata). *Environmental Biology of Fishes*, 99(1), 95-104. doi: 10.1007/s10641-015-0458-5
- Øverli, Ø., Kotzian, S., & Winberg, S. (2002). Effects of cortisol on aggression and locomotor activity in rainbow trout. *Hormones and Behavior*, 42(1), 53-61.
- Pitman, D. L., Ottenweller, J. E., & Natelson, B. H. (1990). Effect of stressor intensity on habituation and sensitization of glucocorticoid responses in rats. *Behavioral Neuroscience*, 104(1), 28-36.
- Polverino, G., Ruberto, T., Staaks, G., & Mehner, T. (2016). Tank size alters mean behaviours and individual rank orders in personality traits of fish depending on their life stage. *Animal Behaviour*, 115, 127-135. doi: <http://dx.doi.org/10.1016/j.anbehav.2016.03.013>
- R Core Team. (2013). R: A language and environment for statistical computing. Retrieved from <http://www.R-project.org/>
- Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews*, 82(2), 291-318. doi: 10.1111/j.1469-185X.2007.00010.x
- Řežucha, R., & Reichard, M. (2016). The Association Between Personality Traits, Morphological Traits and Alternative Mating Behaviour in Male Endler's Guppies, *Poecilia wingei*. *Ethology*, 122(6), 456-467. doi: 10.1111/eth.12491
- Roche, D. G., Careau, V., & Binning, S. A. (2016). Demystifying animal 'personality' (or not): why individual variation matters to experimental biologists. *Journal of Experimental Biology*, 219, 3832-3843.
- Sih, A., Bell, A., Johnson, J. C., & Ziemba, R. E. (2004). Behavioral Syndromes: An Integrative Overview. *The Quarterly Review of Biology*, 79(3), 241-277. doi: 10.1086/422893
- Sih, A., & Del Giudice, M. (2012). Linking behavioural syndromes and cognition: a behavioural ecology perspective. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 367(1603), 2762-2772.
- Strange, R. J., Schreck, C. B., & Ewing, R. D. (1978). Cortisol concentrations in confined juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Transactions of the American Fisheries Society*, 107(6), 812-819.
- Swaney, W. T., Cabrera-Álvarez, M. J., & Reader, S. M. (2015). Behavioural responses of feral and domestic guppies (*Poecilia reticulata*) to predators and their cues. *Behavioural Processes*, 118, 42-46. doi: <http://dx.doi.org/10.1016/j.beproc.2015.05.010>
- Toms, C. N., & Echevarria, D. J. (2014). Back to basics: searching for a comprehensive framework for exploring individual differences in zebrafish (*Danio rerio*) behavior. *Zebrafish*, 11(4), 325-340.
- Trompf, L., & Brown, C. (2014). Personality affects learning and trade-offs between private and social information in guppies, *Poecilia reticulata*. *Animal Behaviour*, 88, 99-106.
- Vallon, M., Grom, C., Kalb, N., Sprenger, D., Anthes, N., Lindström, K., & Heubel, K. U. (2016). You eat what you are: personality-dependent filial cannibalism in a fish with paternal care. *Ecology and Evolution*, 6(5), 1340-1352.
- Wedemeyer, G. (1972). Some Physiological Consequences of Handling Stress in the Juvenile Coho Salmon (*Oncorhynchus kisutch*) and Steelhead Trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada*, 29(12), 1780-1783. doi: 10.1139/f72-284
- Wilson, D. S., Coleman, K., Clark, A. B., & Biederman, L. (1993). Shy-bold continuum in pumpkinseed sunfish (*Lepomis gibbosus*): An ecological study of a psychological trait. *Journal of Comparative Psychology*, 107(3), 250-260.

## APPENDIX

**Table 4.** Repeatability estimates across acclimatisation (Accl.) trials for all fish as well as domestic and feral populations. The table shows repeatability (R) and adjusted repeatability (RA), standard errors (SE), the 95% confidence interval (CI) and associated P value of each estimate. Significant repeatability estimates are shown in bold.

<i>Emergence ~ 1 (intercept)</i>					<i>Emergence ~ Trial</i>		
Treatment	Accl.	R (SE)	CI	P-value	R <sub>A</sub> (SE)	CI	P-value
All Fish	2	0.545 (0.104)	[0.297, 0.706]	<b>&lt;0.001</b>	0.527 (0.105)	[0.289, 0.696]	<b>&lt;0.001</b>
	5	0.603 (0.089)	[0.400, 0.744]	<b>&lt;0.001</b>	0.529 (0.106)	[0.300, 0.702]	<b>&lt;0.001</b>
	10	0.709 (0.075)	[0.529, 0.822]	<b>&lt;0.001</b>	0.679 (0.081)	[0.491, 0.805]	<b>&lt;0.001</b>
	30	0.461 (0.115)	[0.214, 0.654]	<b>&lt;0.001</b>	0.461 (0.112)	[0.212, 0.652]	<b>0.002</b>
Domestic	2	0.460 (0.154)	[0.110, 0.707]	<b>0.021</b>	0.444 (0.161)	[0.077, 0.698]	<b>0.002</b>
	5	0.576 (0.145)	[0.235, 0.792]	<b>0.002</b>	0.562 (0.147)	[0.211, 0.778]	<b>0.005</b>
	10	0.603 (0.133)	[0.291, 0.809]	<b>0.002</b>	0.603 (0.140)	[0.271, 0.802]	<b>0.003</b>
	30	0.521 (0.153)	[0.180, 0.754]	<b>0.006</b>	0.506 (0.151)	[0.152, 0.743]	<b>0.008</b>
Feral	2	0.613 (0.129)	[0.312, 0.803]	<b>&lt;0.001</b>	0.607(0.127)	[0.295, 0.801]	<b>0.002</b>
	5	0.501 (0.150)	[0.185, 0.742]	<b>0.010</b>	0.494(0.159)	[0.135, 0.749]	<b>0.016</b>
	10	0.740 (0.096)	[0.496, 0.882]	<b>&lt;0.001</b>	0.742 (0.096)	[0.498, 0.875]	<b>&lt;0.001</b>
	30	0.403 (0.165)	[0.000, 0.675]	<b>0.039</b>	0.399 (0.163)	[0.035, 0.653]	0.060



**Figure 7.** Test apparatus for the emergence assay. Fish were introduced into the opaque Perspex starter chamber. After acclimatisation, the trap door could be remotely raised and the time taken for the fish to exit the chamber measured.