BEHAVIOURAL PHENOTYPES: ASSOCIATED LIFE-HISTORY TRAITS AND ENVIRONMENTAL EFFECTS ON DEVELOPMENT

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: 

Abstract

It is widely documented that non-human organisms express individual differences in behavioural patterns. For example individuals can be categorised as bold or shy and when these individual behavioural differences are consistent through time, they are termed behavioural types (BTs). In recent years research has identified that BTs often correlate across contexts/situations and these correlations are referred to as behavioural syndromes. Behavioural types and syndromes (i.e. personality) have also been implicated as major factors shaping population dynamics and the ability to buffer environmental disturbance. Recent theoretical predictions have proposed that BT variation may be underpinned by life-history strategies; however, these predictions have been little studied to date. Moreover, little research has focused upon environmental influences and the ontogeny of personality.

In this thesis I use the Mangrove killifish (*Kryptolebias marmoratus*), a naturally occurring clonal vertebrate, as a model organism. This species presents a powerful tool providing the ability to replicate within and between isogenic genotypes in a controlled manner. Moreover the natural clonality expressed by this species permits environmental effects upon BT plasticity and BT-life-history interactions to be investigated within a developmental framework. In chapter 2, I present microsatellite genotyping results which show that the founding individuals used to propagate a laboratory population at The University of Exeter represent 20 genetically distinct homozygous genotypes.

I additionally address five research questions exploring genotypic, environmental, and developmental effects upon three commonly studied BTs (exploration, boldness and aggression): Firstly; I ask do adult hermaphrodite and secondary males exhibit personality i.e. repeatable BT expression? In chapter 3, I present results showing that both of the sexes express short term personality. Moreover, I show that that genotype is an important factor influencing BTs expressed, regardless of sex, indicating underlying genetic control. Secondly I ask; does genotype level life-

history variation underpin personality trait variation during ontogeny? In chapter 4, I show considerable developmental plasticity in behavioural expression between genotypes but not life-history and I find limited behaviour-life-history relationships during development. Thirdly I ask; does the rearing environment influence life-history and behavioural plasticity? In chapter 5, I show that in comparison to a control treatment, the presence of conspecifics during ontogeny results in an average reduction in behavioural scores; however, life-history was unaffected. In addition, I show that development in a low food environment lowered average exploration and growth rate but had no effect on boldness or aggression. Furthermore, fish exposed to a predation risk simulation during ontogeny exhibited similar behavioural scores as the control, yet this treatment generated BTs i.e. personality. My fourth question asks; does the parental rearing environment (utilised in chapter 5) influence behavioural expression in the next generation? In chapter 6, I show that transgeneratonal effects of each parental rearing environment influenced life-history but had a minimal effect upon behaviour in the next generation. Finally I ask; does kin or familiarity influence plasticity in associations and aggression? In chapter 7, I show that genotypes have the ability to discriminate kin and familiars and modulate aggression and association accordingly.

These results support the concept that developmental and environmental induced plasticity may be more important than life-history in shaping behaviour. Furthermore, although adults exhibit personality and genotypic effects appear important, genotype interacts with environmental/experiential influences to differentially shape behavioural plasticity during ontogeny. I suggest that theoretical predictions regarding life-history may be insufficient to explain the complexity of animal personality in this species. I discuss these results within developmental and epigenetic frameworks with reference to the ecological significance of these patterns within this species and the animal kingdom as a whole.

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Declaration

The work in this thesis has involved collaborations with Patrick Hamilton (PH) and Rebecca Greesley (RG). Their contributions are indicated below. Darren Croft was involved in all of the studies.

Chapter 2: PH provided advice and assistance with microsatellite genotyping protocols.

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General Introduction

Chapter 1

This thesis examines environmental influences, genotype plasticity and the ontogeny of behaviours that are commonly referred to as 'personality traits'. In this chapter I provide an overview of animal personality and explore some of the current state-dependent models and environmental influences that have been suggested to generate and maintain personality traits within non-model animal populations. I then move on to discuss empirical examples that have documented environmental influences upon personality trait expression and how these relate to fitness, before finally outlining the structure of this thesis.

1.1 Individual variation: meaningful or just noise?

It is well documented that behaviour varies from individual to individual irrespective of sex, size and age (Reale et al. 2007) and it is this variation which forms the basis for behavioural evolution via natural selection (Darwin 1859). Historically within behavioural ecology, individual variation is viewed as scatter surrounding an optimum mean for a given species or population (Reale et al. 2007). In recent years, however, research has begun to explore behavioural variation and ubiquitous phenomena have emerged, revealing that individual differences tend to be non-randomly distributed along axes (i.e. bold-shy, aggressive-submissive etc). Moreover, this variation has been documented to be temporally consistent (repeatable) (Bell et al. 2009) which has prompted a recent surge of interest investigating what are commonly referred to as behavioural types or 'personality traits' because of their analogy to human personality (Gosling 2001). The presence of personality trait variation has in turn been shown to have fitness consequences (Cote et al. 2008; Dingemanse and Reale 2005; Gosling 2001; Pruitt et al. 2008; Smith and Blumstein 2008; Smith and Blumstein 2010) and be heritable (Dingemanse et al. 2002; Drent et al. 2003; Reale et al. 2009; Sinn et al. 2006; van Oers et al. 2004b), suggesting that personality is likely to be adaptive and

governed by natural selection (Dingemanse and Reale 2005; Reale et al. 2007). Behavioural types have also been shown to have consequences for population and community dynamics being, for example, related to dispersal (Fraser et al. 2001), migration (Chapman et al. 2011), and reproductive success (Godin and Dugatikin 1996). Variation can therefore be considered as an important component of a population and far from just noise surrounding the adaptive mean.

1.2 Defining animal personality

While the study of animal personality is a rapidly growing area of research, consistent terminology is lacking within the literature, with individual behavioural variation commonly referred to as behavioural types, temperament, coping styles, axes, traits, constructs, strategies and personality (Gosling 2001; Reale et al. 2007; Sih et al. 2004b). Several integrative overviews have synthesised our understanding and in turn have attempted to consolidate terminology, concentrating on the terms behavioural type and behavioural syndrome (Bell 2007; Sih et al. 2004a; Sih et al. 2004b). The terms behavioural type (BT) and behavioural syndrome and the general term; animal personality are therefore used throughout this thesis for continuity.

It is therefore important to clearly define BTs and behavioural syndromes in order to form a basis for this thesis. Simply, BTs refer to individual differences in behaviour that are consistent (repeatable) over time (Bell 2007), for example some individuals are consistently bold while others are consistently shy (Bell et al. 2009). In addition, behavioural syndrome refers correlations between BTs at the population or species level, for example boldness often co-varies across contexts or situations, with bold individuals remaining bold in both the presence of conspecifics and predators (Sih et al. 2004a). Moreover, behavioural syndromes can additionally explain covariance

between functionally different BTs, for example bold individuals are often dominant, neophilic, aggressive, and exploratory (Bell 2007; Reale et al. 2007; Sih et al. 2004a; Sih et al. 2004b). The animal personality literature is rapidly growing and it is evident that BTs and behavioural syndromes occur in multiple species across a wide range of taxonomic groups including mammals (Lantová et al. 2011; Martin and Reale 2008; Reale and Festa-Bianchet 2003), fish (Bell 2005; Webster et al. 2009; Wilson and Stevens 2005), birds (Dingemanse et al. 2004; Dingemanse et al. 2003; Drent et al. 2003), reptiles (Carter et al. 2010; Stapley and Keogh 2005), amphibians (Sih et al. 2003), molluscs (Sinn et al. 2008; Sinn and Moltschaniwskyj 2005) and arthropods (Johnson and Sih 2005; Schuett et al. 2011; Sih and Watters 2005). Importantly, studies have shown that ontogeny may be characterised by non-repeatable BT expression (Bell and Stamps 2004; Sinn et al. 2008). In light of this, I make clear distinctions between consistent individual differences (BTs) and inconsistent individual behavioural expression. In particular, when consistent behavioural expression is absent within a study, I refer to behaviours expressed at any given point in time simply as either behaviour or behavioural expression.

1.3 Behavioural syndromes: constraint or adaptation?

The presence of correlated suites of behaviour (behavioural syndromes) in multiple species suggests that BTs are not expressed independently. It has been suggested that cross-context covariance of BTs is the product of correlation selection (Sih et al. 2004b) and/or the same genes or hormones (pleiotropy) acting on several targets (Ketterson and Nolan 1999). For example, hormones can influence behavioural expression via multiple target tissues that may be local to (autocrine), adjacent to (paracrine), or distant from the source of secretion (endocrine) (Ketterson and Nolan 1999). Suites of correlated BTs may therefore be difficult to uncouple throughout

evolution because the underlying mechanisms underpinning these correlations would require genetic and/or hormonal restructuring. This concept has been termed 'the constraint hypothesis' (Bell 2005; Gerlach et al. 2001). Interestingly, studies have also documented the absence of behavioural syndromes (Bell 2005; Brydges et al. 2008; Coleman and Wilson 1998; Dingemanse et al. 2007) giving rise to an alternative view that selection may favour correlations between BTs only under certain circumstances i.e. specific habitats. Correlations between behaviours may therefore be a product of selection and not a constraint upon it and this has been coined 'the adaptive hypothesis' (Bell 2005; Gerlach et al. 2001).While the constraint hypothesis has received extensive investigation with examples observed in multiple organisms i.e. behavioural correlations documented (e.g. Arnqvist and Henriksson 1997; Duckworth 2006; Garamszegi et al. 2009; Huntingford 1976; Reale and Festa-Bianchet 2003; Reale et al. 2000; Sih et al. 2003; Wilson and Godin 2009), the adaptive hypothesis has received relatively limited direct scientific investigation (but see Bell 2005; Brydges et al. 2008; Dingemanse et al. 2007 for examples). It does, however, appear that differing selective pressures within different habitats have the potential to uncouple correlations and thus permit adaptive expression of BTs. For example research has documented that in three-spine sticklebacks an evolutionary history in the presence or absence of predation predictably affects behavioural syndrome structure. For example within six predator sympatric populations correlations were documented between BTs. In contrast, within six populations that have an evolutionary history in the absence of predators, these correlations were not present (Dingemanse et al. 2007).

1.4 BT repeatability

There are several widely accepted methodologies to assess BT consistency through time, however, there are two fundamentally different approaches depending

upon the research question. Firstly, research may be interested in individual stability, irrespective of other individuals within the sample and use a measure such as the individual stability statistic (ISS) (Asendorpf 1990; see Sinn et al. 2008 for an animal personality example of this approach). Secondly, consistency may be estimated using repeatability statistics that assess individual rank order stability over time relative to other individuals within the population or sample (Bell 2009). These repeatability estimates, in contrast to ISS statistics, are the most commonly used within the animal personality literature (Bell 2009). Estimation of repeatability can be achieved using a variety of statistical approaches, for example Kendall's concordance coefficient is one possibility (see Briffa 2008 for a discussion), however, this has been rarely adopted within the animal personality literature. In contrast, the intra-class correlation coefficient is the most commonly utilised measure of repeatability (Bell et al. 2009). The intra-class coefficient is defined as the proportion of the total variance explained by differences among groups (Lessells and Boag 1987; Sokal and Rohlf 1995) and can be calculated using the following formula:

$$R = \frac{\sigma_{\alpha}^2}{\sigma_{\alpha}^2 + \sigma_{\varepsilon}^2}$$

Where σ_{α}^2 is the between group variance and σ_{ε}^2 is the within group variance. The sum of σ_{α}^2 and σ_{ε}^2 is the total phenotypic variance (Nakagawa and Schielzeth 2010).

From a pure optimality school of thought, the concept of individual consistency through time or across contexts appears to be somewhat counter intuitive, because we would predict BT plasticity to maximise fitness. For example, while bold or aggressive individuals may increase resource acquisition in the presence of conspecifics, bold and aggressive behaviour in the presence of predators is likely to increase mortality. Repeatability measures, however, do not directly consider individual plasticity through time or contexts, in fact both consistency and plasticity may be mutually expressed

within a given population or sample (Briffa et al. 2008). For example, if all individuals change their behaviour in a similar manner through time or across contexts their rank order, relative to others in the sample, may be maintained and thus repeatability estimates are high. One important aspect of repeatability measures is that these estimates are the product of the variation within and between-individuals in the sample. Repeatability estimates using the intra-class coefficient are therefore only documented when there is between-individual variation present. Repeatability estimates can in turn be low for two reasons; when between-individual variation is low or within-individual variation is high (Nakagawa and Schielzeth 2010). This concept can be explained by considering a population of three individuals (white (1), grey (2) and black (3)), as depicted in figure 1.1, that are sampled for their boldness over time. We can see that individual 1 remains bolder than individuals 2 or 3 i.e. the rank order of each individual is maintained over time In addition, it is clear that individual 1's boldness increases throughout development, whereas individuals 2 and 3 express similar more stable behavioural expression. In this instance we would observe a relatively high repeatability estimate even though individual 1 could be considered as being more plastic. This is because the variation among individuals is high; while within individual variation is low. The take home message from this is that repeatability must be estimated in a manner that is appropriate for the research questions being addressed. If plasticity is expected to occur then repeatability estimates may be useful to investigate whether individuals maintain their level of behavioural expression in relation to others within the sample or population. If on the other hand, research is assessing individual behavioural inflexibility then ISS may be more relevant.

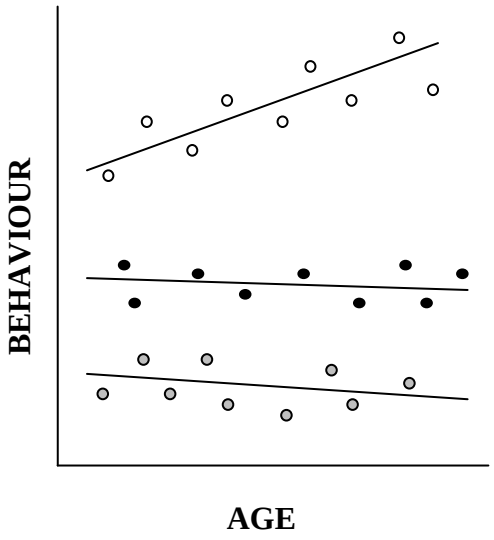


Figure 1.1. Represents consistent individual differences in behavioural expression through time (i.e. date or age), even though individual differences in plasticity are also present. Each point represents a BT value (i.e. boldness) at a given time for each of three individuals ((1) white, (2) black and (3) grey). Each line represents the fitted regression line for each individual. Regression lines highlight that individuals maintain their relative rank order differences, and are thus considered as being repeatable (consistent) in their BT expression. Individual 1 also exhibits a different slope elevation, i.e. exhibits plasticity in BT expression (see the following section and figure 1.2 for more details regarding plasticity) (figure re-drawn from Dingemanse and Wolf 2010)

1.5 Plasticity: concepts and measurement

As mentioned, plasticity is likely to occur during BT expression, indeed current thinking is moving away from the notion that BTs are completely fixed and unchanging with more studies focusing upon the potential for individual level plasticity (Biro et al. 2010; Briffa et al. 2008; Dingemanse et al. 2007; Frost et al. 2007; Lee and Berejikian 2008; Magnhagen and Bunnefeld 2009; Magnhagen and Staffan 2005; Quinn and Cresswell 2005; Sinn et al. 2008). In fact, some studies are beginning to focus upon individual differences in plasticity as an interesting BT, in and of itself (Boyce and Ellis 2005; Reale et al. 2009)

The study of phenotypic plasticity has a long history within the life-history literature (Pigliucci 2001). Historically, phenotypic plasticity eludes to the ability of a single genotype to produce multiple phenotypes in response to environmental conditions (Pigliucci 2001). Life-history research has in turn generated statistical frameworks that have long been used to explore phenotypic plasticity i.e. the reaction norm approach (Pigliucci 2001; Stearns 1992). Within the reaction norm approach, replicates within a single genotype (i.e. clone) are reared along an environmental gradient (i.e. salinity), which permits direct investigation of the extent of genotype variation in phenotypic plasticity. This approach, in particular, permits, via random regression modelling, a linear (or non-linear (Scheiner 1993)) phenotypic response to be studied as a function of an environmental covariate of interest (see Dingemanse et al. 2010; Nussey et al. 2007 for discussions).

Within these random regression models, the fitted random regression lines provide two informative measures for each genotype (or alternatively individuals): the intercept (also termed elevation), and slope. Intercepts are interpreted as the average behaviour expressed by each genotype/individual, while the slope explains plasticity in response to the environmental gradient (refer to figure 1.2 for examples of possible norms of reaction). This analytical framework therefore provides information of both genotype/individual differences (intercept) and genotype/individual plasticity (slope) simultaneously (Dingemanse et al. 2010). Furthermore, variance estimates generated in these random regression models can additionally be utilised to calculate repeatability (intra-class correlation coefficient) (Nakagawa and Schielzeth 2010). This approach is therefore highly applicable to addressing key questions within the animal personality framework.

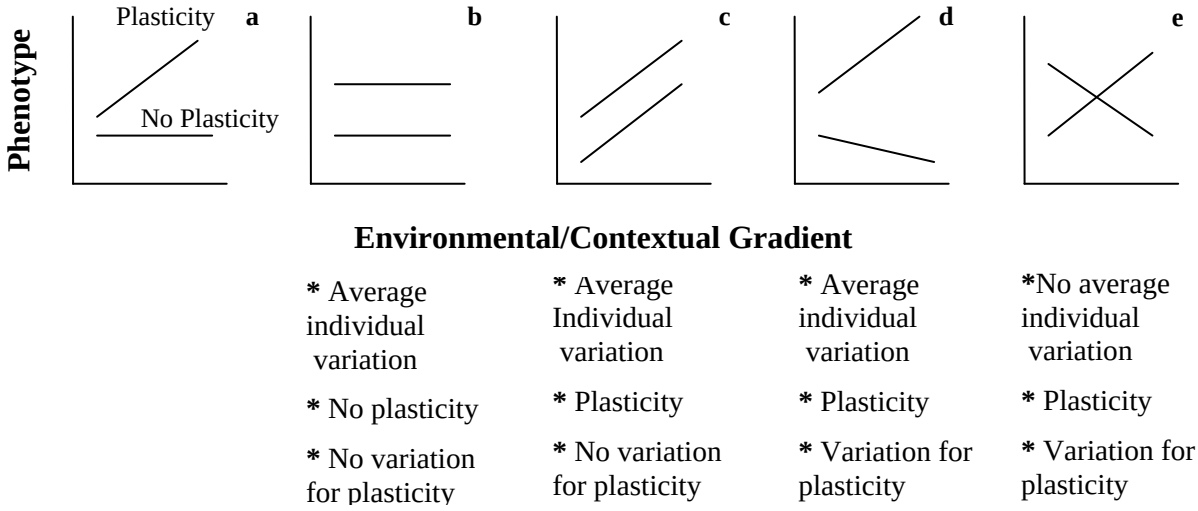


Figure 1.2.. Properties of linear reaction norms using an environmental or contextual covariate (this gradient may also refer to time i.e. within a trial or age), a) represents the concept of plasticity and no plasticity, b) represents a situation where individuals (or genotypes) represented by each line, differ in their intercept i.e. average individual variation, but do not differ in plasticity i.e. identical slope elevations. c) represents a situation where individuals vary in their intercepts as in graph b, however while individuals exhibit plasticity denoted by the non-horizontal regression lines, there is not differences in plasticity, i.e. same slope elevation. d) represents a situation where individuals differ in their intercepts, and both exhibit plasticity, however, these individuals express variation in plasticity i.e. variation in slope elevation. Finally e) is similar to d) except that average individual differences are not observed, resulting from the crossing reaction norms. (figure re-drawn from Pigliucci 2001).

1.6 Empirical examples using the reaction norm approach

Although the reaction norm approach has yet to be fully embraced within the animal personality literature, recent studies have highlighted the strength of this method. One such study utilised the reaction norm framework in relation to temperature effects upon BT expression (Biro et al. 2010). This study documented that in yellow damsel fish (*Pomacentrus moluccensis*) an increase in temperature, within normal daily ranges (+3ºC), resulted in increased individual boldness and aggression scores. However, while individuals increased their boldness and aggression scores, individual slopes ran parallel (as shown in figure 1.2c) highlighting that, although individuals exhibited temperature induced BT change, there was no between-individual variation in BT plasticity. In contrast, individuals were found to differ in their activity responses, with some

individuals exhibiting increased activity while others decreased activity in response to temperature, resulting in between-individual plasticity variation (i.e. crossing reaction norm slopes as depicted in figure 1.2e). In another study, house mice (*Mus musculus*) and eastern chipmunks (*Tamias striatus*) were found to exhibit individual plasticity variation in activity patterns within a single open field assay, that is variation in slopes over the sampling period, with individuals becoming more or less active during the course of the trial (Montiglio et al. 2010). Research can therefore benefit from the implementation of the reaction norm approach and thus our understanding of animal personality is set to greatly advance in the coming years.

1.7 Explaining the maintenance of personality variation and consistency

Historically, fixed genetic determinants of behaviour have been used to explain consistency in genetically encoded traits with several processes documented to maintain variation within populations. Variation may, for example, be maintained via frequency-dependent selection (Roff 1998), environmental heterogeneity (Mangel 1991) and a balance between weak selection and mutation (Santiago 1998). In recent years multiple theoretical models have tackled aspects of animal personality and these have generated several testable predictions (see Dingemanse and Wolf 2010 for a review of recent models developed). These theoretical models have focused upon three main concepts (i) state-dependent explanations, (ii) feedback mechanisms between state and BTs that generate consistent individual differences in behaviour and (iii) state-independent explanations of BT variation and consistency i.e. frequency-dependent selection (see Wolf and Weissing 2010 for a discussion of these modelling approaches).

1.7.1 State-dependent individual differences

State refers to any feature that is characteristic of an individual (sensu Houston and McNamara 1999). Individuals for example, differ in their size, morphology, life-history strategy, physiology, energy reserves, environment experienced etc (Houston and McNamara 1999). State-dependent explanations for BTs focus upon how between-individual variation in these states influence costs and benefits associated with specific behavioural decisions (Houston and McNamara 1999; Wolf and Weissing 2010). States can be relevant in several ways, for example, an individual with low energy reserves may be more willing to take risks in the presence of a predator (Damsgard and Dill 1998), or elevated parasite burden may influence activity, anti-predator responses and escape ability in prey species (Poulin 1993). While states can be short lived i.e. labile, they may also be stable over extended time periods or completely irreversible i.e. gender in mammals. If states exhibit temporal stability they potentially present an adaptive explanation for both consistent individual differences and BT plasticity (Wolf and Weissing 2010). Moreover, a single state has the potential to underpin multiple associated BTs in synchrony and thus may explain behavioural syndromes (Dingemanse and Wolf 2010; Wolf and Weissing 2010). Although in recent years multiple state-dependent models have been formulated (see Dingemanse and Wolf 2010 for a review), the following sections will focus upon two specific models and their predictions (Stamps 2007; Wolf et al. 2007b) which are in part the focus of this thesis. I will additionally discuss some models addressing feedback loops between state and behaviour because these are particularly relevant to the models of Wolf (2007b) and Stamps (2007) as well as their predictions.

1.7.2 Growth-mortality tradeoffs – a verbal model (Stamps 2007)

It is well documented that individuals differ in their growth rates (Bjorklund et al. 2003; Kirkpatrick and Lofsvold 1992; Mangel and Stamps 2001; Stamps et al. 1998) and growth rate consistency has been linked to fitness (Mangel and Munch 2005). Consistent individual differences in growth therefore presents a candidate state that is likely to influence multiple behaviours associated with food acquisition (Stamps 2007). Stamps (2007) therefore proposed a state-dependent model founded upon these concepts which suggests a trade-off between growth and mortality could favour the maintenance of BT variation. In this scenario, fast growth is predicted to generate bolder, more exploratory and aggressive BTs which facilitate resource acquisition, thereby maintaining this developmental trajectory over extended time periods. If individuals can sustain higher growth rates by expressing risk prone BTs (i.e. greater resource acquisition) they will in turn be capable of reaching maturity at an earlier age than a risk-averse, slow growing individual. However, while risk prone BT expression increases food intake, this BT may also result in elevated mortality risk and thus, if different growth dependent strategies have equal lifetime fitness (i.e. produce the same number of offspring) then these strategies can coexist within a population (Stamps 2007). This model was additionally extended to incorporate an additional state: metabolic rate (Biro and Stamps 2008).

While little research has directly tested the hypothesis of Stamps (2007), the link between growth rate/mortality risk and personality traits in animal personality is supported, although tentatively, in a number of studies (reviewed by Huntingford and Adams 2005). For example, research has shown that in comparison to wild fish, hatchery reared fish artificially selected for fast growth rates tend to be bolder (Sundstrom et al. 2004), more aggressive (Metcalfe et al. 2003) and risk prone (Biro et

al. 2004; Biro et al. 2006; Biro and Post 2008). However, within these examples the cause and effect relationship between growth and BT variation is unclear. For example, it is difficult to determine if selection for high growth is a driver of BT expression or if relaxed selection pressures in the captive environment (e.g. high food, reduced selection pressures) are influencing both high growth and bold/exploratory/aggressive BTs. In addition, recent research suggests that growth variation may not be sufficient to explain BT variation. For example, Conrad & Sih (2009) documented that newly emerged stealhead (*Oncorhynchus mykiss)* exhibiting bold/active BTs showed increased feeding rates during behavioural assays, yet these BTs did not predict growth rate or survival probability during the first three months of life. Adriaenssens & Johnsson (2011) also found the opposite pattern to those suggested by Stamps (2007), with shy fish growing at faster rates compared to bold fish. Moreover, Bell et al, (2011) documented that, contrary to Stamps' (2007) prediction, wild caught sticklebacks exposed to predator odour cues exhibited increased juvenile somatic growth rates. This study suggested that, because many piscivorous predators are gape limited and predate heavily on smaller individuals, elevated growth may permit fast transition between a small, high risk size classes to large, lower risk size classes. Further research investigating growth rate variation as possible underpinnings of BT variation, within species that have not been artificially selected for high growth rate is, however, necessary to fully investigate this potential mechanism.

1.7.3 Residual reproductive potential- a simulation model (Wolf et al. 2007b)

The second theoretical model that I focus on here, formulated by Wolf et al. (2007b), implicates a second life-history trade-off as a potential driver of BT variation and maintenance. Instead of a growth-mortality relationship, this model focuses on current vs. future fitness, measured as residual reproductive potential. This model is

grounded within the asset protection principle, within which, individuals that have much to lose are more risk averse to protect their acquired assets and thus maximise fitness (Clark 1994). In Wolf et al's, (2007b) model; individuals either reproduce in the first year or delay reproduction until the second year and it was discovered that delayed reproducers exhibited shy, non-aggressive strategies and explored thoroughly, whereas early reproducers were bold, aggressive, superficial explorers. Within delayed reproducers, shy BTs were suggested as a mechanism to increase survival probability to meet reproductive expectation in the second year. This model has additionally received some empirical support; for example, Careau et al. (2009) found that inter-specific exploration was positively correlated with age at first reproduction in a comparative study of 19 muroid rodent species. Intra-specific empirical support for this model is still, however, lacking in the literature but potential links are likely. The predictions generated by this model are therefore in need of empirical investigation within a species to determine if residual reproductive potential is related to BT variation.

1.7.4. Negative or positive feedback?

While both of the above models present clear testable predictions, they are both based upon relatively labile states and thus present some potential issues that require resolution. This contention relates directly to negative feedback loops which erode variation in states and associated BT expression (see Dingemanse and Wolf 2010 for a discussion). In relation to Wolf et al's (2007b) proposal, as individuals grow and reproduce, they will reach a point where they have low residual reproductive potential and thus we would expect BTs to homogenise with age (McElreath et al. 2007). This negative feedback may thus fail to explain individual variation and consistency, because we would expect behavioural strategies to change with changing life-history. Similarly Stamps'(2007) proposal could potentially be invalidated by negative feedback, because

compensatory growth, also termed catch up growth (Mangel and Munch 2005), can erode size variation (Arnold et al. 2007a) and influence BTs (Krause and Naguib 2011).

In response to Wolf et al's (2007b) model, McElreath et al. (2007) suggested an alternative mechanism; positive feedback, as a potential explanation for BT variation. In particular individuals that explore may acquire assets such as resources and knowledge that may positively affect survival. This positive feedback would in turn be expected to reinforce and stabilise differences in assets and thus BT variation, which was recently supported by theoretical modelling (Luttbeg and Sih 2010; Wolf et al. 2008). However, while positive feedback is a possible alternative, Wolf et al. (2007a) suggested that negative feedback will only destabilise labile state variation and associated BTs when risky behaviour generates a large increase in assets that contribute to future reproductive potential. In contrast, if assets are not accumulated, and instead utilised in current, as opposed to future reproductive effort, initial individual state differences could be maintained (Wolf et al. 2007a).

Modelling approaches also provide a possible resolution to negative feedback in relation to Stamps' (2007) proposal. In this instance, models by Rands et al. (2003; 2008) suggest that negative feedback may be absent when social foraging is beneficial i.e. anti-predator benefits. These models indicate that although bold, risk prone individuals (i.e. greater energy requirements) would be more likely to leave a shelter first and return last, both individuals would forage together and thus initial state differences could be maintained without confounding effects of negative feedback and thus Stamps' prediction may hold. In contrast, shy individuals have been shown to exhibit higher physiological stress responses compared to bolder individuals and may have higher metabolic rates and thus higher energy requirements (Carere et al. 2003;

Hoglund et al. 2008; Martin and Reale 2008; Martins et al. 2007; Veenema et al. 2003). It is therefore, paramount that the predictions of Stamps (2007) and Wolf (2007b) be investigated to determine the potential of these labile state-BT relationships.

1.8 The ontogeny of animal personality

To date the vast majority of research exploring animal personality has focused upon adults or within specific developmental stages, generating a strong bias in the literature, which may be potentially problematic. For example, multiple behaviours are age dependent; such as aggression, which usually increases prior to the onset of sexual maturity (Delville et al. 2006). It therefore seems intuitive that BTs which are regularly reported to co-vary with aggression i.e. boldness (Bell 2007; Reale et al. 2007; Sih et al. 2004a; Sih et al. 2004b) may also be age-dependent. Furthermore in humans, research has also documented that the consistency of the big five personality dimensions tends to increase with age (Roberts and DelVecchio 2000), reaching a plateau at approximately 50 years of age after which only small changes occur (Caspi et al. 2005). In contrast within the animal personality literature, test-re-test intervals tend to be relatively short (personal observation) and thus it is not surprising that consistency is regularly observed. Intuitively, larger periods between samples are likely to yield less consistent estimates and this has been substantiated in recent years (Bell et al. 2009). Long term research which investigates personality across multiple developmental stages is therefore needed to provide insight into long term repeatability and the ontogeny of BTs.

While there are some insights into early rearing environmental effects upon personality expression (see the following section below for a discussion), very little work has simply characterised the ontogeny of personality in controlled laboratory

settings without environmental influences being the main focus of research. For example, only three studies have implicitly characterised BT expression, repeatability/stability and behavioural syndrome structure throughout ontogeny while controlling for environmental effects during development. Sinn et al, (2008) for example, found that in dumpling squid (*Euprymna tasmanica*) there were no correlations between boldness in a feeding context and boldness in a threat context at each stage of development investigated, highlighting context independent expression of behaviour. This study also highlighted that the behaviours studied exhibited different levels of plasticity, with bold individuals being more plastic in threat contexts while shy individuals were more plastic in the feeding context. In contrast, Bell and Stamps (2004) found that during ontogeny, two populations of threespined sticklebacks (*Gasterosteus aculeatus*) differed in their behavioural consistency and behavioural syndrome structure. In particular, high predation individuals exhibited little behavioural consistency, however, correlations between behaviours were found. This suggests that although individual behaviour was not consistent, coordinated plasticity of functionally different behaviours occurred (boldness, activity and aggression), thereby maintaining behavioural syndrome structure at specific stages of development. In contrast, Brodin (2008) found evidence for developmental consistency (BTs) and behavioural syndromes (boldness and activity) in larval damsel flies (*Lestes congener*) which persisted in to adulthood. This result is especially intriguing when we consider that larval and adult phases of life in damsel flies occur in different habitats/environments with different selection regimes. It is therefore likely that consistency in commonly studied BTs and the presence of behavioural syndromes is species, behavioural trait and/or age specific.

Further detailed investigations incorporating developmental aspects of personality are now needed to determine how animal personality is expressed

throughout life, whether transitions between specific life stages influence BT expression and behavioural syndrome structure, and how experiential effects are incorporated in to the adult phenotype (Stamps and Groothuis 2010a; Stamps and Groothuis 2010b). Developmental perspectives are therefore likely to provide new insights in to how, if and when BTs are expressed and whether plasticity is likely to be adaptive. Furthermore, early life is usually characterised by high growth and thus developmental perspectives present an ideal opportunity to investigate state dependent models of BT variation and maintenance.

1.9 Rearing environment effects upon BT expression

While we know relatively little about the basic ontogeny of animal personality, environmental influences during early life stages, are known to shape the expression personality traits (see Stamps and Groothuis 2010a; Stamps and Groothuis 2010b for discussions). It is therefore likely that differing environmental conditions experienced during ontogeny have large impacts upon BT expression and behavioural syndrome structure in later life (see Stamps and Groothuis 2010a; Stamps and Groothuis 2010b for discussions). In addition, because personality research is heavily biased towards adults, understanding developmental processes that shape adult BTs are necessary to fully comprehend the complexities we observe in the natural world. At the current time, there has been some progression in understanding experiential and rearing environmental effects on personality in non-human organisms, however, these examples still remain a relatively small percentage in the literature (Stamps and Groothuis 2010a).

Research investigating early rearing environmental effects upon personality development have generally focused upon model organisms, such as rodents (see Laviola and Terranova 1998 for a review). For example, the composition of the early

social environment i.e. litter size, has been shown to influence the development of emotionality, with large litters generating higher anxiety levels (Dimitsantos et al. 2007). In addition, body size has been linked to higher levels of boldness and exploration in rats (Rodel and Meyer 2011). Maternal behaviour in rodents such as arched-back nursing and grooming have also been well documented to influence multiple aspects of personality expression (Caldji et al. 1998; Francis et al. 2000; Francis et al. 1999; Menard et al. 2004). Cohen et al, (2008) also reported that six mouse genotypes exposed to a predator (cat) odour cue at a single stage of ontogeny, exhibited higher anxiety and startle responses compared to their before exposure responses.

In contrast to the above examples, investigations using non-model species are less common in the literature. One such study, investigating steelhead (*Oncorhynchus mykiss*), compared the effect of the rearing environment (barren, stable structure and variable structure) on exploration and documented that structural additions to the environment increased exploration scores but only when the positioning of these structural components were stable throughout development (Lee and Berejikian 2008). Chapman et al, (2010) also found that temporal variability in food provisioning during development in the Trinidadian guppy (*Poecilia retuculata*) generated bold and exploratory individuals when compared to those reared in a stable feeding environment. In addition, Carere et al. (2005a) documented that sibling competition and food availability in great tits (*Parus major*) generated higher exploration and aggression tendencies. Moreover, experimental immune challenge during specific developmental stages in mallard ducks (*Anas platyrhynchos*) has been shown to influence adult activity (Butler et al. 2011). All of these studies combined indicate that environmental variability i.e. resource abundance, environmental structure, the social environment,

stressful events and physiological challenges during ontogeny, have the potential to influence plasticity of behavioural expression.

It is clear that environmental influences during ontogeny have the potential to shape aspects of personality, however, greater understanding of environmental influences upon BTs and behavioural syndromes are needed to determine how environmentally induced plasticity manifests during ontogeny in non-model organisms. As mentioned previously, the reaction norm approach presents an exciting framework for addressing key developmental aspects of personality expression. Future work is therefore primed, with well established methodological and statistical approaches, to investigate experiential/developmental effects. In particular, manipulation of the rearing environment permits us to begin to understand how differing experiential effects and ontogenetic processes interact and shape animal personality. Furthermore, research investigating the timing of specific experiences upon the expression of personality traits will permit insight into developmental stages that are more susceptible to environmental conditions and how these influence plasticity (Stamps and Groothuis 2010a; Stamps and Groothuis 2010b).

1.10 Maternal effects and personality

In addition to ontogeny and experience, maternal effects are another mechanism by which personality may be influenced. Maternal effects describe how the maternal phenotype and/or the maternal environment influence the phenotype of the next generation (Mousseau and Fox 1998). Maternal effects are favoured when maternal environmental cues are reliable and offspring environments can be predicted with some certainty. Modulation of offspring phenotypes may therefore be a form of what has been termed ‘adaptive transgenerational plasticity’ which has the potential to override the

direct effects of transmitted genes (Marshall and Uller 2007). Although maternal effects are commonly viewed as adaptive responses permitting offspring to buffer local environmental stressors (Mousseau and Fox 1998), examples of non-adaptive maternal effects are also documented e.g. offspring disperse in to environments that are dissimilar to the maternal environment, exhibiting a reduction in fitness (see Marshall and Uller 2007 for a discussion). Maternal effects have received extensive interest in relation to life-history and it is widely documented that they can have long term influences upon morphology and behaviour (Strasser and Schwabl 2004), in some instances being identifiable in grand-offspring (Hafer et al. 2011).

Recent work within the animal personality framework has begun to document that maternal effects have the potential to act upon personality trait expression at several stages of development. For example, variation in androgen concentrations deposited within avian yolk have been related to boldness (Groothuis et al. 2008) and aggression (Eising et al. 2006). Furthermore, female embryo position, in relation to male embryos in-utero, have been related to aggressiveness, exploration, activity and other behaviours (reviewed by Ryan and Vandenbergh 2002). Organisms which exhibit no parental care can also influence behaviour in the next generation by adjusting oviposition site decisions in relation to environmental cues. Kolbe and Janzen (2001) for example, document that neonatal snapping turtles (*Chelydra serpentine*) differed in their dispersal patterns and survival depending on the density of vegetation at the nest site. In addition to pre-natal maternal effects, BTs may also be modulated by mothers during neonatal stages of development. It has been shown, for example that variations in maternal care (Caldji et al. 1998) and parental mediated neonatal nutrition (Arnold et al. 2007b) can influence offspring personality. Further research exploring maternal effects upon personality research is, however, needed to determine how different environments

experienced by a mother influence BT expression in the next generation. Moreover, exploring the fitness consequences of maternal effects upon BTs and behavioural syndromes will be an exciting avenue of research in the coming years.

1.11 Empirical research: environmental consequences of personality

Within the following sections I will review some of the empirical literature that has investigated environmental influences upon BT expression and behavioural syndrome structure with specific emphasis upon selection and fitness. This section aims to place the previously discussed sections into context in relation environmental effects upon personality and their fitness consequences in natural populations.

1.11.1 Environmental variation and selection - Predation risk

In natural habitats, populations are subjected to different predator assemblages that vary both spatially and temporally. The influence of predation risk and the resulting selective forces acting upon prey populations has received extensive scientific study and has been implicated in life-history differences between differing predation risk localities (Reznick and Endler 1982; Reznick et al. 1996). Elevated predation risk has also been noted to have an effect upon morphology (Bronmark and Miner 1992; Poleo 1993; Relyea 2004), colouration (Endler 1980; Endler 1982; Endler 1995), behaviour e.g. shoaling tendency (Magurran and Seghers 1990; Seghers 1974), and sexual behaviour e.g. sneaky mating strategies (Godin 1995), among others. These have all been proposed as mechanisms to reduce potential risk of mortality from predator species. It is therefore not surprising that predation risk has also been noted to have considerable influences upon BT expression and behavioural syndrome structure.

Bell and Stamps (2004) and Bell (2005) for example, found that two populations of three-spine sticklebacks differing in predation risk (high and low), exhibit different BT expression. In addition, high predation populations were found to exhibit strong correlations between activity, boldness and aggression, however, these correlations were absent in low predation fish. Similarly, Dingemanse et al, (2007) investigated predation risk as an influence upon aggression, boldness and exploration in 12 populations of threespined sticklebacks and found support for predation risk as a selective force for the generation and maintenance of behavioural syndromes. Conversely, cannibalism (another form of predation) can occur at high conspecific densities, generating variation in risk. It is therefore not surprising that cannibalism has additionally been implicated in behavioural syndrome development within populations (Smith et al. 2009).

These results indicate selection for tight behavioural correlations is likely to occur in high predation localities whereas, when risk is limited, BTs may be uncoupled throughout evolution (adaptive hypothesis). The influence of predation risk has been further supported experimentally by investigating BTs and behavioural syndrome structure before and after exposure to predation (Bell and Sih 2007). Bell and Sih (2007) observed no correlation between aggression and boldness prior to exposure; however, after exposure correlations between BTs were evident. In this instance predation removed very bold individuals from the population (selection) and this coupled with individual plasticity in aggressive behavioural responses contributed to the observed post exposure correlation. It was suggested that stress may generate a trade-off, resulting in tighter correlations between BTs. Alternatively it was suggested that individual differences in anti-predator tactics may be shape these patterns. For example, shy, schooling fish may be selected for low aggression, while bold; predator inspectors that rely on personal information may be permitted to express relatively high

aggression. These possibilities, however, require further investigation to unravel mechanistic aspects of tight BT correlations in predator sympatric populations.

In addition to selection for tight correlations, we may also predict that predators would select against risk prone BTs. This prediction has been supported in domesticated trout with bold, risk prone individuals suffering higher mortality (Biro et al. 2004). Moreover, bold and active fish have been noted to be more at risk from human harvesting practices, resulting from elevated interactions with fishing gear (Biro and Post 2008). Selection against bold, risk prone individuals would therefore be expected to generate lower mean boldness expression in predator sympatric populations. Multiple studies have, however, found the opposite trend with higher mean levels of boldness in high and not low risk populations (Brown et al. 2007a; Brown et al. 2005; Brown et al. 2007b; Magnhagen and Borcherding 2008; Sih et al. 2003). These results although unexpected, can be interpreted in relation to trade-offs between risk and lost feeding opportunities (Brown et al. 2005), or lost mating opportunities (Lima and Bednekoff 1999). It is therefore likely that high predation locations select for an increased acceptance of risk and thus individuals are comparatively bolder than their low risk counterparts (Brown et al. 2005).

1.11.2 Environmental variation and selection - Ecology

Although the evidence of predation risk as a driver of behavioural syndrome formation in compelling, it is important to note that predation risk is not the only ecological factor that may generate correlations, or lack thereof. For example, Brydges (2008) documented behavioural syndromes in only one of four predator sympatric populations, and suggested additional ecological factors are likely to uncouple correlations between BTs. Environmental conditions are, for example, temporally and

spatially heterogeneous and are noted to select for variation in life-history and morphological traits (e.g. Coltman et al. 1999). It is therefore likely that specific ecological conditions will select for different BTs and behavioural syndromes. Riechert and Hall (2000) for example, document that different environments that vary in resource availability (desert - low resources and riparian - high resources) differentially selected for aggressiveness and boldness in spiders (*Agelenopsis aperta)*. In desert habitats, competition for limited resources resulted in aggressive and bold BTs with the opposite pattern in riparian habitats. This difference was additionally documented to be the result of selection using transplant experiments (Riechert and Hall 2000). It is therefore important that additional environmental conditions be thoroughly investigated in the coming years to understand the role of predation and ecology, as well as their interactive effects, upon behavioural syndrome structure and BT expression.

1.11.3 Temporal fluctuations in selection

While specific ecological conditions may select for specific BT combinations, few studies have investigated temporal variation in selective pressures and the resulting temporal changes in BTs. Dingemanse (2004) for example investigated exploration in wild great tits across three successive years in relation to selection (measured as annual survival between breeding seasons). It was documented that survival was related to exploration tendency and that selection pressures, coinciding with beech (*Fagus sylvaticus*) masting crop failure, differentially affected male and female BTs. In years of decreased food availability exploratory/aggressive females survived better which was linked to competitive advantage. During years of high food availability exploratory and aggressive females were selected against, proposed to result from increased mortality resulting from maladaptive aggression. Males, in contrast showed the opposite patterns; in years of high food availability exploratory/aggressive males survived best. This was

suggested to result from aggression providing a dominance advantage, which in turn assisted territory holding potential. When resources were limited and competition relaxed (i.e. greater mortality at the population level) it was suggested exploratory/aggressive males were selected against i.e. higher net costs associated with their BT that exceed the benefits of aggression in this selective regime. Boon et al, (2007) also found that in years of high food availability (white spruce masting; *Picea glauca*) female red squirrel (*Tamiasciurus hudsonicus*) activity was positively correlated with offspring growth, however, in years of limited resources; negative correlations were observed. This was linked to variation in the cost of activity relative to resource acquisition potential between years of high and low abundance. Nest survival was additionally unrelated to activity and negatively associated with maternal aggression, however; over winter survival of offspring was positively related to maternal aggression. This is likely to have been the result of aggression BTs being heritable and thus greater over winter survival was observed in offspring from aggressive mothers, but only when resources are low (i.e. high competition). Future work investigating resource availability is therefore set to provide insight in to the evolution and maintenance of population specific BT characteristics.

In addition to fluctuating selection coinciding with food availability, predator density variation across years have additionally been documented to exert differential effects on different BTs. Reale & Festa-Bianchet (2003) documented that in years of high cougar (*Puma concolor*) density, big horn ewe (*Ovis Canadensis*) mortality increased substantially. During high predation year's boldness permitted a survival advantage in younger individuals, while old and docile ewes were found to suffer higher predation. In contrast, during years of low predator density selection was relaxed. This study highlighted that, although predator induced selection was evident; BT evolution

was unlikely to have long term consequences because old individuals have low residual reproductive potential. The authors do, however, suggest that other predators with differing hunting techniques may select against different BTs and thus further long term investigations would be fruitful.

1.11.4 Species and population differences

In addition to environmental variation experienced within a species, studies have identified that exploratory behaviour and neophobia is related to species differences in migration and residency (Mettke-Hofmann et al. 2005a; Mettke-Hofmann et al. 2009). For example, Mettke-Hofmann et al. (2005a) documented that migratory Garden warblers (*Sylvia borin*) were not repeatable in exploration of, or neophobia towards, novel objects during a feeding context when assayed in a familiar environment. It was suggested that context-specific behaviour expression or environmental variation experienced by migratory species may result in flexibility. This is, however, unlikely because other migratory species have been documented to exhibit individual consistency (Chapman et al. 2011; Sih et al. 2003; Sneddon 2003). In contrast, within the same experimental assay, resident Scandinavian warblers (*Sylvia melanocephala mormus*) were found to exhibit behavioural consistency (i.e. exhibited personality) which may be adaptive within the permanent local environment (Mettke-Hofmann et al. 2005a). In addition, when investigating spatial neophobia in an unfamiliar environment it has been shown that migratory warblers are less neophobic compared to residents (Mettke-Hofmann et al. 2009). It is possible that the observed reduction in neophobia during spatial exploration of a novel environment relates to regular encounters with multiple novel environments during migration. This suggestion was additionally supported by research investigating resident and nomadic parrot species (Mettke-Hofmann 2000; Mettke-Hofmann et al. 2005b). In addition, Mettke-Hofmann

et al., (2002) also document that dietary specialisations and environmental complexity in parrots have the potential to influence personality in multifaceted ways that are both context and species-specific. Greater insights into environmental influences are now needed to determine how general these trends are across the animal kingdom and how specific environmental factors shape species-specific personality expression.

1.11.5 Social influences

In addition to ecological factors, the social environment is likely to influence the expression of BTs. However, the vast majority of studies to date have assayed specific BTs within a solitary context, and these assays have, in general been conducted with limited regard to the study species' social tendency under natural conditions (Brown and Braithwaite 2004; Brown et al. 2005; Brown et al. 2007b). This approach may therefore be problematic because the vast majority of study organisms are prey species that naturally form social groups. The formation of social groups has a long history of research within behavioural ecology, with sociality being documented to convey multiple benefits to individual group members i.e. anti-predatory effects (see Krause and Ruxton 2002 for a discussion). It therefore seems intuitive that in the absence of conspecifics during standardised assays, the resulting BT scores documented may be at best misleading, and at worst inappropriate estimates of individual behaviour under natural conditions. Understanding the role of the social environment, which is transient both temporally and spatially, is therefore necessary to understand behavioural expression, repeatability and BT co-variation. Surprisingly very few studies have directly investigated the influence of social partners during BT assays. In recent years researchers have begun to tackle this problem and have shed some light upon the role of the social environment in BT plasticity.

Webster et al, (2007) for example, found that larger group size in three spine sticklebacks reduced time to resume activity following an aerial attack. This potentially resulted from multiple anti-predatory benefits related to conspecific presence (Krause and Ruxton 2002). van Oers et al, (2005b) additionally found that latency to feed was influenced by conspecific BTs in great tits (*Parus major*). Moreover, the social group has been documented to influence boldness in a series of experiments using perch (*Perca fluviatilis*) (Magnhagen 2007; Magnhagen and Bunnefeld 2009; Magnhagen and Staffan 2005). These experiments revealed that within randomly assigned groupings; shy fish associated more within their own BT than bold fish, however, when associating with bold conspecifics shy fish spent longer in the open (Magnhagen and Staffan 2005). In extension of this, it was later documented that correlations between risk taking and exploration were only observed when behaviour was adjusted to incorporate the behaviour of other group members, indicating group influences on behavioural syndrome structure (Magnhagen 2007). In a final experiment, the authors revealed that conspecific presence resulted in behavioural plasticity i.e. fish were bolder than when tested in a group, however, small within group differences were found to be the result of BTs inherent to the individual that manifested in both asocial and social contexts (Magnhagen and Bunnefeld 2009).

In addition to studies investigating conspecific presence or absence, research has also investigated the fitness consequences of social groups and their constituent BTs. Dyer et al, (2009) for example documented groups of mixed boldness phenotypes had higher foraging success compared single BT shoals, indicating group composition effects on fitness. Bold fish were suggested to rely on shy, cautious individuals for predator detection, whereas shy fish exploited bold individual's foraging abilities, indicating a potential producer-scrounger system (Barnard and Sibly 1981). Nomakuchi

et al, (2009) also uncovered a positive correlation between exploration of a novel maze and tendency to utilise demonstrator conspecifics trained to solve the maze for a food reward. This indicated that exploratory individuals in novel environments readily exploit demonstrators, potentially increasing fitness.

Although BT composition within a shoal can convey benefits as highlighted by Dyer et al., (2009), it may also generate costs that negatively influence individual fitness. Sih and Watters (2005) for example, documented that groups comprised of aggressive and active individuals of water striders exhibited lower mating success and thus fitness, in comparison to less aggressive and less active groups. This was attributed to keystone hyper-aggressive males that caused female mediated group fission. In addition to group BT composition, gender composition of social partners has additionally been found to influence BT expression. Piyapong et al. (2010) investigated the effect of male and female presence upon boldness expressed by each of the sexes in guppies following aerial predation simulations. It was predicted that males would be bolder in the presence of females to maximise the opportunity for reproductive interactions. Females were in contrast, predicted to be shy in the presence of males to avoid harassment, which exerts substantial negative effects upon female fitness (Magurran and Seghers 1994; Ojanguren and Magurran 2007). Females were found to confirm predictions; however, males did not differ from female group members, this is suggested to result from male boldness being influenced by female risk perception

Two studies have also explored social network structure in relation to BTs in both laboratory (threespined sticklebacks: Pike et al. 2008) and natural populations (guppies: Croft et al. 2009). Pike et al, (2008) found that networks constructed of bold individuals had interactions that were low in strength and evenly distributed, whereas

shy networks were characterised by assortative interactions between few individuals. Within mixed BT groups this pattern was also found, indicating assortment based on BTs may be a general pattern even though fitness is potentially maximised by associating with the opposite phenotype (Dyer et al. 2009). Croft et al, (2009) also found similar results in wild guppies with high scoring BTs having few, weak interactions. All of these studies indicate that the presence of social partners, BT composition and gender composition of the social group can have large influences upon the expression of behaviour and consequently fitness. In addition, these studies highlight the importance of considering a species' social behaviour prior to developing BT assaying protocols because when assaying group forming species, BTs sampled in isolation are likely to underestimate natural individual differences in behaviour. Further work investigating BTs and how these relate to social decisions and resulting fission-fusion dynamics will additionally be rewarding in the future.

1.12 Study organism

In this thesis I utilise a laboratory population of the mangrove killifish (*Kryptolebias marmoratus,* formally *Rivulus marmoratus*) as a model organism. *K. marmoratus* is an inshore dwelling species with a widespread geographic distribution throughout Central/South America and Florida, that is congruent with the distribution of the red mangrove (*Rhizophora mangle)* (Taylor 2000). This species is a naturally occurring, self-fertilising simultaneous hermaphroditic fish (Taylor et al. 2001) and self-fertilisation is achieved via the ovotestis, a reproductive organ containing both testicular and ovarian tissue (Sakakura et al. 2006). This permits internal fertilisation of eggs within the gonadal lumen following ovulation, and prior to oviposition (Sakakura et al. 2006).

In contrast to sexual reproduction which generates high heterozygosity, self-fertilisation over successive generations reduces genetic diversity and generates inbred genotypes (Sato et al. 2002) that are homozygous across most, if not all of the genome (Mackiewicz et al. 2006a). In addition to self-fertilisation, this species has also been documented to occasionally outcross with rare males, generating recombinant genomes that revert to homozygosity following several generations of selfing (Lubinski et al. 1995; Mackiewicz et al. 2006a; Mackiewicz et al. 2006b; Mackiewicz et al. 2006c; Tatarenkov et al. 2010; Taylor et al. 2001). This near unique reproductive system in a vertebrate, presents a powerful model organism that is characterised by multiple genetically distinct genotypes that are each homozygous within their respective lineage (Kallman and Harrington 1964; Lubinski et al. 1995; Mackiewicz et al. 2006a; Mackiewicz et al. 2006b; Mackiewicz et al. 2006c; Tatarenkov et al. 2007; Tatarenkov et al. 2009; Vrijenhoek 1985). Replicating both within and across multiple homozygous genotypes therefore permits the investigation of genotypic plasticity in response to environmental conditions. In addition, 2 genotypes of *K.marmoratus* have also been documented to exhibit variation in life-history traits including growth rate, age at first reproduction, and egg laying rate (Grageda et al. 2005). This species is therefore suited to reaction norm approaches, and particularly to the aims and objectives of this thesis.

1.13 Ethics

Fish originating from the Florida population were collected in 2007 in accordance with collection permits issued to Dr. S. Taylor by the State of Florida. Fish were collected from streams, ditches and pools using funnel traps or collected directly from emergent log refuges (see Taylor et al. 2008a for examples of log refuges). *K.marmoratus* have the ability to survive out of water for up to 66 days in moist conditions (Taylor 1990), during which, aerial respiration occurs via an epidermal

capillary network (Grizzle and Thiyagarajah 1987). Fish were therefore transported (both wild caught Florida fish, and fish supplied by Valdosta State University) (maximum 2 days) in 500ml plastic water bottles (1 fish/bottle) containing 20ml synthetic salt water and a paper towel to retain moisture. For transport all correct export and import documentation/permits were collated using specialist UK and USA ornamental fish distribution and logistics companies. During transport no mortality occurred and all fish fed successfully 1 hour after arrival in the laboratory.

All fish were visually checked for signs of ill health or abnormal behaviour expression on a daily basis between the hours of 8.00-9.00 during each study presented in this thesis. In addition, all behavioural assays, marking and rearing protocols were approved by the School of Psychology Ethics Committee at the University of Exeter and all work was undertaken under a U.K. Home Office license held by Dr Darren Croft (see appendix A 1.1). No adverse effects of behavioural assays/rearing environments were observed during each of the studies and mortality was low being <1%. This is considerably lower than the expected 5 - 9% mortality documented for this species during the early stages of development within stock aquaria, under captive a standardised captive rearing condition (Edenbrow unpub data). Following completion of each study, 1-2 individual representatives of each genotype per study were retained as 'breeding' individuals for generation of fish for the next experiment. The remaining fish were euthanized using MS-222 in accordance with U.K. Home Office regulations.

1.14 Chapter overview

The six data chapters presented in this thesis explore genotype level BT plasticity during multiple stages of life. In particular I investigate behavioural consistency and BT plasticity within a developmental framework and explore the role of

the rearing environment and genotype upon BT developmental plasticity. In addition, I investigate plasticity across a single generation following parental environmental manipulation. These key concepts are also explored within the encompassing umbrella of state-dependent BT expression, to address the current theoretical predictions generated from the models of Wolf (2007b) and Stamps (2007). I additionally explore plasticity of BTs (sociality and aggression) in relation to kin and familiarity and sequential hermaphroditsm.

The general foundation of the reaction norm approach for investigating phenotypic plasticity, is the utilisation of a study organism that exhibits clonality (i.e. homozygosity) (Pigliucci 2001). The possibility of out-crossing in natural populations of *K.marmoratus*, however, presents the possibility that wild caught individuals exhibit some heterozygosity. In addition, assuming that wild-caught individuals are genetically independent may also be problematic, because multiple individuals collected together or randomly, may be representatives of the same genotype. Recent research has also documented that human error during propagation within a laboratory, across successive years has resulted in approximately 20% of *K.marmoratus* laboratory genotypes being accidentally mixed (Tatarenkov et al. 2010). It is therefore important that when establishing a new laboratory population of this species, from both laboratory and wild caught populations, that genotyping is implemented to determine the precise composition of the genetic stock. In Chapter 2; I present a study within which I genotyped 24 founder individuals housed at The University of Exeter, using microsatellite analysis. The aim of this study were two fold, firstly to document genetic independence of founder fish, and secondly to quantify within individual homozygosity.

Sex differences in the animal personality literature have generated varied results and when one sex has been documented to be more aggressive or bold, sexual size dimorphism (Brown et al. 2007b) or hormonal profile (Archer 1991) have been proposed as likely mechanisms. In Chapter 3, I investigate adult sex differences (hermaphrodite and secondary male) and repeatability of behaviour (i.e. BTs) by replicating within and across 10 independent homozygous genotypes. Replication within a genotype provided a sample of males and hermaphrodites that are essentially monozygotic twins (within their respective genotype) which differ in their sexual phenotype. This approach therefore provides methodological control for genetic variation presenting a powerful approach to determine how the sexual phenotype influences behavioural expression and repeatability.

Developmental perspectives of animal personality are relatively rare in the literature and thus much work is needed to understand the ontogeny of consistent individual differences in BTs and behavioural syndromes (Stamps and Groothuis 2010a; Stamps and Groothuis 2010b). Furthermore, current theoretical predictions suggest life-history as a potential state underpinning BT expression (Stamps 2007; Wolf et al. 2007b). In Chapter 4, I investigate genotype variation in behavioural plasticity, repeatability and behavioural syndrome structure during ontogeny using 20 genetically independent genotypes that were identified in chapter 2. I additionally investigate relationships between genotype level growth rate, reproductive measures and behavioural expression during ontogeny, to investigate current theoretical predictions relating to state-dependent underpinnings of BT variation.

In Chapter 5, I describe the role of the rearing environment and its effects upon the ontogeny of behavioural expression and life-history strategy. I replicate within and

across 5 independent genotypes, and rear replicates within three ecologically relevant rearing environments. The rearing environments utilised have all been noted to influence BT expression: predation risk (Brown et al. 2007a) and the presence of conspecifics (Carere et al. 2005a), or life-history strategy: restricted food rations (Lin and Dunson 1999). Behavioural and life-history responses within each environment were then compared to a control treatment to investigate how experiential effects contribute to genotypic and developmental plasticity of commonly studied personality traits.

Chapter 6 continues directly from chapter 5 and describes the influence of both the maternal environment and genotype, and how these influence transgenerational behavioural expression and life-history strategies. In this study I rear a single offspring laid by each maternal replicate (generated in chapter 5), to determine if and how the maternal environment influences behavioural and life-history expression in the next generation.

Chapter 7 takes a slightly different approach investigating plasticity in sociality and aggression in response to the immediate environment. In this chapter I investigate responses to stimulus conspecifics that are either familiar/unfamiliar (controlling for genotype effects) or unfamiliar kin/non-kin.

Chapter 8 takes the form of a general discussion of the results presented in chapters 2-7, with specific emphasis upon their implications for understanding how experiential and developmental influences shape behavioural expression of commonly studies BTs. Finally I propose some exciting areas of future research based directly upon the findings of this thesis.

Genotyping Wild Caught and Lab Reared Genotypes of *Kryptolebias marmoratus* Housed at The University of Exeter

Abstract

The mangrove killifish is one of only two known naturally self-fertilising isogenic vertebrate species. While predominant self-fertilisation generates highly inbred genotypes, rare outcross events with rare males in natural populations results in recombination of parental DNA. When establishing a laboratory population it is therefore essential that both within genotype homozygosity and between genotype independence be investigated before research commences. In this study I conduct a genetic microsatellite inventory of 24 presumed to be independent/homozygous genotypes (11 wild caught, and 13 captive bred) to quantify intra-genotype homozygosity and inter-genotype genetic independence. Results suggest that a single genotype exhibited heterozygosity at a single locus, with the remaining genotypes being homozygous at all loci investigated. These results support previous research that widely document high levels of homozygosity in this species. While 18 genotypes were found to be genetically independent, I find that two groups of three founder individuals were replicates of two genotypes. One of these groups of three founder fish originated from a wild population, potentially indicative of sampling bias facilitated by kin assortment in natural habitats. The remaining group of three founder fish originated from Valdosta State University, which indicates human error during captive propagation and maintenance. The results reported highlight the importance of genotyping presumed genotypes prior to establishment of a new laboratory population regardless of a specimen's origin. In addition, these results highlight the need for rigorous husbandry and propagation protocols to minimise potential error. Finally high homozygosity and inter-genotype genetic variation within this species presents a powerful model organism for behavioural ecology research.

2.1 Introduction

Sexual reproduction is the primary mode of propagation in most taxonomic groups that, as a process, generates heterozygous populations resulting from recombination of parental DNA (Feldman et al. 1996). Within sexually reproducing species hermaphroditsm is a widely documented reproductive strategy, with numerous examples across the animal kingdom (see Ghiselin 1969 for examples) and multiple instances documented in the Teleost fishes alone (see Sadovy de Mitcheson and Liu 2008 for a review). In contrast, mixed mating strategies in which males and hermaphrodites exist in the same population with no functional female gonochorists, termed androdioecy, is a relatively rare phenomenon (Mackiewicz et al. 2006b; also see Weeks et al. 2006 for a review).

Androdioecy has only been documented in three vertebrate species of fish within the *Rivulidae;* the mangrove killifish, and two sister species *Kryptolebias ocellatus* (Costa et al. 2010; Tatarenkov et al. 2009) and *Kryptolebias caudomarginatus* (Costa et al. 2010). Although populations of these three species are constructed of males and hermaphrodites, only *K.marmoratus* and *K.ocellatus* have been documented to self-fertilise (Tatarenkov et al. 2009). Self-fertilisation in *K.marmoratus* is achieved by a reproductive organ known as an ovotestis within which testicular and ovarian tissue is interwoven with no distinct separation (Sakakura et al. 2006). This permits internal fertilisation of eggs within the gonadal lumen following ovulation, and prior to oviposition (Sakakura et al. 2006)

In contrast to sexual reproduction, in which levels of heterozygosity are high, self-fertilisation over successive generations reduces genetic diversity and generates inbred genotypes (Sato et al. 2002) that are homozygous across most, if not all of the

genome (Mackiewicz et al. 2006a). These homozygous genotypes are in turn commonly referred to as clones, genotypes, strains or lineages. In *K.marmoratus* evidence for high levels of homozygosity was initially documented nearly 50 years ago by transplanting tissues (fin, spleens and hearts) between individuals (i.e. sib to sib, parent to offspring and between geographically separated individuals) (Kallman and Harrington 1964). This study found that individuals within a genotype (parents, offspring and siblings) accepted grafts successfully, whereas grafts between individuals from different locations failed, indicating different genetic structure between donor and recipient. In recent years microsatellite analysis has further documented high homozygosity levels in this species resulting from a long history of self-fertilisation (Mackiewicz et al. 2006b; Tatarenkov et al. 2007; Tatarenkov et al. 2009; Vrijenhoek 1985). Furthermore, these molecular studies have substantiated that populations are characterised by numerous genotypes that are genetically distinct from one another. The formation of multiple genotypes that are genetically distinct, yet homozygous within a lineage, therefore presents a powerful model organism applicable to multiple scientific disciplines.

The utilisation of organisms with uniform genetic backgrounds is by no means a new application in scientific research, for example, highly inbred mouse models have been adopted as the organism of choice within the medical sciences for over a century (Beck et al. 2000). These model organisms have long been recognised as a valuable tool because research can benefit from minimising variation between measurements within repeated assays, and between replicates within a study (Tatarenkov et al. 2010). While mouse models are extremely useful tools within science, the nature of artificial breeding and selection to generate specific phenotypes of interest, to address specific questions (Beck et al. 2000), is potentially problematic for ecology and evolution based research.

Artificial breeding/selection is particularly problematic because these approaches may result in co-selection of multiple non-target traits (Groothuis and Trillmich 2011). For example, when replicating selection lines within the same species, the genes contributing to the phenotype of interest are likely to differ depending upon the starting population utilised. The resulting phenotypes generated may therefore be the product of population specific gene combinations and thus artificial induction of phenotypic differences are likely to generate inconsistent results between selection lines (Groothuis and Trillmich 2011). Selection for specific trait combinations may also generate differing thresholds of environmental sensitivity, having severe implications for the study of BT plasticity (Stamps and Groothuis 2010a). One additional problem is that selection line generation tends to result in bimodal distributions of BTs i.e. the two lines fall at the extremes of the personality continua (e.g Drent et al. 2003; van Oers et al. 2004a). These bimodal distributions may bias our understanding of BT expression because they do not represent the continuous personality variation observed in the multitude of species observed to date (see Groothuis and Trillmich 2011 for a detailed discussion of artificial selection and associated problems). In contrast, the presence of naturally occurring homozygous genotypes in *K.marmoratus* present a powerful model within ecology and evolution because these genotypes are a product of natural selection and thus the discussed issues are minimised/absent.

While high levels of homozygosity, resulting from self-fertilisation, is a common phenomenon within populations of *K.marmoratus*, studies have documented that the ratio of primary males (develop as functional males with no female function) or secondary males (hermaphrodites that loose female function, becoming functional males at later life stages) to hermaphrodites varies considerably by geographical region (Turner et al. 1992). Moreover researchers have discovered that these populations differ

in the levels of homozygosity and heterozygosity present (Tatarenkov et al. 2007; Taylor et al. 2001). Florida locales for example, are characterised by <1% male occurrence and are characterised by high levels of homozygosity (Davis et al. 1990; Turner et al. 1992). In contrast Twin Cays, Belize exhibits approximately 20-25% male occurrence and exhibits high levels of heterozygosity (Davis et al. 1990; Turner et al. 1992). These population differences in male: hermaphrodite ratios have been suggested to facilitate the generation of recombinant heterozygous genotypes, resulting from out-crossing events (Lubinski et al. 1995; Mackiewicz et al. 2006a; Mackiewicz et al. 2006b; Mackiewicz et al. 2006c; Taylor et al. 2001). Further laboratory work has confirmed that male-hermaphrodite out-crossing occurs and thus male density as the prime mechanism for generating population differences in genetic architecture (Mackiewicz et al. 2006a).

This species' mode of reproduction can therefore be defined as selfing with occasional outcross events, followed by the resumption of selfing. Out-crossing events (that may be rare in some populations) are therefore likely to facilitate the generation of recombinant genomes that become homozygous following recommencement of self-fertilisation (Sato et al. 2002). In particular with each successive generation of selfing, heterozygosity reduces by 50% (Tatarenkov et al. 2010). For example, starting with a fully heterozygous individual that is propagated across 10 generations, levels of heterozygosity will reduce to approximately 0.195%, and by 15 generations be as low as 0.006% (Tatarenkov et al. 2010).

The potential for out-crossing in the wild in certain locales, however, makes the use of wild caught individuals potentially problematic if homozygous genotypes are to be used for experimentation. It is therefore imperative that new individuals collected

from the wild be genotyped to assess both the level of homozygosity and also genetic independence of each founder, from which genotypes are to be propagated. It may also be assumed that laboratory lines of *K.marmoratus* will undoubtedly be homozygous resulting from years of propagation from single individuals. While strictly speaking this is true, the potential for human error during propagation over multiple years' means that it is also essential that genotypes supplied from other institutions be genotyped to assure that mixing of stock during propagation/husbandry protocols has not occurred (Tatarenkov et al. 2010). This study aimed to genotype (microsatellite analysis) all founder individuals (N=24) represented by 11 hermaphrodites collected from Florida in 2007 and 13 hermaphrodites supplied from Valdosta State University, Georgia USA in 2008. This approach allows both the assessment of founder individual homozygosity as well as the genetic independence of each founder, from which independent genotypes can be propagated (refer to table 2.1 for genotype specific information).

2.2 Methods

2.2.1 DNA extraction

DNA was isolated from each F_0 individual (F_0 describes the founder generation) representing each of the 24 presumed independent genotypes housed at The University of Exeter (See Table 2.1. for genotype specific information). These individuals were culled using MS-222 and stored in 100% ethanol, following propagation of 20-30 offspring housed as stock/future breeding individuals. From each of these preserved individuals $1mm^2$ sections of the caudal fin was transferred to 1.5 ml eppendorf tubes, to which 500µl of 10% Chelex® 100 sodium form solution (Bio-Rad Laboratories Inc) containing 20mg/ml Protinase K (Qiagen)) was added. Tubes were mixed using a vortex mixer for 1 minute and incubated at 55˚C for 75 minutes. During this incubation period tissue extracts were further mixed for 1 minute periods at 15 minute intervals

throughout the tissue digestion period, again using a vortex mixer. Mixing was carried out to facilitate the binding of polar cellular components to the Chelex beads while leaving non-polar nuclear DNA suspended in the solution. Following the digestion period samples were further incubated at 100˚C for 15 minutes to denature the Protinase K and thus halt further digestion. Samples were then cooled and stored at -18˚C until amplification of microsatellite loci.

2.2.2 Primers

Eight of 36 microsatellite primers originally developed and isolated from seven inbred genotypes of *K.marmoratus* by Mackiewicz et al (2006a) were utilised for documenting both independence of genotypes and levels of homozygosity for each founder fish. The eight microsatellite primers selected were chosen because these have been shown to exhibit high levels of variation between individual lineages of *K. marmoratus* sampled from the Florida population (Mackiewicz et al. 2006a) (see table 2.2 for details of primers employed). Fluorescently labelled primers (5') were obtained from Sigma Genosys and were labelled with three colour variants. The colours utilised were black (WellRED D2-PA), green (WellRED D3-PA) and blue (WellRED D4-PA). Either forward or reverse primers were modified with fluorescent labels as detailed in table 2.2, and colour labels were assigned to primers to ensure that size ranges did not overlap within a colour variant (table 2.2.). These primers were then utilised in Polymerase Chain Reactions (PCR) to amplify each of the 8 Loci of interest.

Table 2.1. Genotype designation code/number, location sampled, geographic origin, supplier (VSU = Valdosta State University, Georgia, USA and WC= Wild Caught) and the year genotypes established in captivity for all founder individuals housed at The University of Exeter.

Clone Line	Clone Number	Location collected	Geographic Origin	Supplier	Year established
HON2	1	Bay Islands, Utila	Honduras	VSU	1996
HON7	2	Bay Islands, Utila	Honduras	VSU	1996
HON9	3	Bay Islands, Utila	Honduras	VSU	1996
HON11	4	Bay Islands, Utila	Honduras	VSU	1996
R2	5	Bay Islands, Roatan	Honduras	VSU	2000
50:91	6	Twin Cayes	Belize	VSU	1991
DAN	7	South Pelican Beach, Dangaria	Belize	VSU	1992
DAN2K	8	4-5km South of Pelican Beach, Dangaria	Belize	VSU	2000
RHL	9	Reckley Hill Pond, San Salvador	Bahamas	VSU	2001
SS:LL	10	Little Lake, San Salvador	Bahamas	VSU	2001
ENP02-2	11	Homestead Canal, Flamingo (Everglades)	Florida	VSU	2002
VOL	12	Mosquito Lagoon, Potato Island	Florida	VSU	1995
SLC8E	13	Nuclear Power Plant (St Lucie County)	Florida	VSU	1995
LK2	14	Long Key	Florida	WC	2007
LK4	15	Long Key	Florida	WC	2007
LK6	16	Long Key	Florida	WC	2007
LK7	17	Long Key	Florida	WC	2007
LK13	18	Long Key	Florida	WC	2007
LK15	19	Long Key	Florida	WC	2007
BP3	20	Bogie Rd., Big Pine Key	Florida	WC	2007
BP11	21	Bogie Rd., Big Pine Key	Florida	WC	2007
NNKN1	22	No Name Key, North	Florida	WC	2007
NNKN5	23	No Name Key, North	Florida	WC	2007
NNKN10	24	No Name Key, North	Florida	WC	2007

2.2.3 PCR amplification of DNA and sequencing

Two separate PCR cycles were utilised to successfully amplify all loci with each primer corresponding to PCR cycle 1 or 2, see table 2.2 for corresponding primers and PCR programme. PCR programme cycle 1 employed the following protocol: 95˚C for 5 minutes, followed by 30 cycles of 30s at 95˚C, 30s at 50˚C and 60s at 72˚C and a final extension period of 10 minutes at 72˚C. PCR cycle 2 utilised the following programme: 95˚C for 5 minutes, followed by 35 cycles of 30 seconds at 95˚C, 90s at 55˚C and 3 minutes at 72˚C and a final extension period of 10 minutes at 72˚C. The reaction mix contained 0.2mM of each dNTP, 1xCR buffer (Bioline), 0.5µM of each primer, 3mM $MgCl_2$, 0.5 U Red*Taq* (Bioline), 1µl template DNA and water to a final volume of 10µl. PCR fragments were analysed using a DNA sequencer (CEQ 8000 Genetic Analysis System, Beckman Coulter) and fragment sizes determined using CEQ 8000 Genetic Analysis System, Version 9.0 (Beckman Coulter).

Table 2.2. Primer sequences (Mackiewicz et al. 2006a); fluorescent labelling and PCR cycles utilised to amplify polymorphic microsatellite loci in the Mangrove killifish and the expected size fragments (bp).

Loci	Repeat Motif	PCR Cycle	Primer Sequence 5-3'	Size Range (bp)	Primer labelled /colour
R3	$(ACAG)_{24}$	1	F: AAT TTT ATG TAT CTG GAC ACA GG	126-186	Forward
			R: TAA TAC ACT TCT ACA GCC AAG GT		Black
R5	$(AATC)_{15}$	1	F: CAT CAT CAC TGT CAC CAT ATT T	290-310	Forward
			R: TGG ACC TAT TTG TGT GTC TTT A		Blue
R10	$(AAG)_{27}$	2	F: GAA ACA TGT CCT CAT ACT CCA	210-240	Forward
			R: TAA ACC TCT GTT ATC TGC TGC		Blue
R11	$(AAAG)_4AAGG(AAAG)_{16}$	2	F: CTG CAC TAA GTG GAT CTG TTC	166-186	Reverse
			R: TTG TTA CAC CAA TCA TTA CCC		Blue
R22	$(ACAG)_{10}$	1	F: CTC GCT GCT ACT ATT GCT G	166-202	Reverse
			R: CCG TAT GGG TTG TTC TTT T		Green
R25	$(AAAC)_{18}$	1	F: TGG CTA ACC AAA CAA ACA GA	100-132	Reverse
			R:CAT GAG TAG GGT TGT CCT GT		Green
R37	$(ATCT)_{44}(GTCT)_{11}$	1	F: CTG ATC CCT GAA CTA AAT CCT A	268-340	Forward
			R: GCA TTC ATT GAT GTT CTA CTT G		Green
R38	$(AAAG)_3AGAG(AAAG)_{16}$	2	F: TGC CTC CAA ACC AGT CTA	190-210	Forward
			R: CCA CCA ATG GAC TGA GAA		Black

2.3 Results

Microsatellite analysis indicated that 23 of the 24 genotypes exhibited homozygosity at all eight microsatellite loci (table 2.3). Genotype LK13 (No: 18) was, however, found to be heterozygous at one loci exhibiting two differing sized fragments at loci R3 (table 2.3).

When exploring genotype genetic independence, 18 of the 24 presumed independent genotypes were genetically distinct from one another (table 2.3). In contrast two separate groups of three genotypes were identical at all microsatellite loci assayed (table 2.3, No's: 7-9 (DAN, DAN2K, RHL) and 22-24 (NNKN1, NNKN5, NNKN10), respectively). Interestingly and quite surprisingly founder individuals 7-9 were supplied by Valdosta State University and arrived individually packaged with different genotype designations as indicated in table 2.1. I therefore expected these individuals to be genetically independent founders derived from three separate established laboratory propagated lineages. Genotypes 22-24 were all collected from the same geographic location (No Name Key, North) in Florida in 2007 (table 2.1).

2.4 Discussion

The microsatellite analysis revealed that 18 of the 24 founder individuals housed at The University of Exeter were genetically distinct from one another i.e. a minimum of 1 loci difference. The remaining 6 founder individuals represented two independent genotypes, each containing three individuals that were genetically identical at all 8 loci. While one of these groups of three founder fish were collected from Florida in 2007 (NNKN1, NNKN5, NNKN10), the second group of three fish originated from the captive laboratory population at Valdosta State University (RHL, DAN, DAN2K). These results suggest that founder individuals housed at Exeter represent a total of 20

unique genotypes of *K.marmoratus*. Each of these 20 genetically independent lines were additionally found to be homozygous at all 8 microsatellite loci amplified, with the exception of one genotype (LK13) which exhibited heterozygosity at one locus.

2.4.1 Inter-genotype variation

The occurrence of 6 founder individuals that were presumed to each originate from genetically distinct lineages, actually being representatives of only two independent genotypes, exemplifies the importance of genotyping fish. Furthermore these results highlight that genotyping is important regardless of origin, prior to founding a new laboratory population. With regards to genotypes RHL, DAN and DAN2K, supplied by Valdosta State, these results are particularly alarming because researchers may inappropriately assume genotypes be independent upon delivery. In this instance it is likely that mixing and/or miss labelling of genotypes occurred during laboratory propagation prior to dispatch. In addition, it is also possible that an error occurred at the time of packaging fish ready for transportation. The results presented here also mirror those of Tatarenkov et al (2010) whom completed a genetic survey of laboratory stocks worldwide, using 21 genotypes of *K.marmoratus*. In particular Tatarenkov and co-workers (2010) report as many as 20% of the laboratory stock of *K.marmoratus* have been mislabelled, accidently mixed or incorrectly propagated from incorrect lineages. This high occurrence of human error has multiple implications for research that is founded on the presumption of genetically independent genotypes. In particular, experimentation may utilise multiple genotypes that are in fact representative of the same genetic construct and thus pseudo-replication is potentially common. In addition when genotypes are shared with co-workers in other laboratories the potential mixing of genotypes prior to dispatch has serious implications for reproducibility of results across laboratories especially when specific genotypes are the focus of specific

Table 2.3. Results of microsatellite sequencing and the resulting sizes of fragments observed at each microsatellite loci. Fragment data enclosed in red rectangles represent heterozygote loci; Genotype data enclosed in black rectangles represent non–independent genotypes. - - represent missing data.

	Bands Observed (bp) / Locus							
Genotype	**R3**	**R5**	**R10**	**R11**	**R22**	**R25**	**R37**	**R38**
HON2	177, 177	294, 294	206, 206	171, 171	197, 197	134, 134	321, 321	207, 207
HON7	185, 185	298, 298	234, 234	171, 171	199, 199	138, 138	333, 333	207, 207
HON9	185, 185	298, 298	224, 224	197, 197	215, 215	130, 130	281, 281	203, 203
HON11	177, 177	294, 294	206, 206	181, 181	195, 195	138, 138	341, 341	203, 203
R2	157, 157	290, 290	284, 284	177, 177	187, 187	130,130	309, 309	290, 290
50:91	125, 125	314, 314	218, 218	193, 193	187, 187	116, 116	329, 329	195, 195
DAN	157, 157	306, 306	- -	181, 181	187, 187	104, 104	313, 313	195, 195
DAN2K	157, 157	306, 306	- -	181, 181	187, 187	104, 104	313, 313	195, 195
RHL	157, 157	306, 306	- -	181, 181	187, 187	104, 104	313, 313	195, 195
SS:LL	153, 153	302, 302	196, 196	197, 197	171, 171	108, 108	313, 313	211, 211
ENP02-2	155, 155	288, 288	226, 256	180,180	203, 203	100, 100	304, 304	194, 194
VOL	161, 161	290, 290	224, 224	171, 171	169, 169	108, 108	297, 297	189, 189
SLC8E	149, 149	302, 302	224, 224	185, 185	173, 173	108, 108	325, 325	199, 189
LK2	161, 161	306, 306	228, 228	193, 193	195, 195	108, 108	289, 289	189, 189
LK4	149, 149	302, 302	244, 244	189, 189	219, 219	104, 104	289, 289	191, 191
LK6	153, 153	290, 290	204, 204	189, 189	199, 199	104, 104	297, 297	189, 189
LK7	153, 153	290, 290	202, 202	189, 189	199, 199	104, 104	297, 297	189, 189
LK13	**149, 161**	306, 306	278, 278	185, 189	195, 195	108, 108	293, 293	181, 181
LK15	157, 157	302, 302	214, 214	185,185	199, 199	- -	293, 293	177, 177
BP3	149, 149	290, 290	244, 244	177, 177	203, 203	104, 104	293, 293	185, 185
BP11	153, 153	290, 290	206, 206	177, 177	199, 199	104, 104	301, 301	- -
NNKN1	157, 157	294, 294	208, 208	167, 167	207, 207	108, 108	273, 273	189, 189
NNKN5	157, 157	294, 294	208, 208	167, 167	207, 207	108, 108	273, 273	189, 189
NNKN10	157, 157	294, 294	208, 208	167, 167	207, 207	108, 108	273, 273	189, 189

research areas. I therefore suggest that laboratories need stringent propagation and husbandry protocols to be implemented to ensure that errors are minimised. Furthermore, intermittent genotyping of stock will permit errors to be identified earlier and thus appropriate steps can be taken to restore the genetic validity of a laboratory population.

With regards to founders NNKN1, NNKN5 and NNKN10, these individuals were collected from the same geographical location as denoted by the NNKN genotype designation. This result suggests that each of these genotypes were siblings/clone mates potentially originating from the same parental individual in the wild. While we have little information regarding the environmental conditions at this specific collection location, it could be argued that environmental conditions limited the potential for dispersal. For example some species, such as Trinidadian guppies, may be isolated within pools for extended periods during the dry season (ME. pers. observation). In relation to *K.marmoratus*, however, this is unlikely to have driven this sampling bias because this species can survive out of water for up to 66 days in moist conditions (Taylor 1990), due to an epidermal capillary network that permits respiration via the skin surface (Grizzle and Thiyagarajah 1987). This specialist adaptation allows this species to emmerse within the terrestrial habitat, with individuals being commonly found in leaf litter, fossorial niches (Taylor 2000), and within emergent logs (Taylor et al. 2008b). If individuals hatched from eggs oviposited and subsequently isolated within a pool, they therefore have the ability to disperse from the oviposition site via emmersion behaviour.

An alternative explanation here may implicate kin assortment. Kin discrimination abilities have been widely documented in multiple species of Teleost fish

(see Brown and Brown 1996b; & Ward and Hart 2003 for reviews) and thus assortment based upon kin is possible in this instance. In relation to *K.marmoraus* however, I am unaware of any published research that has documented kin discrimination or assortment in *K.marmoratus*. In chapter 7, I explore kin effects on social behaviour and discrimination abilities in the laboratory, and find supporting evidence for this explanation. This suggests kin assortment as a possible mechanism for this location specific sampling bias, however, further investigation in the wild is necessary to further elucidate this possibility. In light of the above discussed non-independence of 6 presumed independent genotypes, I randomly selected the offspring of one founder individual from each of these two groups of three (NNKN1 and DAN) and these individuals were used for further propagation protocols.

2.4.2 Intra-genotype variation

The results of microsatellite analysis also reveal that 19 of the 20 independent genotypes (excluding within genotype replications discussed above) were homozygous at all eight microsatellite loci sampled. These results support the widely documented occurrence of high homozygosity in this species due to a long history of self-fertilisation in both wild Florida populations (Taylor et al. 2001) and in laboratory derived populations (Kallman and Harrington 1964). I did, however, find that one genotype (LK13) was heterozygous at the R3 locus; however this genotype was homozygous at the remaining seven loci, which still indicates high homozygosity within this genotype. These results generally support the isogenic nature of this species and its suitability for use as a model organism for research that requires replication within homozygous genotypes.

While I was unable to determine the exact cause of heterozygosity in LK13, there are some potential explanations for this occurrence. The observed heterozygosity at R3 for this genotype may for example, indicate a relatively recent outcross event in the wild. As mentioned previously out-crossing events followed by resumption of selfing reduces heterozygosity by 50% per generation (Mackiewicz et al. 2006a) and thus it is possible that this genotype has yet to propagate through sufficient generations for full homozygosity to be achieved. Although this is the most likely cause for heterozygosity at this locus, it is also possible that this genotype was fully homozygous in the past and subsequent *de novo* mutation (mutation within one germ cell) generated heterozygosity at this locus within this founder individual. In particular Tatarenkov et al (2010) suggest that mutation rates range from $7x10^{-4}$ to $3x10^{-3}$ per locus, per generation and thus *de novo* mutation cannot be ruled out as a potential explanation in this instance.

While LK13 was heterozygous at one locus, this low amount of heterozygosity in this founder individual does not mean that this genotype is unsuitable for use in experimentation. On the contrary, this genotype was still homozygous at 7 of 8 loci indicating high levels genetic uniformity. Furthermore, all genotypes were propagated across two subsequent generations prior to commencement of the first study, to remove maternal effects, and thus heterozygosity in this genotype is expected to have reduced further between the F_0 and F_2 generations. It is therefore likely that heterozygosity was reduced further prior to experimentation.

These results support previous research indicating that homozygosity resulting from self-fertilisation over successive generations is characteristic in this species. Furthermore these results indicate the importance of not only genotyping wild caught

individuals when setting up a new laboratory population, regardless of their origin, but also the importance for rigorous record keeping and husbandry practices to ensure genotypes remain independent from one another during propagation. The results reported have therefore permitted a laboratory population to be founded at The University of Exeter, within which all potential genotype discrepancies were identified prior to establishment. The documented high levels of homozygosity in this laboratory population present a powerful model organism for multiple scientific approaches. In particular studies investigating phenotypic plasticity of both life-history and BTs are especially suitable avenues of research utilising this species. This organism presents a powerful tool because it is possible to replicate within a genotype across multiple environmental treatments to explore genotype variation and plasticity in relation to environmental influences, while controlling for genetic variation. Furthermore, replication across multiple genotypes allows general responses to be investigated in relation to the rearing or social environment. The documentation of 20 independent genotypes therefore forms the foundation for each study presented in the following chapters. In the next chapter I investigate sex differences in average behavioural types, and behavioural type repeatability between adult secondary males and hermaphrodites.

Sequential Hermaphroditsm and Personality in a Clonal Vertebrate: The Mangrove Killifish

Abstract

Individuals are regularly documented to consistently differ in their behavioural types (BTs). For example, some individuals are consistently bold whereas others are consistently shy. Within the human personality literature, the big 5 personality dimensions are commonly documented to be sex-specific with testosterone suggested to underpin aggressiveness. Moreover, aggression has been documented to correlate with other BTs in several species. In non-human animals recent research suggests sex-specific BT expression may be influenced by ecology, mating system and sexual selection. While most research on sex-specific personality has focused on dioecious species, I explore short term repeatability of behaviour (over a 7 day period) and sex differences in BT expression in adults of a sequential hermaphrodite; the mangrove killifish. I replicate within 10 isogenic genotypes and investigate sex differences (hermaphrodite and secondary male) in three BTs (exploration, boldness and aggression). This approach allows sex differences in BT expression to be investigated while controlling for genetic variation. In this study I found that secondary male and hermaphrodite individuals are consistent in their behaviour i.e. exhibit BTs (personality) over a 7 day period. I also documented that while the sexes exhibited similar average BT scores, genotypes differed in their average levels of aggression, suggesting genetic control of this BT. Finally, adult secondary males were significantly more repeatable than adult hermaphrodite in their boldness BTs, potentially supporting proposals relating to sexual selection.

3.1 Introduction

The study of animal personality is a rapidly growing sub-discipline of behavioural ecology, which focuses upon consistent individual differences in behavioural types (BTs), for example some individuals are consistently bold while others are consistently shy (Bell 2007; Reale et al. 2007; Sih et al. 2004b). These BTs have additionally been found to influence fitness (Cote et al. 2008; Dingemanse and Reale 2005; Gosling 2001; Pruitt et al. 2008; Smith and Blumstein 2008; Smith and Blumstein 2010) and be heritable (Dingemanse et al. 2002; Drent et al. 2003; Reale et al. 2009; Sinn et al. 2006; van Oers et al. 2004b), suggesting that personality is likely to be adaptive and governed by natural selection (Dingemanse and Reale 2005; Reale et al. 2007). BTs have also been shown to have consequences for population and community dynamics (Chapman et al. 2011; Fraser et al. 2001; Godin and Dugatkin 1996).

In addition to consistency, BTs are also widely documented to co-vary across differing contexts or situations with bold individuals remaining bold in both the presence of predators and conspecifics (Sih et al. 2004b). Moreover, multiple BTs have been found to co-vary, for example bold individuals are often more aggressive than their shy counterparts (Huntingford 1976). The presence of correlated BTs across contexts has been suggested to be a constraint upon the independent expression of BTs, and is thought to result from genetic correlations between traits (Sih et al. 2004b) and/or the same genes or hormones acting on several targets i.e. pleiotropy (Ketterson and Nolan 1999). In contrast, some studies have also documented no correlations between BTs suggesting that in some instances behaviours are independently expressed i.e. the adaptive hypothesis (e.g. Bell 2005; Coleman and Wilson 1998; Reale et al. 2000).

Within the human personality literature, sex differences are regularly reported in the big 5 personality dimensions: openness, conscientiousness, extraversion, agreeableness, and neuroticism (McCrae and Costa 1999). Males for example, generally take more risks (Byrnes et al. 1999) exhibit greater assertiveness, while being less trusting and anxious (Costa et al. 2001; Feingold 1994). Furthermore, males tend to be more aggressive than females (see Archer 2006 for a discussion) which is thought to be driven by sex differences in testosterone and/or serotonin levels (see Archer 1991; Nelson and Chiavegatto 2001; Nelson and Trainor 2007 for reviews). The non-human animal personality literature, in contrast, suggests that sex differences in average BT expression may be influenced by multiple factors. For example, while males have been found to be bolder in some species (Dugatkin 1988; Johnsson et al. 2001; Piyapong et al. 2010), in others, no sex differences are observed (e.g. Carere and van Oers 2004; Fraser et al. 2001; Kurvers et al. 2009; Reddon and Hurd 2009). Furthermore, sex-specific aggression and boldness has been shown to be influenced by predation risk and motivation for access to mates or resources (Archard and Braithwaite 2011; Brown et al. 2007b). Moreover, sex differences in aggression have also been found to be mating system specific with males tending to be more aggressive in polygynous species (e.g. Bakker 1986; Johnsson et al. 2001), whereas, females may be more aggressive in polyandrous or monogamous mating systems (e.g. Arnott and Elwood 2009; Goymann et al. 2004; Swenson 1997).

In recent years, there has been growing interest into the mechanisms generating and maintaining consistent individual differences in behaviour (Dall et al. 2004; Dingemanse and Wolf 2010; Schuett et al. 2010; Stamps 2007; Stamps and Groothuis 2010a; Stamps and Groothuis 2010b; Wolf et al. 2007b). In a recent review article, sexual selection was proposed as a likely mechanism maintaining sex differences in

personality traits (see Schuett et al. 2010 for a review). Although personality has received minimal attention in relation to sexual selection, boldness has been shown to influence female mate-choice in guppies, with bolder males attaining higher reproductive success than shy males (Godin and Dugatikin 1996). In addition BT repeatability, also termed consistency, has been proposed to be a sexually selected trait (Schuett et al. 2010). In this scenario, if a male exhibits consistent behaviour in one context and consistency predicts future behaviour expression, females may gain fitness benefits from choosing males based upon their repeatability. Male consistency may therefore be a reliable cue during mate choice, from which females can predict a partner's behaviour (Nakagawa et al. 2007; Schuett and Dall 2009; Schuett et al. 2010).

To date BTs have generally been studied within dioecious species; however, many organisms exhibit various forms of hermaphroditsm (Ghiselin 1969) with multiple examples within the Teleost fishes alone (Sadovy de Mitcheson and Liu 2008). In this study, I take an innovative approach using a naturally occurring isogenic vertebrate, *K.marmoratus* as a model organism. I investigate whether size matched hermaphrodite and secondary male phenotypes are repeatable in their behaviour and differ in their repeatability estimates. Moreover, I assess whether the sexes exhibit differences in average BT scores. By using 10 isogenic genotypes I am able to control for genetic variation and tease apart the effect of both genotype and sex upon BT expression. I investigate three commonly studied BTs; namely exploration of a novel maze, boldness following an aerial predation simulation, and aggression towards a mirror image. Unfortunately resulting from small sample size (i.e. only 10 genotypes utilised) I was unable to investigate correlations between BTs in this study.

3.2 Methods

3.2.1 Study organism

While hermaphroditsm is a relatively common phenomenon in the Teleost fish (Sadovy de Mitcheson and Liu 2008) androdioecy, a mating system within which functional males and simultaneous hermaphrodites exist in the same population, is extremely rare (Weeks et al. 2006). Within the documented examples of androdioecy even fewer species have been documented to contain self-fertilising, simultaneous hermaphrodites that additionally outcross with functional males (primary and/or secondary). In vertebrates this strategy has only been observed in two related species: *K.marmoratus* and a sister species; *K.ocellatus* (Tatarenkov et al. 2009).

Within *K.marmoratus*, repeated self-fertilisation over successive generations has been documented to remove genetic diversity, generating homozygous, isogenic genotypes (Mackiewicz et al. 2006a). In addition, occasional outcross events between hermaphrodites and males has been shown to facilitate recombination of parental DNA, following which, selfing returns a lineage to homozygosity (Lubinski et al. 1995; Mackiewicz et al. 2006a; Mackiewicz et al. 2006b; Mackiewicz et al. 2006c; Taylor et al. 2001). This interesting reproductive strategy therefore generates complex population genetic architectures, within which multiple genetically independent homozygous genotypes exist (Sato et al. 2002). In addition to self-fertilisation, the mangrove killifish also exhibits sequential hermaphroditsm i.e. sex change, where self-fertilising hermaphrodites lose female function, becoming secondary males (Harrington 1968; Harrington 1971). These secondary males are brightly coloured compared to hermaphrodites, exhibiting an orange body colouration and no caudal ocellus (see figure 3.1). Males and hermaphrodites within a single genotype can therefore be classified as monozygotic twins that differ in their sexual and morphological phenotype. This species

therefore presents an exciting model with which sex differences may be investigated while controlling for genetic variation.

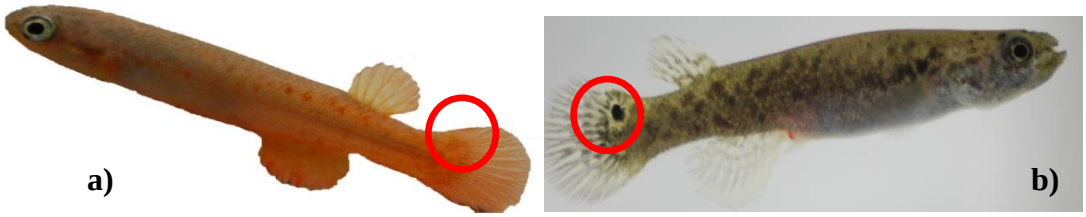


Figure 3.1. Photographs of a) male and b) hermaphrodite sexual phenotypes. Males are characterised by orange carotiniod colouration and hermaphrodites characteristically exhibit a brown mottled colouration. Red circles highlight the presence (hermaphrodite) or absence (male) of the caudal ocellus

3.2.2 General protocols

All fish were housed in 20ppt synthetic salt water (Instant Ocean^{TM}) at 25˚C ± 0.5˚C in a 12hr light 12hr dark cycle and fed ad-libitum with artemia naupili (*Artemia salinas*). Twenty F_2 fish representing 20 homozygous genotypes were reared from hatching (15 day variation in hatching date across aquaria and ± 7 days within aquaria), within stock tanks (394 x 250 x 140mm) containing 3 litres of salt water. All fish were maintained within genotype specific aquaria and all fish exhibited hermaphroditic phenotypes at approximately three months of age (mottled brown colouration and fertilised egg production). At approximately 600 days of age some hermaphrodites began to develop orange body colouration which has been documented to be a robust indicator of secondary male formation, during which female function ceases (Sakakura and Noakes 2000). Stock tanks containing hermaphrodites and secondary males, were maintained until 650 days, at which point equal numbers of secondary males and hermaphrodites (adults) from each tank were selected. To control for size variation between the sexes within each tank I selected pairs of hermaphrodites and secondary males that were size matched (± 3mm) and were all 650 days old ± 7 days (i.e.

maximum of 14 day variation in hatching date). Genotypes produced variable numbers of secondary males and thus the number of replicates per genotype was uneven (see table 3.1 for the number of individual representatives per genotype used in experiments) providing a total number of 30 secondary males and 30 hermaphrodites for experiments. Fish were individually housed for two months in standard aquaria (28 x 17.5 x 16cm) containing 2 litres of salt water and a sparse layer of gravel. This two month isolation period was chosen to minimise social influences upon behavioural expression because conspecific presence during ontogeny has been found to influence behaviour in this species (see Chapter 5 for details). In addition, high density aggregations in the wild have been documented to result in reduced aggression in this species (Taylor 2000). This two month isolation period also permitted egg production to be monitored (at the time of tank cleaning every 15 days) and secondary males were found to produce no eggs during this time confirming the loss of female function. After completion of the isolation period, behavioural assays were conducted for exploration, boldness and aggression. No mortality was documented during the study and all fish were successfully assayed for behavioural expression on days 1-3 and 7-9 (see below for more details).

Table 3.1. Table showing the number of males and hermaphrodites sampled for each genotype.

Genotype	**Genotype Number**	**N Hermaphrodites**	**N Secondary Males**
BP11	1	5	5
BP3	2	6	6
HON2	3	1	1
LK13	4	2	2
LK15	5	4	4
LK4	6	4	4
LK6	7	2	2
LK7	8	4	4
NNKN1	9	1	1
DAN	10	1	1
N=		**30**	**30**

3.2.3 Behavioural assays

Boldness, exploration and aggression assays were separated by 24 hours to ensure independence of data points and limit carry over effects between assays. Exploration was assayed on day one, boldness on day two and aggression on day three and these three assays were repeated seven days later. Firstly, each fish was randomly assigned to a testing order on day one post hatching (prior to the exploration assay) and this order was maintained for each behavioural assay (exploration, boldness and aggression) within each of testing period (days 1-3 and days 7-9). This was completed because I was primarily interested in the temporal stability of individual behavioural variation (i.e. BTs). Maintaining experimental testing orders within/between assays across each testing period therefore limited the potential for additional experimentally induced within-individual variation that would confound repeatability estimation. Each trial was recorded using a Sony ExwaveHad black and white video camera fitted with a Computar Vari Focal 5-50mm F1.3 lens. Ethovision XT version 6.0 (Noldus Information Technology) was used to record each fish's movements within exploration assays.

Exploration was assayed using a white novel maze measuring 60 x 60 x 15 cm (see figure 3.2a). A white maze was utilised to maximise contrast between the background and the test fish to permit individual tracking using Ethovision software. The arena was divided in to 24 equal zones (excluding the acclimatisation enclosure). Fish were introduced to the test tank via the acclimatisation enclosure (containing a sparse layer of white gravel), which was surrounded by a white opaque barrier. Following 5 minutes acclimatisation, individuals were released by raising the opaque barrier. Behaviour was observed for a 10 minute period, during which time I recorded the distance travelled (mm), mean velocity (mm/second), and the number of unique

zones entered. While fish were exploring the novel maze, gravel was removed from home tanks and replaced with removable identical structural components of the boldness trial arena i.e. gravel disc and mesh partition, which were positioned within the home tank to simulate the boldness trial arena as depicted in figure 3.2b. Fish were housed in these 'mock' arenas for a period of 24 hours following the exploration assay to limit potential effects of structural novelty during the subsequent boldness trial which may confound boldness measures (Reale et al. 2007). Unfortunately, due to technical problems during ~30% of boldness assays, it was not possible to score individual activity following the drop test, as completed in chapters 4, 5 and 6. However, as shown in chapter 4 and appendix A4.1; both a single measure of TTM and PCA scores (TTM + activity) yield qualitatively similar results. The use of a single boldness measure (TTM) in this study is therefore comparable with the results using PCA scores in subsequent chapters in this thesis (see chapter 4 for further details).

The boldness test arena measured 28 x 17.5 x 16cm and was divided in to two sections along the length (see figure 3.2b). Following introduction to the boldness arena all individuals were given a 5 minute acclimatisation period after which time a weight suspended 13cm above the drop zone was released in to the arena breaking the water's surface. The inclusion of a separate drop zone ensured the weight never had the potential to strike and injure the test fish. Following the simulated aerial predation event, I recorded time (seconds) to first movement (TTM) (categorised as movement of >1 body length). Each trial lasted a total of 5 minutes (starting directly after the aerial predation simulation) which was sufficient for all individuals to regain activity. During the boldness trial I removed 'mock' boldness structures from the test fish's home tank and replaced these with structural components of the aggression arena (see figure 3.2c for structural components inserted and their positioning within the home tank).

Individuals were then housed in these ‘mock’ aggression arenas for a period of 24 hours prior to aggression assays.

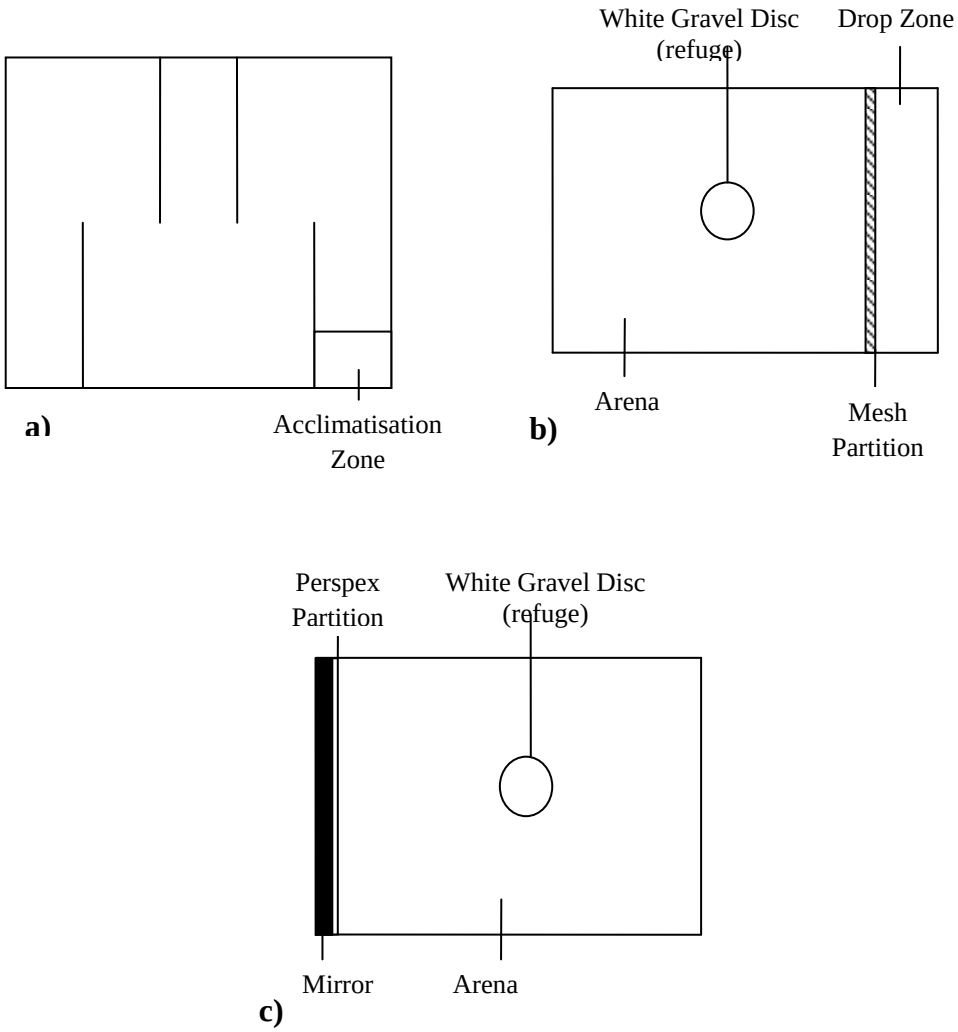


Figure 3.2. Schematic diagram of a) exploration trial arena (lines represent opaque partitions within the arena), b) boldness trial and c) aggression trial arena. The boldness arena incorporated a drop zone measuring 3cm wide (separated by white mosquito netting). The aggression trial arena incorporated a 3mm thick Perspex partition behind which a mirror was fitted. In front of this mirror a removable partition was placed, to permit the mirror to be revealed following a 5 minute acclimatisation period.

The aggression test arena, measuring 28 x 17.5 x 16cm, was divided in to two sections along the length using a clear Perspex partition. 1cm behind this partition, a mirror was fixed. A remotely removable opaque partition, allowing the mirror to be revealed to the test fish, was positioned in front of the mirror and behind the Perspex

partition. (see figure 3.2c). Following introduction into the aggression arena all individuals were given a 5 minute acclimatisation period after which I waited until fish moved in to the furthest half of the arena from the mirror zone, before raising the opaque barrier. An interaction zone was predefined in front of the mirror (2 times the mean standard length of the fish (9cm)). A 10 minute trial then began when the test fish entered the interaction zone. During each trial I recorded the total time spent within the interaction zone together with the frequency of bites and tail flicks directed towards the mirror image. Bites and tail flicks were summed to generate a measure of total aggression. Total aggression (at each sample point) was then used to calculate aggression rate/minute based upon the duration of time spent within the interaction zone. Aggression rate was square-root normalised and used as a response variable in subsequent analysis.

3.2.4 Statistical analysis

3.2.4.1 Assessing the most appropriate measure of exploratory behaviour

Within the animal personality literature multiple statistical approaches are utilised to quantify individual differences in behaviour. One commonly used approach is to quantify multiple behavioural measures within a single assay which presents potential statistical problems. For example, when each measure is analysed using separate statistical tests, the potential for type 1 error increases alongside the number of tests employed (see Budaev 2010 for a discussion). This has lead some researchers to record a single measure per assay e.g. number of zones entered in a novel maze (pure exploration) (e.g. Korpela et al. 2011). In contrast, when multiple measures are recorded, an alternative and commonly used method is Principal Component Analysis (PCA). PCA is a method that permits identification and reduction of correlated variables into composite scores (for each individual) that explain variation in the data (see

Lantová et al. 2011; Sinn et al. 2008 for an example). These individual PCA scores can be utilised as response variables in further analysis, minimising multiple statistical testing problems (see Budaev 2010 for a discussion).

In some instances, researchers have found that multiple measures of pure exploration correlate with activity to form a single behavioural construct (e.g. Lantová et al. 2011; Smith and Blumstein 2010; Smith et al. 2009). In contrast, some research has found that general activity is unrelated to pure measures of exploration, even when collected in a single assay (Biro et al. 2010). The potential for activity and exploration being two aspects of unrelated behavioural expression therefore presents the need to determine whether or not these behavioural characteristics of individuals may explain a single behavioural construct. In many cases this is simply completed using PCA analytical approaches (as described below), however, a criticism of this approach is that activity may inadvertently contaminate a pure exploration score (K.E. Arnold personal communication). In this chapter (and within analyses for chapters 4, 5 and 6) I observed that exploration and activity loaded on to a single principal component (see below for PCA results), suggesting correlated behavioural expression of these traits. It is, however, important to clarify whether further analysis using a PCA score combining exploration and activity measures, qualitatively explains behavioural expression in a similar manner to a single pure measure of exploration i.e. unique number of zones entered. I therefore addressed this possibility by running separate mixed models for PCA scores (Gaussian error and identify link) and my measure of pure exploration (Poisson error and log link) (see linear mixed modelling section below for model structures and Appendix 3.1 for further details). Briefly, the results of this analysis show qualitatively similar results regardless of whether PCA scores (exploration + activity) or my measure of pure exploration (unique zones entered) were modelled as response

variables (see Appendix A3.1 for analysis results). I therefore utilise PCA scores that combine exploration and activity in to a single composite measure in all exploration analyses from this point forwards (within this chapter and chapters 4, 5, and 6).

3.2.4.2 Exploratory principal component analysis

Variance-covariance matrix similarities were compared between each sample (separated by 7 days) for exploration measures (distance moved etc), using common principal component analysis (CPC). CPC is a method by which complex relationships between matrices are compared, for example two or more matrices may be equal (same sampling error), proportional (i.e. matrix elements multiplied by a constant), have all components in common (CPC model), share some principal components (PCPC), or be unrelated (no common components) (for discussions see Arnold and Phillips 1999; Flury 1988; Phillips and Arnold 1999). The CPC approach uses step-up model building and for each level of the hierarchy a maximum likelihood statistic is calculated. Stepwise approaches, however, increase type 1 error rates and thus I compare AIC values of each type of CPC model using the information theoretic framework (I-T) (Burnham and Anderson 2002; 2004), (see below for I-T methods). I find that covariance matrices for exploration share a common principle component over all data points (CPC model AIC = 7.03, Delta AIC = 0, AIC w_i = 0.91). A single PCA for all exploration assay measures, collected at both sample points was therefore conducted, within which exploration measures loaded strongly on to a single component that explained a high percentage of total variance (component loadings: distance travelled: 0.906, unique zones: 0.904, mean velocity: 0.935, percentage of the total variance explained: 83.7%). PCA scores were extracted from this analysis, for each individual at each sample point and these scores were use in subsequent analyses. Bartlett sphericity and Kaiser-Meyer-Olkin (KMO) tests indicated that exploration matrices were suitable

for use in PCA analysis (KMO= 0.740, Bartlett's test $p<0.001$) (see Budaev 2010 for a discussion). PCA analysis was completed using SPSS v 16.0.

3.2.4.3 Repeatability

Repeatabilities (Lessells and Boag 1987) were calculated for each behavioural assay during ontogeny together with 95% confidence intervals (Faraway 2006) using linear mixed effect models fitted with Gaussian error and identity link functions. To determine if *K.marmoratus* exhibit repeatable behaviour (i.e. BTs), and if the sexes differ in their behavioural consistency, repeatabilities were calculated for each behavioural assay for hermaphrodites and males combined and also for the sexes separately. Each response variable (exploration, boldness and aggression) was fitted within a separate model with individual nested within genotype as random effects, from which variance components were predicted. Variance component predictions were used to calculate repeatabilities at the individual level as: the sum of the variance between individuals and the sum of the variance between genotypes divided by the sum of the variance between individuals and variance between genotypes and the residual variance. Repeatability at the genotype level was calculated as: the variance between genotypes divided by the sum of the variance between individual's and variance between genotypes and the residual variance (see Nakagawa and Schielzeth 2010 and ; Schuett et al. 2011 for details). Confidence intervals (95%) for repeatability estimates were obtained from parametric bootstrapping (N=1000 simulation iterations) and p values estimated using randomisation tests (N=1000 iterations) to determine if repeatability estimates were significantly different from zero (see Nakagawa and Schielzeth 2010 for details). Repeatability estimates were compared between the sexes using effect size calculations, and inferences regarding significance of these differences were completed by calculating 95% confidence intervals (Garamszegi 2006; Nakagawa and Cuthill

2007). In particular, I compared repeatability estimates by calculating pair-wise mean difference in Z-transformed repeatability estimates between each sex for each behaviour separately. Confidence intervals that did not span zero indicate a significant difference between the sexes in their behaviour repeatability at the 0.05 α level (Nakagawa and Cuthill 2007).

3.2.4.4 Linear mixed modelling

Linear mixed modelling was completed using R for statistical computing v2.12.0 (R development core team 2010) using the lmer function within the package lme4 (Bates and Maechler, 2009). Using maximum likelihood estimation I investigate the influence of genotype and sex on BT expression. BT scores for exploration (PCA score), boldness (TTM) and aggression rate/minute were inputted as response variables in each respective model. Sex (secondary male or hermaphrodite), genotype and their interaction were included as fixed categorical effects. In addition, random effect covariates for individual nested within genotype were included to control for repeated measures within individuals and replication within genotypes. All random and fixed effect covariates were included in the global model; I then refitted all possible reduced models by sequentially removing fixed effects (refer to Appendix A3.2 for model structures), from which the best explanatory model for the data was assessed using the I-T approach (see below). Normality of model residuals was confirmed by visually inspecting normal probability plots, while homogeneity of variances was confirmed by plotting residuals versus fitted values (Faraway 2006). Models were fitted with Gaussian error and identity link functions.

3.2.4.5 Mixed model selection and inference

I assessed model goodness of fit for each of my candidate models using the I-T approach as described by Burnham and Anderson (2002; 2004). Models were compared based upon AICc (for small sample sizes), by calculating delta AICc (Δ_i) and Akaike weights (w_i) for each respective model (Burnham and Anderson 2002; Burnham and Anderson 2004). If model weightings revealed no single best candidate model (w_i <0.9) I implemented model averaging of estimates and standard error across models <4 Δ_i of AIC_{min}, as described by Burnham and Anderson (2002; 2004) using the MuMln package (Bartoń 2009). In addition I calculate unconditional 95% confidence intervals around estimates, with confidence intervals excluding zero being considered as significant at the 0.05 α level (Nakagawa and Cuthill 2007).

3.3 Results

30 adult hermaphrodites and 30 adult males were successfully assayed for all three behaviours at both sample points (N=60). When I investigated repeatability of behavioural expression for both male and hermaphrodites combined, I find that boldness and exploration were repeatable at the individual level highlighting that adults of this species exhibit personality. Interestingly, these individual level BTs were not documented at the genotype level (table 3.2). Aggression, in contrast was repeatable at both the individual and genotype levels (table 3.2). When males and hermaphrodites were investigated separately, repeatability estimates showed the same patterns for each sex, with boldness and exploration being repeatable at the individual level and aggression at both the individual and genotype level (table 3.2). Further analysis revealed that there were no significant differences between each sex's repeatability estimates for either exploration or aggression (table 3.3). In contrast I observed sex

differences in boldness repeatability with males exhibiting significantly higher repeatability estimates when compared to hermaphrodites (table 3.3).

Table 3.2. Repeatability estimates (R), p values and 95% confidence intervals for all data combined (overall) and each sex separately at the individual and genotype level for exploration, boldness and aggression.. Estimates denoted in bold script indicate significant repeatabilities, $N_{individual}$ =60, $N_{genotype}$ = 10.

	Behavioural type	**Level**	**R**	***P***	**95%CI**
Overall	Exploration	ID	**0.438**	**0.001**	**0.208-0.627**
		Genotype	0.07	0.105	0.000-0.234
	Boldness	ID	**0.697**	**0.001**	**0.587-0.771**
		Genotype	0.031	0.214	0.000-0.165
	Aggression	ID	**0.591**	**0.001**	**0.405-0.772**
		Genotype	**0.335**	**0.001**	**0.067-0.574**
Male	Exploration	ID	**0.392**	**0.007**	**0.061-0.659**
		Genotype	0.13	0.063	0.000-0.396
	Boldness	ID	**0.865**	**0.001**	**0.741-0.934**
		Genotype	0.25	0.019	0.000-0.507
	Aggression	ID	**0.572**	**0.001**	**0.284-0.763**
		Genotype	**0.307**	**0.006**	**0.067-0.566**
Hermaphrodite	Exploration	ID	**0.488**	**0.002**	**0.147-0.725**
		Genotype	0.016	0.333	0.000-0.124
	Boldness	ID	**0.691**	**0.001**	**0.443-0.848**
		Genotype	0.163	0.048	0.000-0.431
	Aggression	ID	**0.613**	**0.001**	**0.325-0.790**
		Genotype	**0.339**	**0.001**	**0.018-0.598**

Table 3.3. Mean difference between hermaphrodite and secondary male Z-transformed repeatability estimates at the individual and genotype level, and their respective 95% confidence intervals (CI). A positive value is found when males exhibited a higher repeatability score compared to hermaphrodites and a negative value is found when hermaphrodites exhibited a higher repeatability score than males. Only those mean differences denoted in bold script represent differences that were significant at the 0.05 alpha level as denoted by non-overlapping 95% CIs.

Hermaphrodite -Male	**Mean Difference**	**95%CI**
Exploration (Individual)	-0.119	-0.254 - 0.016
Exploration (Genotype)	0.114	-0.020 - 0.250
Boldness (Individual)	**0.463**	**0.328 - 0.598**
Boldness (Genotype)	0.091	-0.044 - 0.226
Aggression (Individual)	-0.063	-0.198 - 0.072
Aggression (Genotype)	-0.036	-0.171 - 0.099

When investigating sex differences in average BTs expressed by each of the sexes, results suggest that two competing models were equally good at explaining exploration data. These models contained the population intercept (null model) and the fixed effect of sex, respectively (Appendix A3.2). Boldness was also explained by two competing models, again represented by the null model and fixed effect of sex, respectively (Appendix A3.2). Genotype was absent from both exploration and boldness competing models, suggesting that there were no difference between genotypes in these BTs (Appendix A3.2). Following model averaging for both exploration and boldness separately, the estimated coefficients were found to be similar for each sex and confidence intervals spanned zero (table 3.4, also refer to figure 3.3a and b). I can thus conclude that the sex of the focal fish had minimal effect upon my measure of either exploration or boldness.

Aggression was explained by 4 competing models containing genotype, the null model, sex plus genotype, and sex, respectively (Appendix A3. 2). This suggests that genotype had a large influence upon aggression BTs, whereas sex had some, although minimal influence. Average model estimates further support this interpretation; in particular hermaphrodites and males had similar aggression scores highlighted by zero spanning 95% CIs (table 3.4, also refer to figure 3.3c). In contrast two genotypes were found to be more (genotype 9) and less (genotype 8) aggressive respectively (table 3.4, figure 3.4).

Table 3.4. Model averaged exploration, boldness and aggression estimates for parameters of importance identified by the IT approach (estimates = effect size in comparison to the reference sex or genotype i.e. males compared to hermaphrodites and each genotype compared to genotype 1). Effects and confidence intervals denoted in bold script represent non-zero CI's and thus significant at the 0.05 α level, $N_{individual}$ =60, $N_{genotype}$ = 10.

	Estimate	**SE**	**Lower CI**	**Upper CI**
		Exploration		
Intercept	0.04	0.17	-0.284	0.375
Male	-0.31	0.20	-0.703	0.08
		Boldness		
Intercept	96.6	8.68	79.6	114
Male	0.50	15.3	-29.5	30.5
		Aggression		
Intercept	0.66	0.08	0.50	0.81
Genotype: 2	0.02	0.10	-0.18	0.21
Genotype: 3	-0.29	0.18	-0.64	0.06
Genotype: 4	-0.09	0.14	-0.35	0.18
Genotype: 5	-0.01	0.11	-0.23	0.20
Genotype: 6	0.08	0.11	-0.14	0.29
Genotype: 7	-0.23	0.14	-0.50	0.04
Genotype: 8	**-0.48**	**0.11**	**-0.69**	**-0.26**
Genotype: 9	**0.48**	**0.18**	**0.13**	**0.83**
Genotype: 10	-0.17	0.18	-0.52	0.18
Male	0.06	0.06	-0.06	0.18

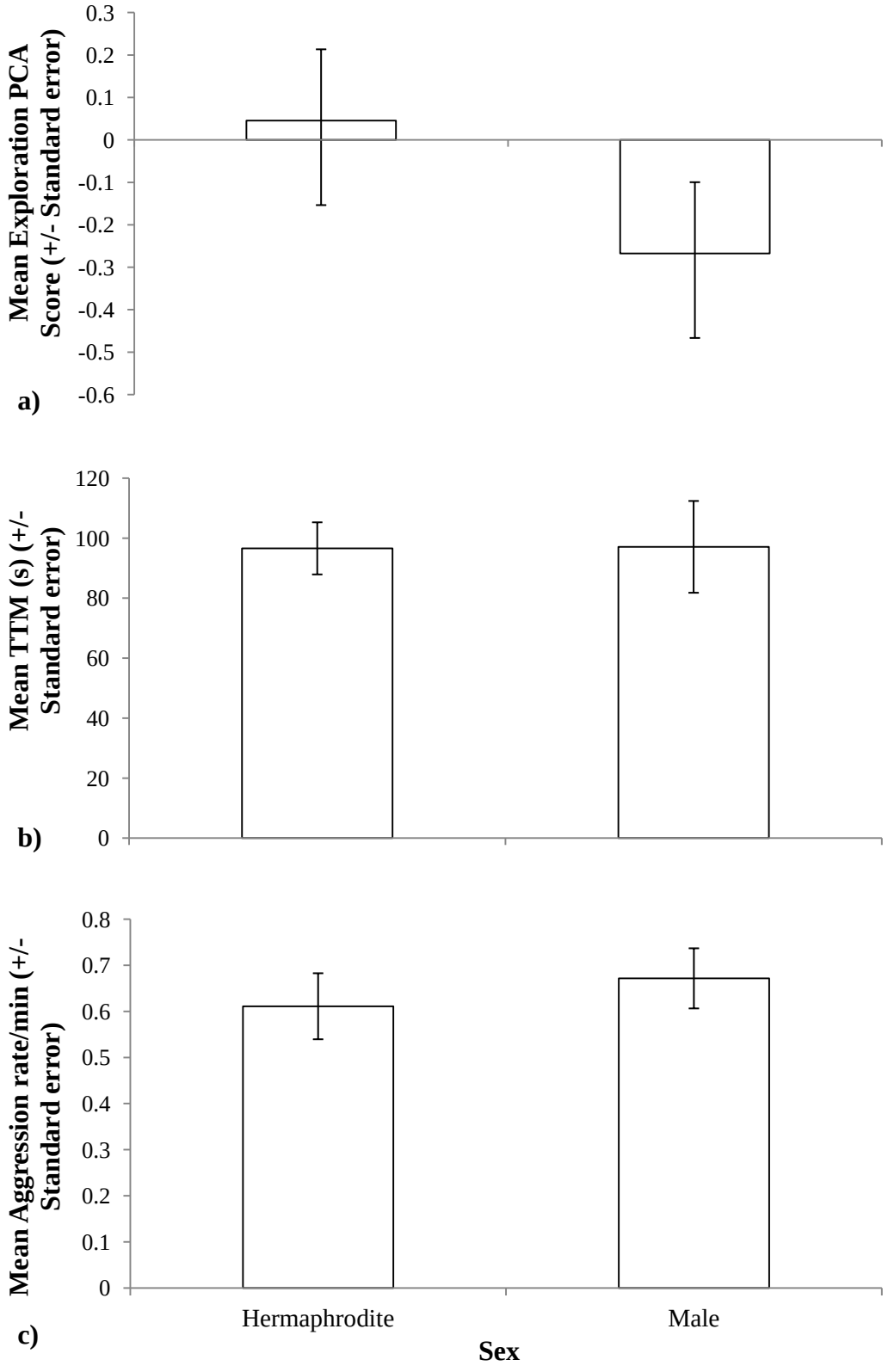


Figure 3.3. Bar charts presenting a) mean exploration PCA scores b) mean TTM (s) (boldness score) and c) mean aggression rate per minute for secondary males and hermaphrodites. Error bars represent standard error, $N_{individual}$ =60, $N_{genotype}$ = 10

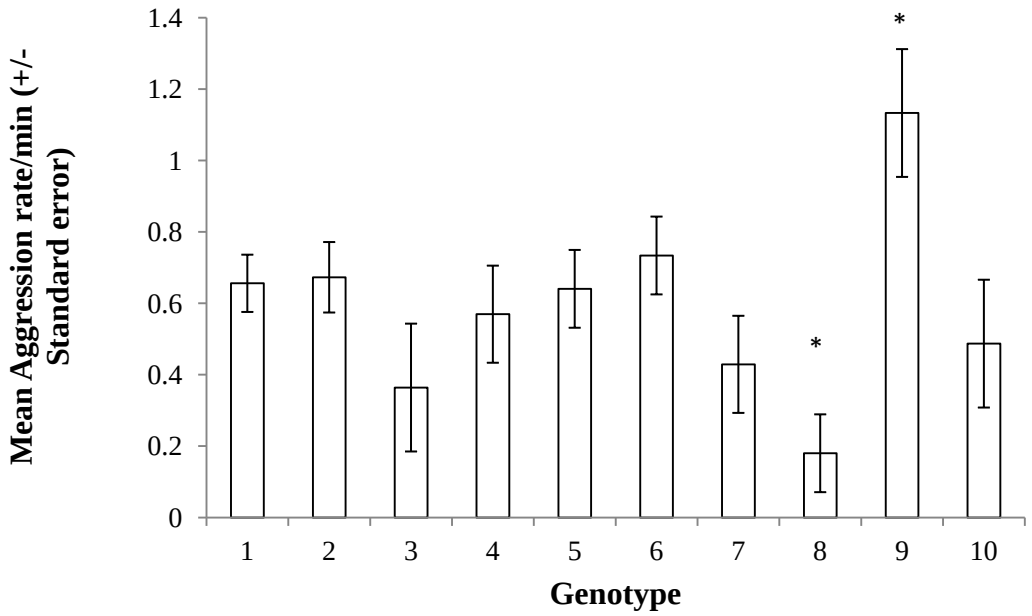


Figure 3.4. Bar charts presenting mean aggression rate per minute for each genotype. Error bars represent standard error and * represents genotypes that had 95% CI's that did not span zero i.e. differed significantly at the 0.05 alpha level in comparison to the reference genotype (genotype 1) for mean aggression rate per minute. $N_{individual}$ =60, $N_{genotype}$ = 10

3.4 Discussion

The mangrove killifish presents a particularly exciting model organism because it is possible to control for genetic variation within a single genotype (i.e. explore genotype specific behavioural expression) and replicate across genetically independent genotypes (i.e. explore general behavioural patterns). In addition, the presence of sequential hermaphroditsm in this species presents an exciting opportunity to begin to understand how sex differences, irrespective of genetic architecture, influence behaviour. In the animal personality literature sex differences have been attributed to multiple factors (when they are observed) including body size (Brown and Braithwaite 2004) and hormonal influences (see Archer 1991; Nelson and Chiavegatto 2001; Nelson and Trainor 2007 for reviews). One issue that is difficult to resolve, however, is that when sex differences in personality are documented the organisms under investigation are sexually reproducing species. Within these species one of the sexes is usually

heterogametic (i.e. males in mammals or females in birds). The presence of heterogamy makes it particularly difficult to determine if observed sex differences in behaviour are driven by multiple sex-linked genes or whether specific hormonal influences are driving the observed patterns. In this study, I controlled for sex differences in genetic structure and body size. Moreover, males have been documented to secrete higher circulating 11-ketotestosterone compared to hermaphrodites (R. Earley personal communication). Controlling for genetics and size in this species therefore permits the direct investigation of how differences in androgens influence the expression of personality between the sexes.

Interestingly I found that average exploration, boldness and aggression did not differ between the sexes, Although average behaviour expressed was not sex dependent, both males and hermaphrodites were found to exhibit short term repeatability (i.e. over the course of 1 week) at the individual level, indicating individual consistency (i.e. rank order was maintained). This result suggests that adults of this species exhibit personality (i.e. the presence of BTs), at least in the short term. In contrast, at the genotype level only aggression was repeatable. When I investigated differences between the sexes in their repeatability estimates, both secondary males and hermaphrodites were found to exhibit similar repeatability estimates for both aggression and exploration. Interestingly, boldness was significantly more repeatable in secondary males compared to hermaphrodites suggesting that high boldness consistency may be an important component of secondary male personality.

Testosterone has been suggested as a driver of sex differences in aggression in humans and other mammals (see Archer 1991; Nelson and Chiavegatto 2001; Nelson and Trainor 2007 for reviews). Furthermore, protogynous sequential sex determination

has been shown to be driven by androgens, specifically 11-ketotestosterone, in multiple fish species (see Devlin and Nagahama 2002 for a review). Although, I did not directly measure androgens, I expected that elevated 11-ketotestosterone secreted in males of this species in comparison to hermaphrodites (R. Earley Personal communication) would result in an increased aggression, boldness and exploration scores, because these BTs tend to be correlated in multiple species (Sih et al. 2004b). *K.marmoratus* hermaphrodites, however, possess ovotestis containing both testicular and ovary tissues (Sakakura et al. 2006) and secrete both 11-ketotestosterone and oestrogens simultaneously in a balance between spermatogenesis and oogenesis (Minamimoto et al. 2006). Furthermore, hermaphrodites of this species are recognised to be, in general, highly aggressive and have been used as a model organism in aggression research (Earley and Hsu 2008; Earley et al. 2000a; Hsu et al. 2009; Hsu and Wolf 1999). The presence of some circulating 11-ketotestosterone in hermaphrodites may therefore be sufficient to explain BT similarities between the sexes observed in this instance, especially if small concentrations of testosterone have a large influence upon these behaviours. Further work directly investigating sex specific hormonal levels and their resulting effects upon aggression and correlated BTs would therefore be rewarding in the future.

I documented behavioural consistency at the individual level suggesting that adults of this species exhibit BTs i.e. personality. In contrast, at the genotype level repeatability of behaviour was not apparent for either exploration or boldness, which was related to high within genotype variation. These results mirror those of a recent study using a clonal insect, the pea aphid (*Acyrthosiphon pisum*) (Schuett et al. 2011) who documented individual but not genotype level BTs. In particular, Schuett et al (2011) documented that each of their studied genotypes differed in the proportion of

BT's produced, indicating that these traits were not genetically fixed in a classic sense, instead it was proposed that response rules may be genetically determined (sensu Houston and McNamara 1999). When I consider that in the current investigation individuals were reared in a social context, within genotype variation may be adaptive in the context of frequency-dependent selection (Bergmuller and Taborsky 2010; Dall et al. 2004). An alternative explanation is that intra-genotype variation could be driven by non-adaptive mechanisms. For example, recent theoretical work using artificial neural networks, has led some theorists to propose that inter-individual differences in behaviour may result from stochastic variation in the stimuli animals experience in early life, which has been termed the "Path Dependence Hypothesis" (Ghirlanda and Enquist 2007; Tosh and Ruxton 2007). Further work exploring within genotype variation in response to differing social environments and experiential effects during ontogeny would therefore be intestesting.

At the genotype level I observed significant repeatability estimates for aggression as well as significant differences between genotypes in their average aggressive tendency. This suggests that there was greater between compared to within-genotype variation for this BT. This species has been widely documented to be aggressive (Earley and Hsu 2008; Earley et al. 2000b; Hsu et al. 2009; Hsu and Wolf 2001; Hsu et al. 2006) and thus significant and consistent differences between genotypes indicates a strong genetic component influencing aggression tendency. In this instance genotype-specific aggression could be related to the serotonergic system. It is well documented that the serotonergic system influences aggression in many species, with multiple examples documented in vertebrates (see Carillo et al. 2009 for a discussion). Moreover, multiple genes have been associated with the serotonergic system and their resulting effects upon aggressive behaviour have been documented

(see Miczek et al. 2001 for examples). Moreover, within humans, length variation in single nucleotide polymorphisms (SNP), in and around the coding regions of the serotonin transporter have been linked to personality variation (Veenstra-VanderWeele et al. 2000). Future work exploring whether differences in aggression are driven by genotype specific differences in serotonin and SNPs would therefore be an exciting avenue of further research.

Repeatability in males was significantly higher than repeatability estimates observed in hermaphrodites. In contrast, there were no significant differences in repeatability in aggression or exploration. While our knowledge of male-hermaphrodite reproductive behaviour in this species is limited, it has been suggested that sexual selection may influence male consistency, if being consistent is a reliable cue upon which females choose potential mates (Schuett et al. 2010). In addition, in guppies boldness has previously been shown to correlate with colour in males. Moreover, females preferred to mate with colourful males and bold males (irrespective of colour) (Godin and Dugatikin 1996). It is therefore possible that boldness and colour are both potential indicators of quality that influence female mate choice (Godin and Dugatikin 1996). The documentation of higher secondary male boldness consistency within this study could therefore indicate the presence of an honest indicator of male fitness that may be governed by sexual selection. While this is possible, I stress that this requires, further work to explore male-hermaphrodite sexual interactions and the traits used during hermaphrodite mate choice.

Unfortunately age variation effects upon BT expression could not be investigated within this experiment because all fish were housed in group aquaria at hatching until 650 days of age (i.e. mixing of ages occurred), and were therefore not

individually identifiable until isolation protocols were implemented. I do not, however, expect age variation to have had a large influence upon behaviour in this study. This is because fish were repeatable in their behaviour over a seven day period (half the age variation between fish).

In conclusion the results of this study suggest that adult secondary males and hermaphrodites of this species show individual level BTs i.e. personality. Moreover, average BT expression is similar between the sexes when genotype and size are controlled. I found limited genotype level repeatability resulting from high within-genotype variation in BTs, potentially resulting from "path dependence" effects or from socially mediated frequency-dependent selection within the social rearing environment. Males were additionally found to be more consistent in their boldness but not aggression or exploration when compared to hermaphrodites. This supports recent proposals relating sexual selection for BT consistency, quantifying the role of personality traits in relation to sexual selection within this species therefore provides an exciting avenue of future research.

Research within the animal personality literature has focused primarily upon adult organisms. Results from adult personality studies are in general similar to those reported in this chapter i.e. individual level repeatabilities documented (Bell et al. 2009), however, relatively little work has investigated animal personality within a developmental framework (but see Bell and Stamps 2004 for examples; Brodin 2008; Sinn et al. 2008). Ontogeny also presents an exciting opportunity to address key predictions arising from state dependent models for the evolution and maintenance of consistent individual differences in BT expression. For example, the main predictions originating from state perspectives of BTs predict that fast growth will generate

exploratory, bold aggressive BTs (Stamps 2007) and that earlier age at first reproduction will generate bold exploratory and aggressive BTs (Wolf et al. 2007). In the next chapter I address the predictions of both and Stamps (2007) and Wolf et al. (2007b) within a developmental approach. Specifically I investigate residual reproductive potential and growth rates as potential drivers of BT expression and their influence upon consistent inter-individual differences in BTs.

Behavioural Traits and Life-History Strategies During Ontogeny in the Mangrove Killifish

Abstract

Consistent differences in behaviour, termed behavioural types (BTs), are well documented in the animal kingdom. Relatively little is known, however, about how, why and when consistency is maintained within populations. In recent years, theoretical work suggests that life-history trade-offs may be an important mechanism driving the maintenance of inter-individual variation in BTs. In this study I use a laboratory population of a clonal vertebrate: the mangrove killifish (*K.marmoratus*) as a model organism. *K. marmoratus* is an internally self-fertilising simultaneous hermaphroditic fish that exhibits within genotype homozygosity. I utilise 20 genotypes to examine behaviour repeatability, behavioural developmental plasticity (boldness and exploration), the development of behavioural correlations, and relationships between life-history strategies and behaviour at five age points during ontogeny. I find that during ontogeny repeatability within each genotype was absent. Moreover, I document that on average, behavioural expression increases during ontogeny and asymptotes during sexual maturity. I also find considerable variation in behavioural developmental plasticity at the genotype level. Moreover, although genotypes exhibit high levels of developmental plasticity and no within genotype repeatability, strong, significant positive correlations between exploration and boldness emerge from day 61 onwards. Furthermore, I observe no difference between genotypes in growth rate and growth was unrelated to behaviour. Contrary to my predictions, I find that while genotypes differ in their age at first reproduction and reproductive output, these differences were unrelated to behavioural expression prior to and following sexual maturity. I discuss these results in relation to mechanisms proposed to drive inter-individual variation in personality and highlight the potential of the mangrove killifish as a model organism for animal personality studies focusing on phenotypic plasticity.

4.1 Introduction

Historically, behavioural ecologists have considered behavioural variation among individuals to be scatter surrounding an optimum mean for a given species or population. Research investigating this variation has documented that, within contexts, individuals tend to be temporally consistent in their behavioural responses i.e. some individuals are consistently bold while others are consistently shy (Bell 2007; Reale et al. 2007). The term behavioural type (BT) (Bell 2007) has become commonly used to define these consistent individual differences, which are analogous to personality (Sih et al. 2004a). Furthermore, multiple BTs have been identified in non-human animals and these include boldness (Coleman and Wilson 1998; Wilson et al. 1993), aggressiveness (Huntingford 1976), reactivity (Koolhaas et al. 1999), neophobia (Cavigelli and McClintock 2003) and exploration (Verbeek et al. 1994) .

In non-human animals, correlated suites of BTs across contexts and correlations between different behaviours have been referred to as 'behavioural syndromes' (Sih et al. 2004a; Sih et al. 2004b). As a phenomenon, behavioural syndromes have been observed in multiple species across a wide range of taxa with examples found in both vertebrates (Bell 2005; Bell and Sih 2007; Dingemanse et al. 2003; Dingemanse and de Goede 2004; Drent et al. 2003; Reale and Festa-Bianchet 2003; Sih et al. 2003; Stapley and Keogh 2005; Webster et al. 2009; Wilson and Stevens 2005) and invertebrates (Johnson and Sih 2005; Sih and Watters 2005; Sinn et al. 2008; Sinn and Moltschaniwskyj 2005). The most widely studied behavioural syndrome is the bold-exploratory syndrome; in which bold individuals tend to be more exploratory, suggesting that these BTs have become linked throughout evolution (Sih et al. 2004b). Research also indicates that behavioural syndromes may be related to fitness (Cote et al. 2008; Dingemanse et al. 2004; Pruitt et al. 2008; Smith and Blumstein 2008; Smith and

Blumstein 2010) and BTs underpinning behavioural syndromes have been documented to be heritable in some species (Dingemanse et al. 2002; van Oers et al. 2004c; van Oers et al. 2004b), although exceptions for some BTs are also evident (e.g. Sinn et al. 2006). Despite the widespread occurrence of BTs and behavioural syndromes across a range of taxonomic groups, relatively little is known about what selects for behavioural syndromes, why individuals exhibit such consistency in BTs, why and when BTs vary across individuals, and how variation is maintained (Sih et al. 2004b). In recent years, it has been suggested that correlation selection (Sih et al. 2004a) or the same genes or hormones acting on several targets (pleiotropy) (Ketterson and Nolan 1999) may constrain plasticity. More recently, life-history trade-offs have been proposed as potential drivers of consistent inter-individual variation in behaviour (Stamps 2007; Wolf et al. 2007b).

It is well documented that life-history strategies vary between individuals (see Biro and Stamps 2008 for a review of the literature; Stamps 2007; Stearns 1992) and thus life-history variation has the potential to underpin individual variation in BTs. Growth-mortality trade-offs in particular have been proposed as mechanisms for BT variation, with variation in innate growth rate suggested to generate systematic differences in behaviour (Stamps 2007). For example, based upon Stamps' (2007) model we may predict fast growing individuals to be more active, exploratory, competitive, and risk prone. This is because these BTs may facilitate food acquisition and are expected to permit individuals to sustain a higher growth rates than less active individuals (Stamps 2007). In addition to growth as a driver of BT differences, current vs. future fitness tradeoffs (residual reproductive potential) have also been proposed to underpin BTs and behavioural syndromes (Wolf et al. 2007b). For example, we could predict individuals with higher residual reproductive potential to be more responsive to

risk to protect their acquired assets (Clark 1994). There is tentative support for both these hypotheses in the literature (see Huntingford and Adams 2005). For example, studies using domesticated fish that have been selected for fast growth tend to be on average bolder (Sundstrom et al. 2004), more aggressive (Metcalfe et al. 2003) and risk prone (Biro et al. 2004; Biro et al. 2006; Biro and Post 2008) compared to wild individuals. However, the cause-effect relationship underpinning these patterns remains unclear, and exceptions have been documented (Laakkonen and Hirvonen 2007). With regard to current vs. future fitness trade-offs, Careau et al. (2009) found that inter-specific exploration (thoroughness) was positively correlated with age at first reproduction in a comparative study of 19 muroid rodent species. However, intra-specific empirical support regarding this relationship is still lacking in the literature, but potential links between residual reproductive potential and BTs are likely (see Careau et al. 2008 for a discussion).

In the current investigation I use a laboratory population of the mangrove killifish (*Kryptolebias marmoratus*) as a model organism to examine behavioural repeatability and plasticity during 151 days of development. In addition, I investigate the development of behavioural correlations and relationships between life-history strategy and behaviour during ontogeny. *K. marmoratus* is an internally fertilising simultaneous hermaphroditic fish species (Taylor et al. 2001) and considered as one of two known isogenic vertebrates (Tatarenkov et al. 2009). This species presents a powerful model organism for behavioural studies because it is possible to replicate at genotype level. Previous work has demonstrated that *K.marmoratus* exhibits genotype variation in life-history traits such as growth rate, age at first reproduction, and egg laying rate (Grageda et al. 2005). This genotype level life-history variation therefore presents an exciting model organism with which I can explore relationships between

life-history strategies and behaviour. In addition, as outlined in Chapter 3 I have previously shown that adult individuals are repeatable in their behaviour suggesting this species exhibits personality i.e. BTs. I focused upon two commonly studied BTs; exploration of a novel maze and boldness following an aerial predation simulation (where a suspended weight is released, breaking the water surface). Based upon previous research I predict that (1) behaviour will be consistent during ontogeny indicating BTs/personality (i.e. Brodin 2008), (2) behavioural developmental trajectories will be similar between genotypes, (3a) genotypes will exhibit different average behavioural scores and (3b) different life-history strategies (Grageda et al. 2005), (4) BTs will correlate at each developmental stage (i.e. Bell and Stamps 2004), and finally (5) life-history variation will correlate with BTs (i.e. Stamps 2007; Wolf et al. 2007b). In particular I predict that growth will correlate positively with both exploration and boldness at each stage of development. In addition, I predict that age at first oviposition would correlate negatively with both exploration and boldness prior to and following sexual maturity, while total number of eggs laid would correlate positively.

4.2 Methods

4.2.1 Study species

K. marmoratus is an inshore dwelling species with a widespread geographic distribution throughout Central/South America and Florida, that is congruent with the red mangrove (*Rhizophora mangle)* (Taylor et al. 2008b). The majority of individuals in wild populations are hermaphroditic, possessing ovotestis (Harrington 1963; Sakakura et al. 2006). Repeated self-fertilisation across generations has given rise to natural populations that are isogenic; formed of homozygous genotypes (Harrington 1963; Kallman and Harrington 1964), although out-crossing events in the laboratory between

hermaphrodites and primary (gonochoristic) males have been documented (Mackiewicz et al. 2006a; Mackiewicz et al. 2006b; Mackiewicz et al. 2006c). This species is commonly found in crab burrows, moist leaf litter, decaying mangrove logs, streams, and ditches (Taylor 2000). Owing to these cryptic habitat preferences, relatively little is known about this species in wild situations (but see Taylor 2000 for a review), with the majority of our current behavioural knowledge arising from studies using captive populations (for example Earley and Hsu 2008; Earley et al. 2000b; Hsu et al. 2009; Hsu and Wolf 2001; Hsu and Wolf 1999; Martin 2007). In captivity *K. marmoratus* reach sexual maturity between 3 and 6 months of age, at approximately 17mm total length (Sakakura and Noakes 2000) and live for approximately 5 years. This species produces fertilised eggs year round with no specific oviposition cycle (Harrington 1963).

In the current study I used individuals from 20 different genotypes (6 individuals per genotype). Genotypes were obtained from two sources; 11 genotypes supplied from Valdosta State University, Georgia, USA that have been captive bred across multiple generations. These fish were descendents of founder individuals collected from 4 geographical locations. The remaining 9 genotypes were collected from wild Florida populations in 2007 and have been maintained in a laboratory setting since collection. Individuals from the Florida genotypes used in this study were representatives of the F_2 generation. For all housing and experimental protocols fish were maintained in 20ppt synthetic salt water (Instant Ocean^{TM}) and at 25˚C ± 0.5˚C in a 12hr light 12hr dark cycle.

4.2.2 Egg collection procedure

'Breeding' individuals (1 per genotype) were individually housed in aquaria (21x13x13cm internal measurements) containing 1 litre of water and fed ad-libitum with frozen adult artemia (*Artemia salinas*). Breeding tanks were inspected twice daily (07.00 and 15.00) for eggs and entire clutches were transferred to transparent 8cm diameter petridishes containing 50ml of salt water. Eggs were inspected daily to monitor for the occurrence of fungal/bacterial infection as well as to identify eggs failing to undergo embryogenesis. Eggs that perished or showed no development after seven days were removed and discarded.

4.2.3 Housing of experimental fish

From hatching to 90 days of age, fish were individually housed in 14 x 7.5 x 10cm aquaria (internal measurements) containing a sparse layer of 2-4mm gravel and 750ml of water and visually isolated using opaque dividers. From days 90-151 individuals were transferred to larger aquaria (28 x 17.5 x 16cm) containing a sparse layer of 2-4mm gravel and 2 litres of synthetic salt water. Each individual was randomly assigned to numbered aquaria and these were randomly positioned on 2 racks containing 5 shelves to control for any aquaria/rack/or shelf effects. Complete water changes were carried out at 15 day intervals. The sparse housing conditions were chosen to represent one of the many habitat types that this species inhabits i.e. small unstructured pools (Taylor 2000). This rearing environment was therefore relevant to the ecology of this species, while also permitting health and welfare to be easily monitored and husbandry protocols be completed.

Fish were fed between 13.00-15.00 on a daily basis and food type was dependent upon fish age. For days 1-3 individuals were fed micro-worms (*Panagrellus*

redivivus) daily (0.1ml of a 1mg/100ml suspension (average number of worms 23.3±2.6)). For the remainder of the study individuals were fed artemia naupili (average: 35.9±3.9 artemia/0.1ml). From day 3 individuals were provisioned 0.2ml of artemia which was subsequently increased by 0.1 ml at fifteen day intervals for the remainder of the study. To control for any order effects due to feeding, feeding order was randomised daily.

4.2.4 Life-history measurements

Upon hatching, and at 30 day intervals, individuals were transferred to a photographing chamber and photographed laterally using a Nikon D60 SLR (18-50mm zoom lens). From the photographs I measured each individual's standard length (tip of the lower jaw to the posterior margin of the hypleural plate) using ImageJ photo analysis software v1.42. At 105 days of age and for the remainder of the study (46 days), tanks were inspected daily for oviposition between the hours of 9.00 and 11.00. During inspections, individuals were removed from their home tank and placed in a small opaque beaker containing approximately 50ml of home tank water. Gravel was agitated to dislodge eggs, which were counted, removed and discarded. For each individual I recorded their age at first laying, and total number of eggs laid.

4.2.5 Behavioural assays

Six replicates from each of the 20 genotypes were assayed for exploration and boldness at five age points during development (days 2, 31, 61, 91 and 151). During the study mortality was low with four individuals dying during ontogeny out of the total sample size of one hundred and twenty individuals. Linear mixed modelling approaches have the ability to handle unbalanced data and thus if each of these four individuals were successfully assayed within a minimum of two age samples (i.e. days 2 and 31)

they were retained within the analysis. Only one individual from genotype 7 died after day 2 and prior to day 31 and this individual was excluded from all subsequent analyses.

In this study I was interested in both behavioural plasticity and repeatability throughout development and thus it was important to limit variation resulting from experimental sequence effects that may have influenced within-individual variance/plasticity. Fish were therefore randomly assigned to an assaying order on the day of hatching and this order was maintained for the remainder of the study. Fish were assayed first for exploration and then for boldness (separated by a minimum of 3 hours) at each age point throughout the study. Trials were video recorded using a Sony ExwaveHad video camera. Ethovision XT version 6.0 (Noldus Information Technology) was used to track each fish's movements and water was changed between each behavioural assay. To limit structural novelty in response to the boldness test arena, I manipulated each test fish's home tank 24 hours prior to both exploration and boldness assays so that it resembled the boldness test arena. This was completed by removing gravel and inserting removable structural components (mesh partition and white gravel disc) in the same configuration as shown in figure 4.1b. One limitation of using Ethovision software is that calibration of the test arena to the video feed is necessary prior to commencement of a trial. Due to the large number of behavioural assays conducted I was unable to assay boldness within the home tank i.e. with structural components inserted. This was because recalibration of Ethovision software to each home tank would have added approximately 10 minutes per trial, adding ~40 hours to each sample point during ontogeny. A single test arena was therefore used and calibration was completed prior to the first assay and these calibrated settings were

monitored throughout each day of testing to ensure that settings were accurate for each assay completed.

Exploration was assayed using a white novel maze which increased in dimensions alongside fish development (see figure 4.1a for dimensions). Arena sizes at each sample point during development were chosen so that the area of each of the 24 zones (see below) were approximately twice the average body length of test fish. To maintain consistency the structure of the maze remained constant throughout the experiment (figure 4.1a), yet to generate novelty the position of the acclimatisation/release zone was altered to a different location for each subsequent trial, presenting fish with a unique starting view of the maze. The arena was divided in to 24 equal zones (excluding the acclimatisation enclosure). Fish were introduced to the test tank via the acclimatisation enclosure (containing a sparse layer of white gravel) which was surrounded by a white opaque barrier. Following a 5 minute acclimatisation period (during which time all fish regained activity) individuals were released by raising the opaque barrier. Behaviour was observed for a 10 minute period, during which time I recorded the distance travelled (mm), mean velocity (mm/second), duration of mobility (seconds) and the number of unique zones entered. At the end of each trial individuals were returned to their mock boldness home tank for a minimum period of 3 hours prior to boldness assays.

The boldness test tank was divided into two sections along the length (see figure 4.1b). The drop zone was constructed to allow a weight to be dropped (to simulate an aerial predation event) while being external to the trial arena (central zone). The inclusion of a separate drop zone ensured the weight never had the potential to strike and thus injure the test fish. Following introduction to the boldness arena all individuals

were given a 5 minute acclimatisation period (during which time all fish regained activity) after which time the weight suspended 13cm above the drop zone was released in to the arena breaking the water's surface. Following the simulated aerial predation event I recorded time (seconds) to first movement (TTM) (categorised as movement of >1 body length), distance travelled (mm) and mean velocity (mm/second) during a 5 minute observation period following the predation simulation. All fish regained activity within the five minute observation period, with the exception of five fish which did not move for the duration of the assay, these fish were given a maximum TTM score of 300 seconds. At the end of each trial, individuals were returned to their home tank containing gravel. Two test arena sizes were used for assaying boldness (see figure 4.1b for dimensions). These two sizes were chosen because they were equal in size to home tanks at the time of testing.

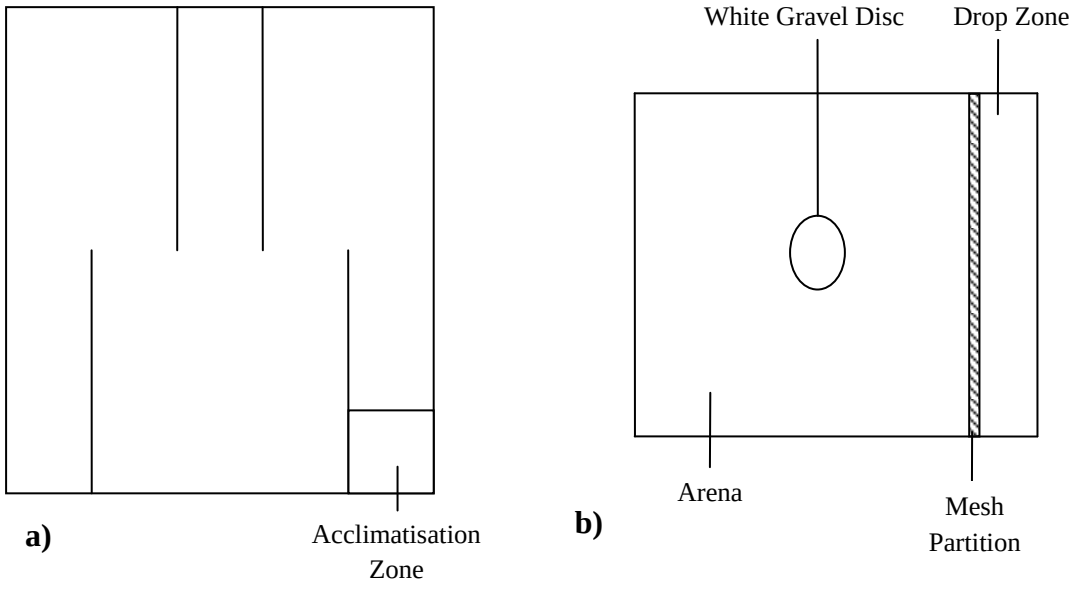


Figure 4.1. Schematic diagram of a) exploration trial arena (dimensions day 2: 7.5x 7.5 x 5cm, day 31: 15 x 15 x 10cm, day 61-151 30 x 30 x 15 cm) and b) boldness trial arena (14 x 7.5 x 10cm) incorporating a drop zone (separated by white mosquito netting) and a central trial arena (measuring 12 x 7.5 x 10 cm for Days 2-61 and 21 x 16.5 x 15.5 cm for days 91 -151). The mock boldness arena (manipulated home tank) was structurally identical to the boldness arena (as shown in schematic diagram b).

4.2.6 Statistical Analysis

4.2.6.1 Assessing the most appropriate measure of boldness behaviour

Within the animal personality literature multiple statistical approaches are utilised to quantify individual differences in behaviour. One common approach is to quantify multiple behavioural measures within a single assay. While this approach is common it also presents some potential statistical issues. For example, when each behavioural measure is analysed using separate statistical tests, the potential for type 1 error increases alongside the number of tests employed (Budaev 2010). This has led some researchers to opt for recording a single boldness measure per assay e.g. time to move (TTM) following a disturbance (e.g. Piyapong et al. 2010) or time to exit a refuge under risk (e.g. Brown and Braithwaite 2004; Chapman et al. 2011). In contrast, when multiple measures are recorded, as is commonly the case, an alternative and common approach is the use of Principal Component Analysis (PCA). PCA is a method that permits identification and reduction of correlated variables into composite scores (for each individual) that explain variation in the data. These individual PCA scores can be utilised as response variables in further analysis, minimising multiple statistical testing problems (see Budaev 2010 for a discussion).

In some studies, researchers have found that measures of boldness correlate with activity to form a single behavioural construct (e.g. Magnhagen and Borcherding 2008; Sinn and Moltschaniwskyj 2005; Smith and Blumstein 2010; Smith et al. 2009). In contrast, other researchers have analysed general activity and boldness separately (e.g. Bell 2005; Bell and Stamps 2004; Brydges et al. 2008), or found that activity and boldness load on to separate principal components i.e. explain different aspects of behaviour (e.g. Conrad and Sih 2009). The potential for activity and boldness being two aspects of unrelated behavioural expression therefore presents the need to determine

whether or not these behavioural characteristics of individuals may explain a single behavioural construct. In many cases this is simply completed using PCA analytical approaches (as described below), however, a criticism of this approach is that activity may inadvertently contaminate a pure boldness score (K.E. Arnold personal communication). In this chapter (see below) and also chapters 5 and 6, I observed that boldness and activity loaded on to single components, suggesting correlated expression of these measures. It is, however, important to clarify if further analysis using PCA scores (TTM + activity) qualitatively explains behavioural expression in a similar manner to a single pure measure of boldness (TTM). I therefore addressed this possibility using the same statistical approach for both PCA and TTM scores (see linear mixed modelling section below and appendix A4.1 for more details). In this analysis, PCA scores (TTM + activity) and my pure measure of boldness (TTM) were modelled as response variables in separate analyses to determine if results were qualitatively different. Briefly, the results of this analysis show qualitatively similar results regardless of whether PCA or TTM scores were modelled as response variables (see Appendix A4.1 for analysis results). I therefore utilise PCA scores that combine TTM + activity into a single composite measure in all boldness analyses from this point forwards (within this chapter and chapters 5 and 6).

4.2.6.2 Growth rate

Specific growth rate between each 30 day time point sample was calculated using the following formula (Priestley et al. 2006):

Specific Growth Rate [%] = $100 \times ((\ln SL_2 - \ln SL_1)/ t)$

Where SL_2 is the standard length at sample 2 ,SL_1 the standard length at sample 1, and t is the time in days between measurements (30).

4.2.6.3 Exploratory principal component analysis

I explored variance-covariance matrix similarities during the study for behaviours collected within each assay (distance moved etc) for both exploration and boldness using common principal component analysis (CPC) (for discussions see Arnold and Phillips 1999; Flury 1988, also see chapter 3 for a more detailed discussion; Phillips and Arnold 1999). I compared AIC values for each CPC model using the information-theoretic framework (I-T) (Burnham and Anderson 2002; 2004) (see below for I-T methods), and I find that covariance matrices for both exploration and boldness share a common principle component over all sample points (see table 4.1a). Bartlett sphericity and Kaiser-Meyer-Olkin (KMO) tests indicated that both exploration and boldness matrices were suitable for use in PCA (see Budaev 2010 for a discussion) (exploration: KMO= 0.745, Bartlett's test $p<0.001$ and boldness: KMO = 0.6, Bartlett's test $p<0.001$). A single PCA for all data collected throughout the course of the study period was therefore conducted for each behavioural assay, and I found that exploration and boldness measures both loaded strongly on to single components that explained high percentage of total variance (see table 4.1b). PCA scores were extracted from the analysis, for each individual at each age point, and these scores were use in subsequent analyses. CPC analysis was completed using the program CPC (Phillips 1998) while PCA analysis was completed using SPSS v 16.0.

Table 4.1. a) results of Common Principal Components Analysis (CPC) for boldness and exploration, model denoted in bold script represents the best fitting CPC model under the Information-Theoretic approach (CPC= common principal components i.e. equal sampling error across variance-covariance matrices, PCPC = partial common principal component i.e. share some but not all principal components, Proportional = matrix elements multiplied by a constant, unrelated = share no common principal components and Equality = complete equality between variance covariance matrices). AIC=Akaike's information criteria, deltaAIC = mean difference between each model and the best fitting and w_i= model weight (explanatory power of each model). b) PCA component loadings and % variance explained for boldness and exploration measures collected throughout ontogeny.

a)

Behavioural Assay	Model	AIC	Delta AIC	w_i
Exploration	**CPC**	**-17.762**	**0**	**0.91**
	PCPC(2)	-12.389	5.4	0.03
	PCPC(1)	-10.81	6.9	0.06
	Unrelated	80	97.7	0.00
	Proportionality	1210.052	1227.8	0.00
	Equality	1417.252	1428.0	0.00
Boldness	**CPC**	**-460.428**	**0**	**0.82**
	PCPC(1)	-457.377	3.0	0.18
	Unrelated	48	508.4	0.00
	Proportionality	308.92	769.3	0.00
	Equality	491.525	951.9	0.00

b)

Behavioural Assay	Behaviour Included	Component Loading
Exploration	Distance Travelled (mm)	0.940
	Unique Zones	0.826
	Mean Velocity (mm/s)	0.871
	Mobility Duration (s)	0.936
	% Variance Explained	**80.04**
Boldness	TTM (s)	-0.738
	Distance Travelled (mm)	0.967
	Mean Velocity (mm/s)	0.965
	% Variance Explained	**80.3**

4.2.6.4 Linear mixed modelling

I calculated repeatabilities for exploration and boldness for individuals within each genotype using ANOVA based repeatability estimates as described by Lessells and Boag (1987). Linear mixed modelling was completed using R for statistical computing v2.12.0 (R development core team 2010) using the lmer function within the package lme4 (Bates and Maechler, 2009). I found that age modelled as a linear covariate with a second order polynomial had better explanatory power than age modelled as a linear covariate only (w_i = 0.99). Using restricted maximum likelihood estimation, I investigated genotype level behavioural developmental trajectory plasticity during ontogeny for exploration and boldness (response variables) with age as a fixed, numeric second order polynomial covariate. Individual and genotype were additionally modelled as random intercepts to control for both repeated measures within individuals and replication within genotypes. In addition, I included age as a random slope effect; permitting developmental trajectories to vary by both individual and genotype (see table 4.2 for model structures). These random intercepts also allowed me to estimate between individual and between genotype variance for each behavioural assay. Furthermore, random slope effects allowed variation in developmental trajectories to be estimated for both individuals and genotypes (Mroczek et al. 2006). I began the analysis with a global model which included all random and fixed effect covariates (model1); reduced models were then refitted, from which the best explanatory model for the data could be determined. The first reduced model (model 2) was refitted without the fixed age covariate to explore overall effects of age (prediction 1). I then refitted a model without the random slope effect for genotype (model 3), to investigate whether genotypes varied in behavioural development during ontogeny (prediction 2). A final model was fitted (model 4) with genotype removed to explore genotype differences in behavioural expression (prediction 3a).

Mixed modelling was also implemented to investigate life-history measures with genotype and individual included as random intercepts and random slopes as described above. These random effects, however, exhibited limited variance in the global model (i.e. variance estimates were close to zero). I therefore fitted models with fixed effect parameters to test prediction 3b and maintained this random effect structure as a statistical control. To analyse growth rate (response variable), I included a linear fixed effect covariate of age (continuous) and a categorical fixed effect of genotype. Reproductive measures (age at first laying and total eggs laid) were also analysed in the same manner; however, only the fixed effect of genotype was included in these models. Normality of BT and life-history model residuals was confirmed by visually inspecting normal probability plots, while homogeneity of variances was confirmed by plotting residuals versus fitted values (Faraway 2006). The structure of each model is presented as R code in table 4.2, models were fitted with Gaussian error structure and identity link function.

4.2.6.5 Mixed model selection and inference

I assessed goodness of fit and model probability for each candidate model using the information-theoretic (I-T) approach as described by Burnham and Anderson (2002; 2004). The I-T approach utilises Akaike's information criteria (AIC) (Akaike 1974) to determine the best relative fitted model in a set of *a priori* constructed candidate models (global and reduced models) from which inferences can be made. Each of the 4 models were ranked based upon AIC (lowest AIC score denoting the best model) and delta AIC calculated. Delta AIC values estimate the relative expected differences between models, from which the level of empirical support can be deduced. In addition to delta AIC, I calculated Akaike weights (w_i) for each model to explore the probability of a model being the best fit to the data and thus containing covariates of

importance (Burnham and Anderson 2002; Burnham and Anderson 2004). For life-history models, I additionally calculated unconditional 95% confidence intervals around fixed effect estimates, with confidence intervals excluding zero being considered as significant at the 0.05 α level (Nakagawa and Cuthill 2007).

4.2.6.6 Correlation analysis

I calculated mean genotype behavioural scores and life-history measures at each age point sampled and these mean values were used in correlation analyses. Correlations between boldness and exploration, within sampling days (prediction 5) were investigated using two tailed Pearson's correlation (SPSS v16.0). To test for significant changes in correlation coefficients between age classes, I used the modified Pearson-Fillon statistic (ZPF) for correlated, non-overlapping correlations which is based upon Fisher's R-Z transformation (Raghunathan et al. 1996). Pearson's correlation was also used to investigate relationships between mean genotype behavioural scores and mean growth rate within each age class (prediction 6). In addition, mean genotype behaviour expressed on day 91 and mean age at first reproduction were investigated to explore how proximity to sexual maturity influenced these traits i.e. are genotypes that on average reproduce earlier, also bolder and/or more exploratory. Furthermore, I investigated correlations between mean genotype behavioural scores on day 151 and mean reproductive measures to explore whether reproductive output and age at first reproduction influenced behavioural expression following sexual maturity. To control for multiple tests I corrected alpha levels using the False Discovery Rate procedure (Benjamini and Hochberg 1995).

4.3 Results

4.3.1. Repeatability

Individuals within each respective genotype exhibited close to zero repeatability estimates for boldness and exploration across 151 days of development with the majority of these estimates being negative (see Appendix A4.2 and A4.3 for repeatability estimates). Negative or low repeatabilities were in general driven by high within-individual, compared to between-individual variation within each respective genotype (see Appendix A4.4 for variance estimates for each genotype) and can therefore be interpreted as zero repeatability estimates (Nakagawa and Schielzeth 2010). Zero repeatability estimates suggest that, during ontogeny, this species does not exhibit personality per se (i.e. BTs), however, variation in behavioural expression was present at each stage of development both between-individuals and between genotypes as can be seen in figure 4.2a and c.

4.3.2. Genotype differences and behavioural type variation

The I-T approach revealed that global models were considerably better at explaining the ontogeny of exploration and boldness behaviours (table 4.2). In particular, Akaike weights suggested that these global models had >99% probability of being the best fit to the data. I can therefore conclude that the inclusion of age as a fixed covariate as well as the random intercept for genotype and associated random slopes, explain the ontogeny of behaviour in this laboratory population. Explanatory parameters in these global models therefore allow inferences to be made. With regards to the fixed effect age covariate; I found that boldness and exploration scores increased during early ontogeny and plateau as fish mature (figure 4.2a). Exploration scores in particular exhibited a sharp increase during these early stages of development and begin to level at approximately 110 days of age (estimated coefficient = 12.4, S.E. = 1.13, 95%CI = 10.1

to 14.6, R^2 = 0.330) (figure 4.2a). Boldness scores in comparison showed a much slower rate of increase yet these scores also plateau at a similar age as exploration behaviours; close to 110 days of age (estimated coefficient = 10.64, S.E. = 1.47, 95%CI = 7.7 to 13.5, R^2 = 0.245) (figure 4.2c). From the random effect parameters I found that there was little variation in genotype intercepts for exploration (<1% of total variance) or boldness (1% of the total variance) i.e. no average genotype differences. In contrast, high variation between genotypes in exploration developmental trajectories (68% of total variance) (figure 4.2b) was observed, while individual developmental trajectories explained less variation (28% of the total variance). For boldness, I also found considerable variation between genotypes in boldness developmental trajectories (92% of the total variance) (figure 4.2d) and minimal individual developmental trajectory variance (5% of the total variance). These results indicate that genotypes exhibited variation in the rate at which exploration and boldness increase during early ontogeny (i.e. plasticity). In addition genotypes varied in both the timing of plateau and subsequent reduction in exploration and boldness scores, prior to and following maturity (see figure 4.2b and d). These patterns were additionally apparent from visual inspection of the genotype means for exploration and boldness measures and genotype R^2 values presented in Appendix A4.2 and A4.3. It is also evident from Figure 2d that boldness slopes cross at higher rates than exploration slopes and this is likely to be driving the higher genotype trajectory variance estimates for this behavioural trait. Together these results indicate that behavioural developmental trajectories are explained primarily by genotype variation during ontogeny.

4.3.3 Behavioural correlations

I observed weak, non-significant correlations between mean genotype exploration and boldness scores on days 2 and 31 (table 4.3) and these correlation

coefficients were not significantly different from one another (ZPF= 0.255, p= 0.399). A significant difference in correlation coefficients between days 31 and 61 (ZPF= -2.16, p= 0.015) was, however, documented suggesting a change in correlation structure occurred between these ages. This difference is mirrored by the emergence of strong significant positive correlations from day 61 onwards (table 4.3). Moreover, correlation coefficients were not significantly different between days 61 and 91 (ZPF= 0.774, p= 0.219) or days 91 and 151 (ZPF= -1.28, p= 0.100).

Table 4.2. Linear mixed model structures for all *a priori* models constructed for exploration scores, boldness scores, and life-history measures (growth rate, age at first reproduction and total eggs laid). Table includes each models respective AIC value, Relative AIC difference (Delta AIC), and Akaike weight (w_i). Model structures are written in R code format, for reference to structure a model example is provided above the table (1 denotes the population average i.e. removal of a fixed or random effect covariate). Models denoted in bold type represent the best fitting model with the highest likelihood and model weight) $N_{genotype}$ =20, N_{total} = 119.

Model Structure Example: Response Variable ~ Fixed effect covariate + (Random slope | Random intercept)

Model N^o	**Mod Structure**	**AIC**	**Delta AIC**	w_i
1 (Global)	**Exploration ~ Age + (Age | Genotype) + (Age | ID)**	**1430**	**0**	**0.98**
3	Exploration ~ Age + (1 | Genotype) + (Age | ID)	1438	8	0
4	Exploration ~ Age + (Age | ID)	1449	19	0
2	Exploration ~ 1 + (Age | Genotype) + (Age | ID)	1467	37	0
1 (Global)	**Boldness ~ Age + (Age | Genotype) + (Age | ID)**	**1468**	**0**	**0.99**
3	Boldness ~ Age + (1 | Genotype) + (Age | ID)	1486	18	0
2	Boldness ~ 1 + (Age | Genotype) + (Age | ID)	1492	24	0
4	Boldness ~ Age + (Age | ID)	1504	36	0
3	**Growth Rate ~ Age + (Age| Genotype/ID)**	**540**	**0**	**1**
4	Growth Rate ~ Genotype+ (Age| Genotype/ID)	575.3	35.3	0
1 (Global)	Growth Rate ~ Age * Genotype + (Age| Genotype/ID)	601.3	61.3	0
5	Growth Rate ~ 1+ (Age| Genotype/ID)	609.2	69.2	0
2	Growth Rate ~ Age + Genotype + (Age| Genotype/ID)	644.8	104.8	0
1 (Global)	**Eggs Laid ~ Genotype + (Age| Genotype/ID)**	**782.2**	**0**	**0.99**
2	Eggs Laid ~ 1+ (Age| Genotype/ID)	792	9.8	0
1 (Global)	**Age First Lay~ Genotype + (Age| Genotype/ID)**	**894.2**	**0**	**0.99**
2	Age First Lay ~ 1+ (Age| Genotype/ID)	912.8	18.6	0.00

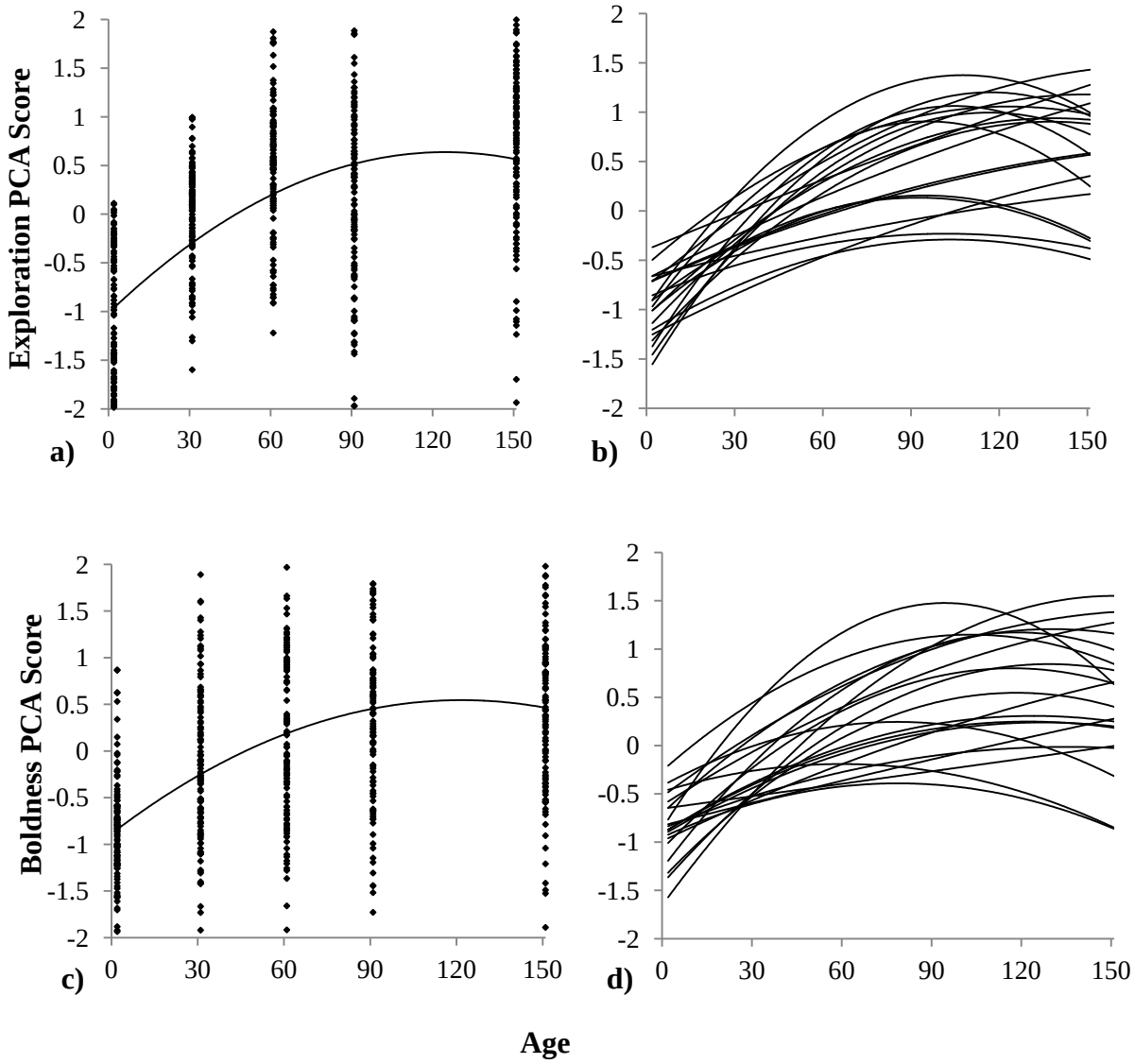


Figure 4.2. Developmental trajectories during ontogeny for (a) population-level and (b) genotype-level exploration PCA scores; each polynomial regression line represents one of the 20 genotypes sampled. (c) population-level and (d) genotype-level boldness PCA scores; each polynomial regression line represents one of the 20 genotypes sampled, $N_{genotype}$ =20, N_{total} = 119.

Table 4.3. Results of Pearson's correlations between mean genotype level exploration and boldness scores and their FDR corrected alpha levels, within each sampling day. Bold type represents significant correlations (significant correlations denoted in bold script).

Day	**N**	**r**	**p**	**FDR corrected alpha**
2	20	0.393	0.087	0.04
31	20	0.324	0.164	0.05
61	20	0.750	**<0.001**	**0.01**
91	20	0.637	**0.003**	**0.03**
151	20	0.814	**<0.001**	**0.02**

4.3.4 Differences in life-history parameters

I found that the fixed effect of age explained growth rate during development (model 3, table 4.2), whereas genotype and genotype by age interactions had no explanatory power (table 4.2). Model 3 (fixed effect of age only) was 35 delta AIC points lower than the next best model and Akaike weights suggest this model had 100% probability of being the best fitting to the data. I can therefore conclude that growth is age dependent; however, genotypes did not differ in their growth rates or growth trajectories. In particular, growth rate exhibited a shallow decrease throughout ontogeny (estimated coefficient = -0.0107, S.E. =0.0021, 95%CI = -0.01 to -0.006. R^2 = 0.477). This is further supported by genotype means for standard length which show similar sizes across all genotypes at each age sampled (see Appendix A4.5). Furthermore these means indicate that the greatest increases in standard length occurred early in development which begins to slow after approximately 60 days.

Age at first laying and total eggs laid were both explained by global models containing genotype as a fixed effect and this model had better explanatory power than the null model (see table 4.2). This suggests that genotypes differed in reproductive life-

history measures which are supported by the model estimates and 95% CIs i.e. non-zero spanning CIs represent significance at the 0.05 alpha level (see Appendix A4.6). In particular, I found that most of the genotypes differed from the reference genotype (genotype 1) having a later age at first laying and a lower number of eggs produced.

4.3.5 Life-history-behavioural type interactions during ontogeny

I observed no correlation between mean genotype level growth rates and either mean exploration or boldness scores (table 4.4). Furthermore, I found that prior to reproductive onset on day 91 (average reproductive onset = 125 days), mean boldness and exploration scores did not correlate with mean age at first oviposition (table 4.5). Similarly average boldness scores following sexual maturity (day 151) did not correlate with mean age at first oviposition or total eggs laid (table 4.5, also see appendix table A4.2, A4.3, A4.5, and A4.6 for genotype mean ± standard deviation of reproductive measures and BT scores). In contrast, a significant correlation was observed between mean exploration scores and age at first oviposition as well as a marginally non-significant correlation with total eggs laid (table 4.5). Following FDR correction, however, these correlations did not retain significance (table 4.5).

Table 4.4. Results of Pearson's correlations between mean genotype level exploration and boldness scores and mean growth rate on days 31, 61, 91, and 151 and their FDR corrected alpha levels (significant correlations denoted in bold script).

Day	**Behaviour**	**N**	***r***	***p***	**FDR corrected alpha**
31	Exploration	20	-0.316	0.175	0.05
	Boldness	20	0.220	0.351	0.025
61	Exploration	20	-0.183	0.440	0.05
	Boldness	20	-0.3369	0.147	0.025
91	Exploration	20	-0.291	0.213	0.025
	Boldness	20	-0.069	0.773	0.05
151	Exploration	20	0.398	0.083	0.025
	Boldness	20	0.083	0.728	0.05

Table 4.5. Results of Pearson's correlations between mean genotype exploration and boldness scores, age at first laying and total eggs laid exhibited on days 91 and 151 of life and their FDR corrected alpha levels (note that no P value retained significance after FDR correction

Day	**Behaviour**	**N**	***r***	***p***	**FDR corrected alpha**
91	Age First Lay – Exploration	20	0.339	0.143	0.025
	Age First Lay – Boldness	20	0.122	0.608	0.05
151	Age First Lay – Exploration	20	0.490	0.028	0.012
	Age First Lay – Boldness	20	-0.090	0.706	0.037
	Total eggs laid – Exploration	20	-0.441	0.052	0.025
	Total eggs laid – Boldness	20	-0.070	0.668	0.05

4.4 Discussion

I found that individuals within each genotype were not repeatable in their behavioural expression during ontogeny, which was driven by low between-individual variation (within each genotype) and high within-individual variation. I also document an overall effect of age on behavioural expression within my standardised rearing environment. In particular fish became more exploratory and bold during early development with a short period of behavioural stabilisation (plateau) followed by subsequent reduction in behavioural scores post sexual maturity. Although these general trends appear to be a common phenomenon within the study species, the rate and timing of these patterns (i.e. plasticity) varied considerably between genotypes, resulting in rank order changes at each stage of ontogeny. Interestingly, although exploration and boldness were highly plastic during ontogeny, strong correlations between these behaviours emerge and were maintained from day 61 onwards. Similarly to genotype level differences in average behavioural expression during ontogeny, I observed no between genotype differences in average growth rate. I did, however, observe some genotype differences in average reproductive measures, namely age at first oviposition and total eggs laid. These variables, however, did not correlate with exploration or boldness.

The observed general age effect upon behavioural expression and low within genotype repeatability estimates suggest that developmental flexibility may be characteristic of this species. From an ecological perspective, individual developmental flexibility is intriguing and may be related to the spatial and temporal heterogeneity of the mangrove habitat in which the species lives. Behaviour developmental flexibility may therefore allow individuals to adapt to local environmental conditions, including predation risk (Bell and Sih 2007) and competitive environments (Carere et al. 2005a).

Although I observed a general age effect upon behaviour, I found that the extent of plasticity varied considerably between my sampled genotypes. I suggest genetic constraints differing between genotypes, as a potential explanation for this result (DeWitt et al. 1998; Pigliucci 2005). These differences are also likely to have ecological consequences, for example, developmentally flexible genotypes may have greater ability to respond adaptively in a variety of environmental conditions (Scheiner 1993; Schlichting 1986). By contrast, less flexible genotypes may be restricted to fewer suitable habitats or find themselves in an environment exceeding their plasticity potential, resulting in environment-behaviour mismatch (DeWitt et al. 1998). Environmental heterogeneity and the interactive influence of genotypic constraints may therefore underpin behavioural variation and potentially maintain variation within populations. It is, however, important to highlight that my laboratory population represents multiple genotypes collected from 4 geographical locations. I am therefore unable rule out population specific causes for the observed variation in behavioural trajectories between genotypes. In particular, research has documented that commonly studied behavioural types i.e. boldness and exploration, can be population specific (Bell 2005; Dingemanse et al. 2007; Sinn et al. 2010), although exceptions are available (e.g. Pruitt et al. 2010). Future work exploring population and genotype specific ontogenic plasticity, their fitness correlates, as well as genotypic responses to specific environmental stimuli in both the laboratory and field, would therefore be exciting avenues of future research.

In addition to these results, previous research has also indicated that behavioural plasticity is complicated and likely dependent on the species under investigation (Bell and Stamps 2004; Brodin 2008; Sinn et al. 2008). Sinn (2008) for example, found that dumpling squid plasticity was dependent upon an individual's BT; with bold individuals

becoming increasingly bold whereas shy individuals maintained their shy phenotype (i.e. were consistent). In contrast, Brodin (2008) found evidence of developmental consistency (boldness and activity) in larval damsel flies which persisted into adulthood. Bell and Stamps (2004) additionally found that stability of BTs was dependent upon the population of origin (high and low predation) in threespined sticklebacks. My results also add another dimension to the growing literature, providing evidence of genotype level differences in behavioural plasticity during ontogeny, highlighting the importance of considering genetics when investigating BTs in a developmental framework. The expression of BTs during development is therefore highly complex, with behavioural consistency likely to be BT, individual, species, population and genotype specific.

My results also document the emergence of strong correlations between behaviour from day 61 onwards i.e. bold genotypes were more exploratory. It is therefore evident that boldness and exploration are linked and expressed in concert from 61 days of age in this species. These results are similar to the findings of Bell and Stamps (2004), who also found that correlations between behaviours are maintained even when the BTs that make up these correlations are themselves plastic. However, in contrast to the work of Bell and Stamps (2004), who noted that behavioural correlations were unstable during transitional phases (during major hormonal restructuring at sexual maturity), I observed the emergence of strong positive correlations between exploration and boldness approximately 40 days prior to, during, and following sexual maturity.

One mechanism that may strengthen behavioural correlations at age 61 days (prior to sexual maturity), is the effect of pleiotropic hormones (Ketterson and Nolan 1999). It is well documented that sex hormones and in particular testosterone influence aggressiveness (Munro and Pitcher 1985), a BT that tends to correlate positively with

both exploration and boldness (Huntingford 1976; Verbeek et al. 1996), however, examples where correlations are absent have also been reported (see Bell 2005; Brydges et al. 2008; Dingemanse et al. 2007 for examples). Pleiotropic effects of sex hormones are additionally implicated by what we know of the sexual development of this species. Research has shown that *K.marmoratus* begin ovotestis development between 34 and 46 days of age, (Sakakura and Noakes 2000) at which point secretion of oestrogen, androgen and progestin begins (Minamimoto et al. 2006). The emergence and maintenance of behavioural correlations therefore seem to mirror sexual development and associated increases in hormone secretion from this reproductive organ. Investigating possible links between sex hormones and behavioural correlations during ontogeny would be a possible future direction that may yield interesting insights in to the development of behavioural correlations.

Contrary to recent proposals regarding growth-BT relationships (Stamps 2007) innate growth rate did not correlate with behavioural scores at each age point. Similar results have recently been reported, for example Conrad and Sih (1985) found that newly emerged steelhead exhibiting bold/active BTs showed increased feeding rates during behavioural assays. These BTs did not, however, predict growth rate or survival probability during the first three months of life in a hatchery environment. In this study, it is possible that the observed low levels of variation in inter-genotype growth rates was insufficient to act as a mechanism contributing to behavioural variation (although considerable variation in developmental trajectories were present). The lack of genotype differences on growth rate also directly contrast with previous research on *K.marmoratus* (Grageda et al. 2005). However, Grageda et al. (2005) compared two genotypes and thus the observed differences may have been the result of the small sample size. In the current investigation individuals were reared in a standard

environment and it is likely that in an unstable or heterogeneous environment the results could be very different. In wild populations environmental conditions are known to affect both life-history (Stamps 2007; Stearns 1992) and BT expression (Boon et al. 2007; Dingemanse et al. 2004; Dingemanse et al. 2009). Environmental influences such as resource availability and competition, among others, may therefore influence the emergence behavioural repeatability. Moreover, these environmental influences may additionally generate the proposed life-history-BT interactions that are absent in this study, as well as differentially affect plasticity. Further investigations testing these possibilities provide an interesting area for future research.

Based upon modelling approaches of Wolf et al. (2007b) I tested the key predictions originating from this research, namely that earlier age at first oviposition would correlate negatively with boldness and exploration, while total number of eggs laid would correlate positively i.e. risk dependent asset protection (Clark 1994). In contrast to these predictions I observed the opposite patterns following sexual maturity for exploration but not boldness. My results suggest that genotypes which on average reproduce later were more exploratory, potentially because payoffs are high if resources are located and devoted to reproduction. In contrast, boldness prior to, or following sexual maturity, was not related to reproduction. In this instance food was not available in the arena (of which all fish were familiar) and thus the motivation to take risks is less likely to directly affect fitness in terms of securing resources. While I observed a moderate positive correlation between exploration and age at first laying (sexual maturity) and a moderate negative correlation between exploration and total eggs laid at day 151, these did not retain significance after FDR correction. Greater replication at the genotype level would therefore be required before I can conclude that exploration tendency is related to residual reproductive potential.

In conclusion I find no support for current theoretical predictions suggesting relationships between BTs and growth rate variation. I do, however, find some evidence that residual reproductive potential may influence behavioural expression although this did not retain significance after correction for multiple tests. My work also demonstrates the potential of *K. marmoratus* as a model species/system for investigating plasticity and how this relates to the development of personality traits i.e. repeatability. In this study I used a standardised and stable rearing environment to assess genotype level differences in behaviour during ontogeny and conclude that genotypes differ in the extent of developmental plasticity. I also find that correlations between exploration and boldness became strong and significant during ontogeny in this species. The natural occurrence of homozygous genotypes within this species presents an robust opportunity to explore the role of both the rearing environment and experiential factors, and how these contribute to BT expression (Stamps and Groothuis 2010a; Stamps and Groothuis 2010b).

The types of environment experienced during ontogeny differ both temporally and spatially. Furthermore, behavioural expression has been documented to differ during ontogeny in threespined sticklebacks depending upon the evolutionary history of high or low predation risk (Bell and Stamps 2004). It therefore seems intuitive that differing rearing environments and resulting experiential differences may have the potential to differentially effect the expression of personality traits i.e. BTs (Stamps and Groothuis 2010a; Stamps and Groothuis 2010b), and may select for relationships between state and behaviour. In the following chapter I address genotype plasticity of behaviour and life-history in response to three ecologically relevant rearing environments that have been suggested to influence either BT expression or life-history.

Environmental and Genetic Effects Shape the Development of Behavioural Traits in the Mangrove Killifish

Chapter 5

Abstract

Consistent individual differences in behaviour termed ‘behavioural types’ (BTs) are well documented in the animal kingdom, for example, some individuals are consistently bold while others are consistently shy. To date our understanding of the mechanisms underpinning consistent individual variation remains limited. Theoretical work suggests life-history trade-offs drive BT variation, however, empirical support is scarce. Moreover, whilst life-history traits are known to be phenotypically plastic, the extent to which such plasticity drives variation in BTs remains to be investigated. Using a natural clonal vertebrate, *K.marmoratus*, I control for genetic variation and investigate phenotypic plasticity in behaviour (exploration, boldness and aggression) and life-history traits in response to three ecologically relevant environments; conspecific presence, low food and predation risk. Both life-history traits and behaviour showed phenotypic plasticity, however, changes in behaviour did not follow theoretical predictions. Conspecific presence had the largest effect on behavioural expression with average behavioural scores and developmental trajectories, being significantly lower when compared to the control treatment. Importantly, whilst predation risk had little effect upon average behaviour expressed, only this treatment generated consistent individual differences i.e. personality. These results highlight the significant role of the environment in behavioural and personality expression. I particular these results suggest that predation risk generates personality and that social experience during ontogeny has a major influence upon behavioural development.

5.1 Introduction

In recent years animal personality has received extensive interest within behavioural ecology (see Sih and Bell 2008 for a review). Animal personalities can be defined as individual behavioural differences that are consistent across time and contexts (Sih et al. 2004b). For example, some individuals are consistently bold while others are consistently shy (Reale et al. 2007; Sih et al. 2004b). These individual differences are regularly termed behavioural types (BTs) (Bell 2007). In non-human animals, correlated suites of BTs across differing contexts/situations and correlations between functionally different behaviours have been referred to as 'behavioural syndromes' (Sih et al. 2004a; Sih et al. 2004b). As a phenomenon, BTs and behavioural syndromes have been observed in multiple species across a wide range of taxa with examples found in both vertebrates (Bell 2005; Bell and Sih 2007; Dingemanse et al. 2003; Dingemanse and de Goede 2004; Drent et al. 2003; Reale and Festa-Bianchet 2003; Sih et al. 2003; Stapley and Keogh 2005; Webster et al. 2009; Wilson and Stevens 2005) and invertebrates (Johnson and Sih 2005; Sih and Watters 2005; Sinn et al. 2008; Sinn and Moltschaniwskyj 2005). Research also indicates that BTs may be heritable (Dingemanse et al. 2002; van Oers et al. 2004c; van Oers et al. 2004b) and influence fitness (Cote et al. 2008; Dingemanse et al. 2004; Pruitt et al. 2008; Smith and Blumstein 2008; Smith and Blumstein 2010). Although BTs are well documented, our understanding of the factors generating and maintaining inter-individual variation within populations and intra-individual consistency remains limited.

Classically, fixed genetic determinants of behaviour have been used to explain consistency and several processes have been documented to maintain variation within populations. For example, variation may be maintained via frequency-dependent selection (Roff 1998), sexual selection (Schuett et al. 2010), environmental

heterogeneity (Mangel 1991) and a balance between weak selection and mutation (Santiago 1998). Although genotypes are influenced by selection across generations, genes are also influenced by environmental factors during ontogeny, which can generate considerable phenotypic variation within and between genotypes (Pigliucci 2005).

Phenotypic plasticity in life-history and behavioural responses are widely documented in the literature (DeWitt et al. 1998; West-Eberhard 1989). In recent years, personality studies have begun to investigate plasticity, and results indicate that while rank order tends to be maintained across individuals i.e. consistency, some plasticity occurs. BT plasticity has been found, for example, in response to temperature (Biro et al. 2010), food availability/competition (Carere et al. 2005a), group composition (Magnhagen and Bunnefeld 2009; Magnhagen and Staffan 2005), and predation pressure (Bell and Sih 2007). Research has additionally documented that BT plasticity is characteristic of ontogeny. In particular, BT developmental plasticity has been found to be population (Bell and Stamps 2004), BT (Sinn et al. 2008) and genotype specific (Edenbrow and Croft 2011). In a developmental sense, plasticity has the potential to provide a mechanism by which individual experience of the environment influences personality expression at later life stages (Stamps and Groothuis 2010a; Stamps and Groothuis 2010b) and thus experiential and environmental influences may generate adaptive personality traits (e.g. Bell and Sih 2007).

Research investigating early rearing environmental effects upon personality development have generally focused upon model organisms, such as rodents (see Laviola and Terranova 1998 for a review). For example, under-nutrition during development in rats and mice has been found to generate increased emotionality, activity, social responsiveness and aggression (Manosevitz and McCanne 1973; Mendl

and Paul 1991a; Mendl and Paul 1991b; Tonkiss et al. 1987; Whatson et al. 1976). In addition, large litter size (Dimitsantos et al. 2007) and maternal nursing behaviour (Caldji et al. 1998; Francis et al. 2000; Francis et al. 1999; Menard et al. 2004) both influences multiple aspects of personality expression in these model organisms. While model organisms have been well studied within the animal personality framework, research investigating non-model species are much less common in the literature. In recent years there has been some progress in dealing with this bias, for example Lee and Berejikian (2008), document that structural components of the rearing environment influence exploration in steelhead. In addition, guppies have been shown to exhibit bolder, more exploratory phenotypes when exposed to temporal variation in food availability compared to individuals reared in a stable feeding environments (Chapman et al. 2010). Carere et al. (2005a) have also documented that sibling competition and food availability in great tit nests generates higher exploration and aggression tendencies. These examples all show that the BTs expressed by individuals have the potential to be influenced by multiple environmental and experiential effects during ontogeny.

In addition to early environmental effects, recent theoretical work proposes that a major driver of BTs are internal state variables (Dall et al. 2004; Stamps 2007; Wolf and Weissing 2010) that differ among individuals and remain consistent for extended periods of time (Mangel and Munch 2005; Stamps 2007). Several internal states have been proposed to be important in BT expression with metabolic rate (Biro and Stamps 2010), life-history strategies i.e. growth rate (Stamps 2007) and residual reproductive potential (Wolf et al. 2007b) all being implicated in recent years. Individuals with higher growth and metabolic rates are subject to higher energetic demands which is predicted to generate bolder, more aggressive and exploratory BTs to increase resource

acquisition (see Biro and Stamps 2010 for a discussion). Environmentally induced directional plasticity in life-history strategies that influence energy requirements may therefore be predicted to generate associated plasticity in personality traits. This hypothesis however, remains to be tested empirically.

In this study I use *Kryptolebias marmoratus* as a model organism to investigate plasticity and repeatability in three commonly studied BTs (exploration, boldness and aggression) in response to three ecologically relevant rearing environments during ontogeny. The use of isogenic organisms allows replicates from a single genotype to be exposed to different environmental/experiential stimuli during development (see Dingemanse et al. 2010; Stamps and Groothuis 2010a; Stamps and Groothuis 2010b for reviews). It is well documented within human, and more recently animal, personality literature that gene polymorphisms have large effects upon the expression of personality (Inoue-Murayama et al. 2011). Replication within homozygous genotypes across environmental conditions therefore controls for genetic variation and thus differential allelic contribution to BT expression (Stamps and Groothuis 2010a). This approach allows me to investigate genotypic constraints upon behaviour and life-history, as well as explore the potential role of different environmental conditions in shaping BTs and BT plasticity during ontogeny.

In this study, I replicate within five genetically distinct homozygous genotypes across three ecologically relevant rearing environments that have been noted to influence BT expression and/or growth rate: conspecific presence treatment (CPT) (Carere et al. 2005a), predation simulation treatment (PST) (Bell and Sih 2007; Brown et al. 2007a; Dingemanse et al. 2007; Magnhagen and Borcherding 2008), and low food treatment (LFT) (Carere et al. 2005a; Lin and Dunson 1999). I additionally use a control

treatment (CT) for comparative analyses. For all individuals in this study I quantify life-history traits (growth rate, age at first reproduction and total eggs laid) and three behaviours (exploration, boldness and aggression) allowing life-history and behaviour to be the investigated in the context of phenotypic plasticity. I predict that (1) PST will result in an higher average boldness, exploration and aggression scores during development as well as fast growth, early reproduction and elevated egg production compared to the control treatment, (2) LFT will result in lower growth rates, delayed reproduction and lower egg production and as a consequence decrease average behavioural scores during ontogeny compared to the control treatment, (3) and CPT will generate increased competition resulting in higher average behavioural scores during ontogeny in comparison to the control treatment. In addition, I investigate whether or not each rearing environment generates behavioural consistency (i.e. repeatability) but make no *a priori* predictions because little work has explored the developmental effects on repeatability *per se*.

5.2 Methods

5.2.1 Study animals

240 individuals representing 5 homozygous genotypes (48 individuals per genotype) were used for experiments. All individuals within a genotype originated from a single parental fish and were from the F3 generation. Genotypes used ((1) BP11, (2) LK15, (3) LK2, (4) LK6 and (5) NNKN1) were descendents of founder individuals collected from Florida in 2007 that have been maintained in a laboratory setting since collection. All fish were housed in 20ppt synthetic salt water (Instant Ocean^{TM}) at 25˚C ± 0.5˚C in a 12hr light 12hr dark cycle.

5.2.2 General housing protocols

Individuals were randomly assigned to one of 4 rearing treatments (CT, CPT, PST, and LFT) for 153 days of development (sexual maturity occurred between ages 110-125 days of age). All fish were housed individually from hatching in the CT, PST and LFT rearing environments, or in groups of three fish in the CPT rearing environment. All fish were maintained in the same sized standard aquaria (28 x 17.5 x 16cm) containing 2 litres of salt water and a sparse layer of gravel throughout the study. Complete water changes were carried out at 15 day intervals and fish were fed between 13.00-15.00 on a daily basis. To minimise waste food and potential ill health due to decaying food within the aquaria, fish were not fed to excess. Instead a standardised amount of age dependent food was provisioned to each fish (amounts used were sufficient for positive growth in all treatments), all food provisioned was consumed in within 5 minutes (see below for age specific volumes provisioned per treatment). On days 1-3 fish were fed micro-worms (*Panagrellus redivivus)* (1mg/100ml suspension (average number of worms 23.3±2.6/0.1ml)) and for the remainder of the study fish received artemia naupili (*Artemia salinas*) (average number artemia/0.1ml: 35.9±3.9).

5.2.3 Rearing treatment specifics

Control Treatment (**CT)***:* Individuals were housed singularly, and on days 0-3 individuals were provisioned with 0.2ml/fish of micro-worms and from days 4-15 individuals were provisioned with 0.2ml/fish of artemia. For the remainder of the study, daily artemia provision increased by 0.2ml/fish at 15 day intervals. To control for any order effects feeding order was randomised daily.

Conspecific presence treatment (CPT): Following hatching individuals were reared in groups of three individuals (of the same genotype) that had hatched within 24 hours of the median aged fish. To structure age points for sampling I took the median

date of hatching and considered this as day 0, therefore all fish within each tank within this treatment group were 1±1 day old. Fish were fed as in the CT; however, the amount of food provided was proportional to the number of fish/aquaria (i.e. 3 times the control group).

Predation simulation treatment (PST): Individuals were housed and fed using the same conditions as those described for CT fish (see above). From day 8, and then at 3 day intervals for the remainder of the study, I pursued individuals with a small net for 1 minute periods to simulate a predation event. This method has been documented to effect boldness, being attributed to increased risk perception (Brown et al. 2007a).

Low food treatment (**LFT)**: Individuals were housed in the same conditions as the CT; however, they were fed at half the food rations of CT fish. While this seems a severe reduction in food availability, resources in the wild are highly variable (Taylor 2000). In addition, fish have been documented to remain in crab burrows for up to 9 months in the wild, during which time food availability is severely limited (Taylor 2000). The experimental food manipulation used in this treatment group is therefore above that experienced in some natural habitats. Importantly, food rationing in LFT fish permitted positive growth in all individuals studied and had no effect upon health or physical condition. Moreover, no deaths were observed during development as a result of this protocol. In addition, LFT fish did not differ in their age at sexual maturity or average egg production compared to the control group (see below) and thus effects upon sexual development were minimal. All rearing environments were also carried out under UK Home Office and University of Exeter ethics approval (see appendix A1.1). All fish were reared within their respective rearing environments for a total of 240 days (permitting egg collection on days 230-235 to investigate maternal environmental effects as presented in chapter 6). Following this study, at 240 days of age, fish were culled using MS-222 in accordance with Home office regulations.

5.2.4 Life-history measurements

Upon hatching, at day 30 and then again at 60 day intervals thereafter, individuals were transferred to a photographing chamber and photographed laterally using a Nikon D60 SLR (18-50mm zoom lens). Using photographs I measured each individual's standard length (tip of the lower jaw to the posterior margin of the hypleural plate) using ImageJ photo analysis software v1.42. When fish were 105 days old, tanks were inspected daily for oviposition between the hours of 9.00 and 11.00 for the remainder of the study (48 days). During these inspections individuals were removed from their home tank and placed in a small opaque beaker containing approximately 50ml of home tank water. Gravel was agitated to dislodge eggs, which were counted, removed and discarded. For each individual I recorded age at first laying and total number of eggs laid.

Egg/reproduction within CPT fish was quantified in the same manner as all other treatment groups' i.e. daily inspection of tanks which permitted the control of any handling effects across treatments. However, because this treatment was constructed using 3 fish per aquaria, I was unable to accurately determine the total number of eggs laid per individual per aquaria. In addition, calculating the mean number of eggs laid per CPT aquaria was unlikely to have accurately described egg production because the age that each of the three fish became sexually mature was expected to vary within each aquaria. Data regarding total eggs laid from this treatment group was therefore excluded from further analysis. To provide a measure for age at first laying within each CPT aquaria, I recorded the median age of fish when the first egg was laid, which provided a measure of the earliest age of sexual maturity within this treatment group. This measure of earliest age at first laying was subsequently analysed together with age at first laying, quantified in all other treatment groups (see below).

5.2.5 Behavioural Assays

Behavioural trials were conducted on days 31-33, 91-93 and 151-153 during the developmental period. Behavioural trials were recorded using a Sony ExwaveHad black and white video camera fitted with a Computar Vari Focal 5-50mm F1.3 lens. Ethovision XT version 6.0 (Noldus Information Technology) was used to track movements of the test fish. I assayed three behaviours separated by 24 hours to limit non-independence of data points i.e. reduction in the potential for carryover effects. In particular, assays were completed on the following days: exploration (days 31, 91, 151), boldness (days 32, 92 and 152) and aggression (on days 33, 93, 153). In this study I was interested in both behavioural plasticity and repeatability throughout development in response to the rearing environment. Maintaining experimental testing orders within/between assays across each testing period therefore limited the potential for additional experimentally induced within-individual variation that could confound repeatability estimation. For the conspecific presence treatment I was unable to identify individuals and thus each of the three fish were tested randomly in sequence on a single day for each respective behavioural assay. I then calculated the mean for each behavioural measure within each behavioural assay on each day of development. Tank means were then used in subsequent analysis.

Exploration was assayed using a white novel maze which increased in dimensions alongside fish development (see figure 5.1a for dimensions). To maintain consistency, the structure of the maze remained constant throughout the experiment (Figure 5.1a), yet to generate novelty the position of the acclimatisation/release zone was altered at subsequent age points presenting fish with a unique starting view of the maze. Consistency of the maze structure was maintained to limit increasing variation in exploration scores (i.e. it is difficult to determine whether one structure is more or less

stressful). This approach permitted a more accurate measure of behavioural consistency throughout the study. The arena was divided in to 24 equal zones (excluding the acclimatisation enclosure). Fish were introduced to the test tank via the acclimatisation enclosure (containing a sparse layer of white gravel) which was surrounded by a white opaque barrier. Following 5 minutes acclimatisation (during which time all individuals regained activity), individuals were released by raising the opaque barrier. Behaviour was observed for a 10 minute period, during which time I recorded the distance travelled (mm), mean velocity (mm/second), duration of mobility (seconds) and the number of unique squares entered. While fish were exploring the novel maze, gravel was removed from home tanks, to which identical structural components of the boldness trial arena were added (figure 5.1b). Fish were housed in these 'mock' arenas for a period of 24 hours following the exploration assay to limit any element of structural novelty during the boldness trials.

The boldness test arena was divided in to two sections along the length (see figure 5.1b). The drop zone (separated by a mesh partition) allowed a weight to be dropped in to this zone while being external to the test zone. Following introduction to the boldness arena all individuals were given a 5 minute acclimatisation period (during which time all individual regained activity) after which time a weight suspended 13cm above the drop zone was released in to the arena breaking the water's surface. Following the simulated aerial predation event I recorded time (seconds) to first movement (TTM) (categorised as movement of >1 body length), distance travelled (mm) and mean velocity (mm/second) during a 5 minute observation period following the predation simulation. All fish regained activity within the five minute observation period, with the exception of one fish on day 91 which did not move for the assay duration and this fish was given a TTM score of 300 seconds. During the boldness trial I

removed ‘mock’ boldness structures from the test fish’s home tank and replaced these with structural components of the aggression arena. Individuals were then housed in these ‘mock’ aggression arenas for a period of 24 hours prior to aggression assays.

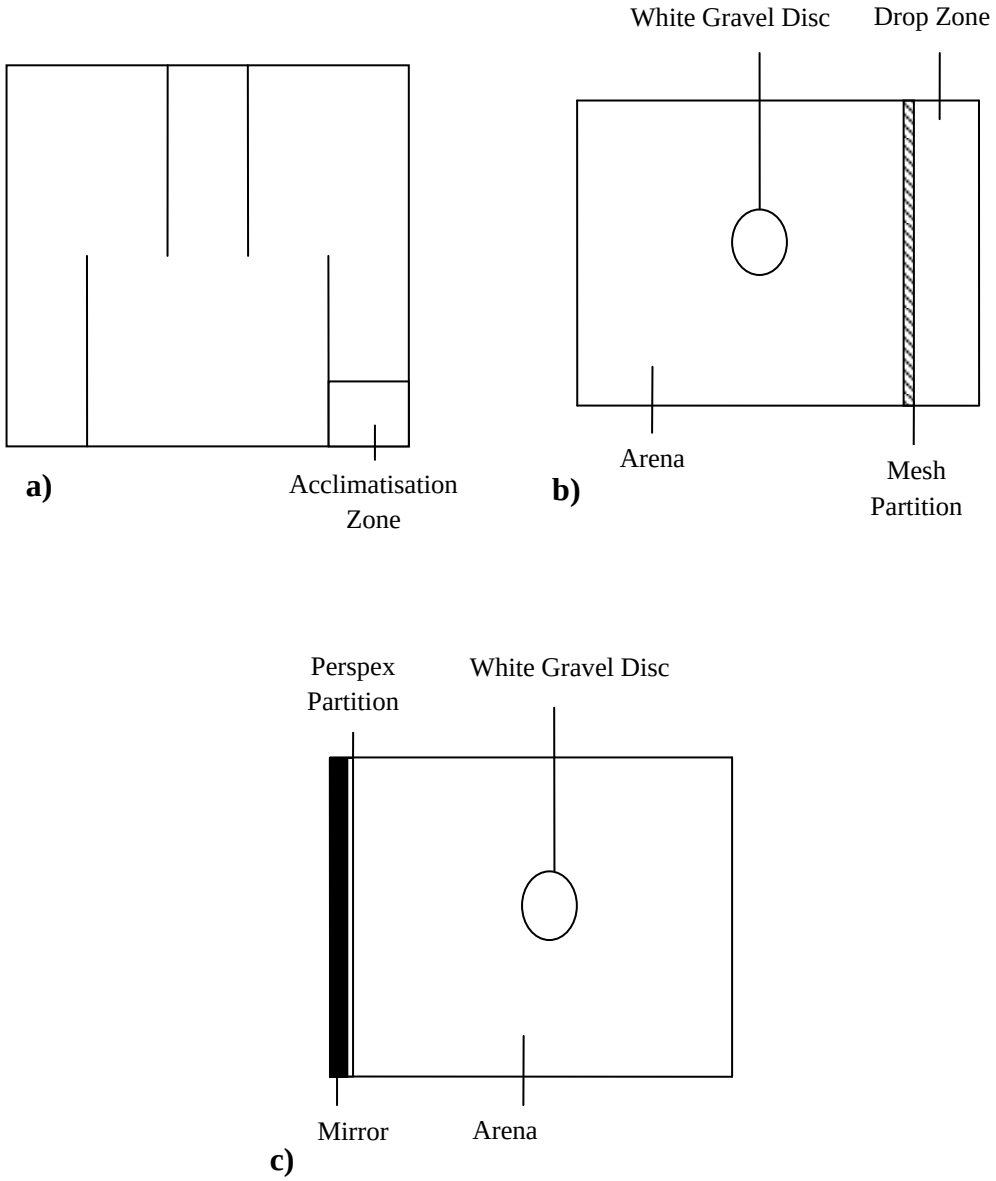


Figure 5.1. Schematic diagram of a) exploration trial arena (dimensions: day 31: 15 x 15 x 10cm, day 90 and 151: 30 x 30 x 15 cm), b) boldness trial and c) aggression trial arena. The boldness arena incorporates a drop zone (separated by white mosquito netting), and trial arena dimensions were 21 x 16.5 x 15.5 cm. The aggression trial arena incorporates a 3 mm thick Perspex partition behind which a mirror was fitted, the trial arena dimensions were 21 x 16.5 x 15.5 cm. In front of this mirror a removable partition was placed to reveal the mirror after a 5 minute acclimatisation period.

The aggression test arena was divided in to two sections along the length using a clear Perspex partition (see Figure 5.1c). 1cm behind this partition, a mirror was fixed. A remotely removable opaque partition, allowing the mirror to be revealed to the test fish, was positioned in front of the mirror and behind the Perspex partition. Following introduction to the aggression arena all individuals were given a 5 minute acclimatisation period after which, I ensured fish were in the furthest half of the arena from the mirror zone, before raising the opaque barrier. An interaction zone was predefined in front of the mirror (two times the mean standard length of control fish at each respective age (31 days = 2cm, 91 days = 4cm, 151 days=5cm). A 10 minute trial then began when fish entered the interaction zone. During each trial I recorded total time spent within the interaction zone together with the frequency of bites and tail flicks directed towards the mirror image. Bites and tail flicks were summed to generate a measure of total aggression. Total aggression was then used to calculate aggression rate/minute based upon the duration of time spent within the interaction zone. Aggression rate was square-root normalised (a common methodology) and used as a response variable in subsequent analysis.

5.2.6 Social/Asocial Boldness Experiment

Recent studies have documented that boldness responses can be modulated in relation to the social environment (Magnhagen and Bunnefeld 2009; Magnhagen and Staffan 2005; Piyapong et al. 2010). During my developmental study, all of my testing protocols were asocial to standardise testing procedures. Changes in the social context between the CPT rearing environment (home tank) and the asocial assaying protocol may therefore have influenced risk perception, resulting low boldness scores that may be an inaccurate representation of individual differences during ontogeny. Following completion of the developmental study (day 200), I ran an additional experiment to

investigate if the asocial testing protocol may have contributed to CPT behaviour. For this experiment, all CPT fish were assayed for boldness as described above, within both a social context (each of the three fish per tank were tested simultaneously with their tank mates) and within an asocial assaying context (each fish tested singularly). These assays were separated by 1 week and social contexts were counterbalanced so that half of the fish received the asocial condition first followed by the social and visa-versa. During trials I scored TTM (s) visually as a measure of boldness for each individual (individual identification was based upon size differences among fish which were apparent at this age). Unfortunately due to programme limitations it was not possible to simultaneously track all three fish's movements within a social boldness assay using Ethovision, and thus activity within this assay was not available for inclusion into a boldness PCA score. This is, however, unlikely to have been a problem because TTM and activity have been found to load strongly on to a single PCA component throughout development (see chapter 4, 6 and below for details) and thus TTM and activity represent a single one-dimensional behavioural construct. In addition, analysing both TTM and boldness PCA scores (TTM + activity) separately revealed qualitatively similar results (see chapter 4 for a discussion and also Appendix A4.1 for results) suggesting that these behavioural measures similarly explain patterns within this behavioural assay.

5.2.7 Statistical Analysis

5.2.7.1 Growth Rate

Specific growth rate between each 30 day time point sample was calculated using the following formula (Priestley et al. 2006):

$$\text{Specific Growth Rate [\%]} = 100 \times ((\ln SL_2 - \ln SL_1)/ t)$$

Where SL_2 the standard length at sample 2, SL_1 is the standard length at sample 1, and t is the time in days between measurements.

5.2.7.2 Exploratory principal component analysis

I explored variance-covariance matrix similarities for exploration and boldness behaviour, collected during the course of the developmental period, using common principal component analysis (CPC) (see Arnold and Phillips 1999; Flury 1988; Phillips and Arnold 1999, also refer to chapter 3 for more detais). Covariance matrices for both exploration and boldness shared a common principle component over all data points (table 5.1a). Bartlett sphericity and Kaiser-Meyer-Olkin (KMO) tests indicated that both exploration and boldness matrices were suitable for use in PCA (see Budaev 2010 for a discussion) (exploration: KMO= 0.653, Bartlett's test $p<0.001$ and boldness: KMO = 0.632, Bartlett's test $p<0.001$). A single PCA for all data collected throughout the course of the study period was conducted for exploration and boldness, and I found that measures for each BT loaded strongly on to single components that explained high percentage of total variance (table 5.1b). PCA scores were extracted from this analysis for each individual at each age point and these scores were use in subsequent analyses. CPC analysis was completed using the program CPC (Phillips 1998) while PCA analysis was completed using SPSS v16.0.

5.2.7.3 Repeatability

Repeatabilities (Lessells and Boag 1987) were calculated for fish during ontogeny together with 95% confidence intervals (Faraway 2006) using general linear mixed effect models fitted with Gaussian error and identity link functions. Repeatabilities were calculated for each behavioural score throughout ontogeny within each treatment group separately, to determine if repeatabilities varied between rearing

Table 5.1. a) Results of CPC analysis for boldness and exploration, model denoted in bold script represents the best fitting CPC model under the I-T approach and b) PCA component loadings and % variance explained for boldness and exploration measures collected throughout ontogeny.

a)

	Model	AIC	Delta AIC	w_i
Exploration	**CPC**	**265.33**	**0.00**	**0.97**
	PCPC1	272.15	6.82	0.03
	PCPC2	277.75	12.42	0.00
	Proportional	346.26	80.93	0.00
	Equality	353.65	88.32	0.00
	Unrelated	356.00	90.67	0.00
Boldness	**CPC**	**280.04**	**0.00**	**0.96**
	CPCP	286.26	6.22	0.04
	Proportional	1171.51	891.47	0.00
	Equality	1378.26	1098.22	0.00
	Unrelated	1382.00	1095.74	0.00

b)

BT	Behaviour	Component Loading
Exploration	Distance Travelled (mm)	0.633
	Unique Zones	0.946
	Mean Velocity (mm/s)	0.933
	Mobility Duration (s)	0.826
	% Variance Explained	71.2
Boldness	TTM (s)	-0.779
	Distance Travelled (mm)	0.964
	Mean Velocity (mm/s)	0.966
	% Variance Explained	82.3

environments. Each response variable (exploration, boldness and aggression) was fitted within separate models with individual nested within genotype as random effects, from which variance components were predicted. Variance component predictions were used to calculate repeatabilities at the individual level (or at the aquaria mean level for conspecific presence fish, owing to the inability to identify individuals during the study) as: the sum of the variance between individuals and the sum of the variance between genotypes divided by the sum of the variance between individuals and variance between

genotypes and the residual variance. Repeatability at the genotype level was calculated as: the variance between genotypes divided by the sum of the variance between individual's and variance between genotypes and the residual variance (see Nakagawa and Schielzeth 2010 and ; Schuett et al. 2011 for details). 95% CIs for repeatability estimates were obtained from parametric bootstrapping (N=1000 simulation iterations) and p values estimated using randomisation tests (N=1000) (see Nakagawa and Schielzeth 2010 for details).

5.2.7.4 Linear mixed modelling

Linear mixed modelling was completed using R for statistical computing v2.12.0 (R development core team 2010) using the lmer function within the package lme4 (Bates and Maechler, 2009). Modelling age as a fixed continuous second order polynomial effect had better explanatory power than age as a linear effect (*wi* = 0.98). In addition to modelling age fixed continuous second order polynomial, I included genotype and treatment as fixed categorical effects and included 1^{st} and 2^{nd} order interactions between these variables within my global model (see appendix A5.1 for model structures). In this study I was primarily interested in treatment effects and thus the interaction between genotype and age was excluded from the analysis because average genotype developmental trajectories may be confounded by treatment effects. Using maximum likelihood estimation I investigate the influence of age, treatment and genotype on behavioural expression and growth rate. Behavioural scores for exploration, boldness and square-root normalised aggression rate, and measures of growth rate and reproduction were used as response variables in each respective model. I additionally included random effect covariates with individual included as a level 1 random intercept to control for repeated measures across ontogeny. Furthermore, individuals were nested within genotype as a level 2 random intercept, to control for

replication within genotypes. All random and fixed effect covariates were included in the global model; I then refitted all possible reduced models to create a candidate set by removing fixed effects (see appendix A5.1 for model structures). Using these candidate sets, the best explanatory model for each response variable was assessed using the Information-Theoretic (I-T) approach (see below). Normality of residuals for all models was confirmed by visually inspecting normal probability plots, while homogeneity of variances was confirmed by plotting residuals versus fitted values (Faraway 2006). Models were fitted with Gaussian error and identity link functions (refer to supplementary material for each model structure).

5.2.7.5 Mixed model selection and inference

I assess model goodness of fit for each of my candidate models using the information-theoretic (I-T) approach as described by Burnham and Anderson (2002; 2004). Models were compared based upon AICc (for small sample sizes), by calculating delta AICc (Δ_i), an estimate of relative difference between the $AICc_{min}$ model and each subsequent alternative model. Δ_i values indicate the level of empirical support for each model with values of <2 indicating substantial support, whereas values of >4 indicate little/no support (Burnham and Anderson 2002). I also calculated Akaike weights (w_i) for each model to explore the likelihood of a model being the best fit to the data and thus containing covariates of importance (Burnham and Anderson 2002; Burnham and Anderson 2004). If model weightings revealed no single best candidate model (w_i <0.9) I implemented model averaging of estimates and standard error across models <4 Δ_i of AIC_{min}, as described by Burnham and Anderson (2002; 2004) using the MuMln package (Bartoń 2009). In addition, I calculated unconditional 95% confidence intervals around estimates, with confidence intervals excluding zero being considered as significant at the 0.05 α level (Nakagawa and Cuthill 2007).

5.2.7.6 Social/Asocial Boldness Experiment

I constructed random coefficient models with log transformed TTM imputed as a response variable (Gaussian error and identity link). I used a nested design for random effects with individuals imputed as a level 1 intercept, genotype as a level 2 intercept, aquaria as a level 3 intercept and context as a level 4 intercept. From this nested hierarchy, variance component analysis of each random effect was completed to decompose the variation explained by each random effect level (between individual, between genotypes, between aquaria and between social/asocial testing contexts).

5.3 Results

5.3.1 Repeatability

When analysing repeatability within each treatment separately, CT, CPT and LF treatments did not generate repeatable behaviour for any of my measured behaviours at either the individual (aquaria level for CPT) or genotype level over 151 days of development (table 5.2). In contrast, PST generated repeatable behaviour at the individual level for boldness and aggression (table 5.2). Moreover, aggression was also found to be repeatable at the genotype level within PST fish (table 5.2). Similarly to CT, CPT and LFT groups, exploration was additionally found to be non-repeatable at either the individual or genotype levels within PST fish. When taken together these results indicate that behaviours were non-repeatable within CT, LFT and CPT treatment groups i.e. no personality expression during ontogeny. My results therefore suggest that PST is an important factor influencing the expression of aggression and boldness BTs (i.e. personality). It is important to note that although repeatability estimates within PST fish were significantly different from zero; these estimates were in general quite low, however, these are within the range of repeatability estimates regularly reported within the animal personality literature (Bell et al. 2009). In addition, it is possible that the

absence of significant repeatabilities reported in CT, LFT and CPT may be related to small sample size, however, this is unlikely because this analysis incorporated 40 measures per treatment at each of the three sample points during ontogeny, which is comparable to sample size ranges from studies reporting significant repeatabilities in different species (e.g. Dingemanse et al. 2002; Jones and Godin 2010; Schuett and Dall 2009; Schuett et al. 2011).

Table 5.2. Repeatability estimates (R) for individuals (CT, PST and LFT) and at the aquaria level (CPT fish: i.e. mean aquaria BT scores). p= p values for each repeatability estimate and 95%CI = 95% confidence intervals surrounding repeatability estimates. Estimates denoted in bold script highlight significant repeatabilities during development. $N_{individual}$=40, $N_{genotype}$ =5 per stage of ontogeny (days 31, 91 and 151 of age), N_{total} = 240.

Data	Response variable	Level	R	*p*	95%CI
Control	**Exploration**	ID	0.104	0.141	.000-.312
		Genotype	0.015	0.261	.000-.109
	Boldness	ID	0.141	0.638	.000-.191
		Genotype	0.056	0.069	.000-.020
	Aggression	ID	0.128	0.091	.000-.318
		Genotype	0.141	0.066	.000-.345
Conspecific Presence	**Exploration**	Aquaria	0.044	0.296	.000-.266
		Genotype	0.092	0.17	.000-.268
	Boldness	Aquaria	0.143	0.071	.000-.337
		Genotype	0.196	0.055	.000-.433
	Aggression	Aquaria	0.048	0.611	.000-.193
		Genotype	0.154	0.064	.000-.394
Predation Risk	**Exploration**	ID	0.099	0.152	.000-.299
		Genotype	0.000	0.523	.000-.077
	Boldness	ID	**0.314**	**0.004**	**.108-.501**
		Genotype	0.119	0.053	.000-.317
	Aggression	ID	**0.343**	**0.004**	**.135-.528**
		Genotype	**0.257**	**<0.001**	**.006-.525**
Low Food	**Exploration**	ID	0.096	0.170	.000-.310
		Genotype	0.081	0.268	.000-.247
	Boldness	ID	0.095	0.146	.000-.294
		Genotype	0.110	0.151	.000-.310
	Aggression	ID	0.142	0.069	.000-.338
		Genotype	0.157	0.059	.000-.389

5.3.2 Behavioural Responses to the Rearing Environment

Exploration was explained by two competing models (<4 Δ_i from the $AICc_{min}$ model). The average model included first order fixed effects of genotype, age and treatment and second order interaction of age by treatment (see Appendix A5.1). Results indicated that, in comparison to CT, both CPT and LFT were on average significantly less exploratory, while PST fish showed no significant difference compared to CT fish (table 5.3, figure 5.2, also refer to Appendix A5.2 for treatment level means). An interaction between age and treatment revealed that exploration developmental trajectories (i.e. treatment level mean age related behavioural expression during ontogeny) were significantly lower in CPT fish when compared to CT fish (table 5.3, figure 5.3a). Figure 5.3a, and genotype means for distance travelled (Appendix A5.2) also highlight that while exploration increased throughout ontogeny in each of the CT, PST and LFT groups, CPT fish exhibited an exploration decrease following sexual maturity.

Three models for boldness competed for the best model position within my candidate set (see Appendix A5.1). The average model for boldness contained all first order effects and the second order interactions of age by treatment and genotype by treatment. Age had a strong influence upon boldness (table 5.3) with population level scores increasing during ontogeny. Similarly to exploration, CPT fish were on average less bold in comparison to CT fish, however, these estimates were spanned by large CIs that crossed zero, resulting from high standard error estimates which may indicate sample size as an issue (table 5.3, figure 5.2, also refer to Appendix A5.3 for treatment means). This reduction in average boldness is, however, supported by the observed significant interaction between age and treatment. In particular, I observed that the boldness developmental trajectoriy (i.e. mean treatment level age related behavioural

expression during ontogeny) for CPT fish was significantly lower than the CT group (table 5.3, figure 5.3b, also refer to Appendix A5.3 for treatment by age mean values). In contrast, fish reared within the PST and LFT groups did not differ significantly from CT fish in their average boldness scores (table 5.3, figure 5.2) or their average developmental trajectories (table 5.3, figure 5.3b).

Two models competed for the best model position for aggression behaviour within my candidate set (see Appendix A5.1). The average model contained first order effects, and second order interactions between age and treatment. Age had a significant effect on population level aggression throughout ontogeny (table 5.3). Similarly to exploration and boldness, I found that CPT reared fish were on average significantly less aggressive than CT fish, while PST and LFT fish exhibited no significant difference (table 5.3, figure 5.2, also refer to Appendix A5.4 for treatment means). During ontogeny however, aggression rate increased during development within CPT fish (table 5.3). Visual inspection of figure 5.3c and treatment by age mean values (Appendix A5.4) further elucidates this trend with aggression rate increasing during early ontogeny, which peaked around sexual maturity, followed by a reduction after approximately 110 days of age. No effect of the LFT or PST was found upon aggression; with both treatments showing similar average aggression rate and aggression expression during development when compared to CT fish (see table 5.3, figure 5.2 and 5.3c, Appendix A5.4).

5.3.3 Genotype effects

On average genotypes differed in their mean exploration, boldness and aggression scores (table 5.3). Genotype by treatment interactions were not included in the average model for either exploration or aggression (Appendix A5.1) indicating that

each genotype exhibited similar levels of plasticity in response to the experimental rearing environments. In contrast, genotypes showed different levels of plasticity for boldness scores when compared to the CT (i.e. genotype x environment effect). In particular, all genotypes exhibited reductions in average boldness in response to CPT and LFT, however, the extent of genotype average level reductions varied (table 5.3). Genotype responses to PST were also variable; however, while some genotype responses were close to zero i.e. no plasticity, others exhibited an increase in risk dependent boldness plasticity in comparison to the baseline CT treatment (table 5.3).

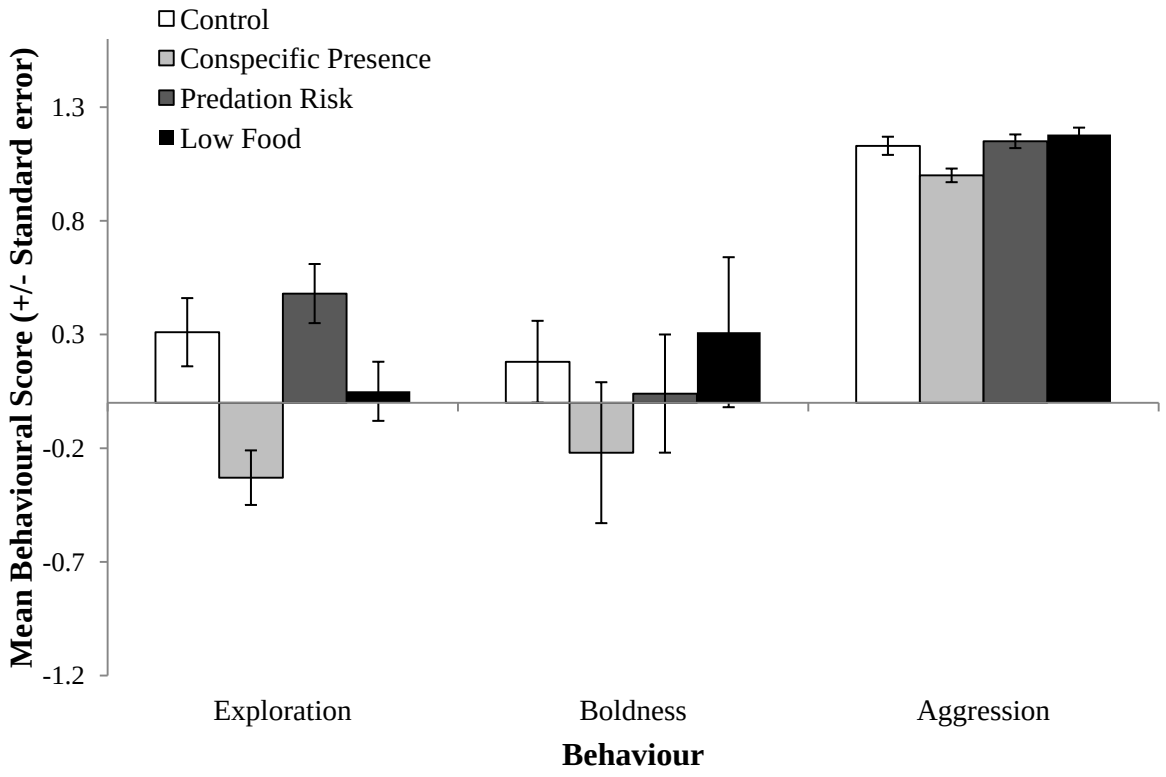


Figure 5.2. Bar chart presenting mean behaviour score estimates (exploration and boldness = mean PCA scores and aggression = mean aggression rate/minute) expressed by each treatment group throughout the entire developmental period (151 days). Error bars represent standard error, $N_{total} = 240$.

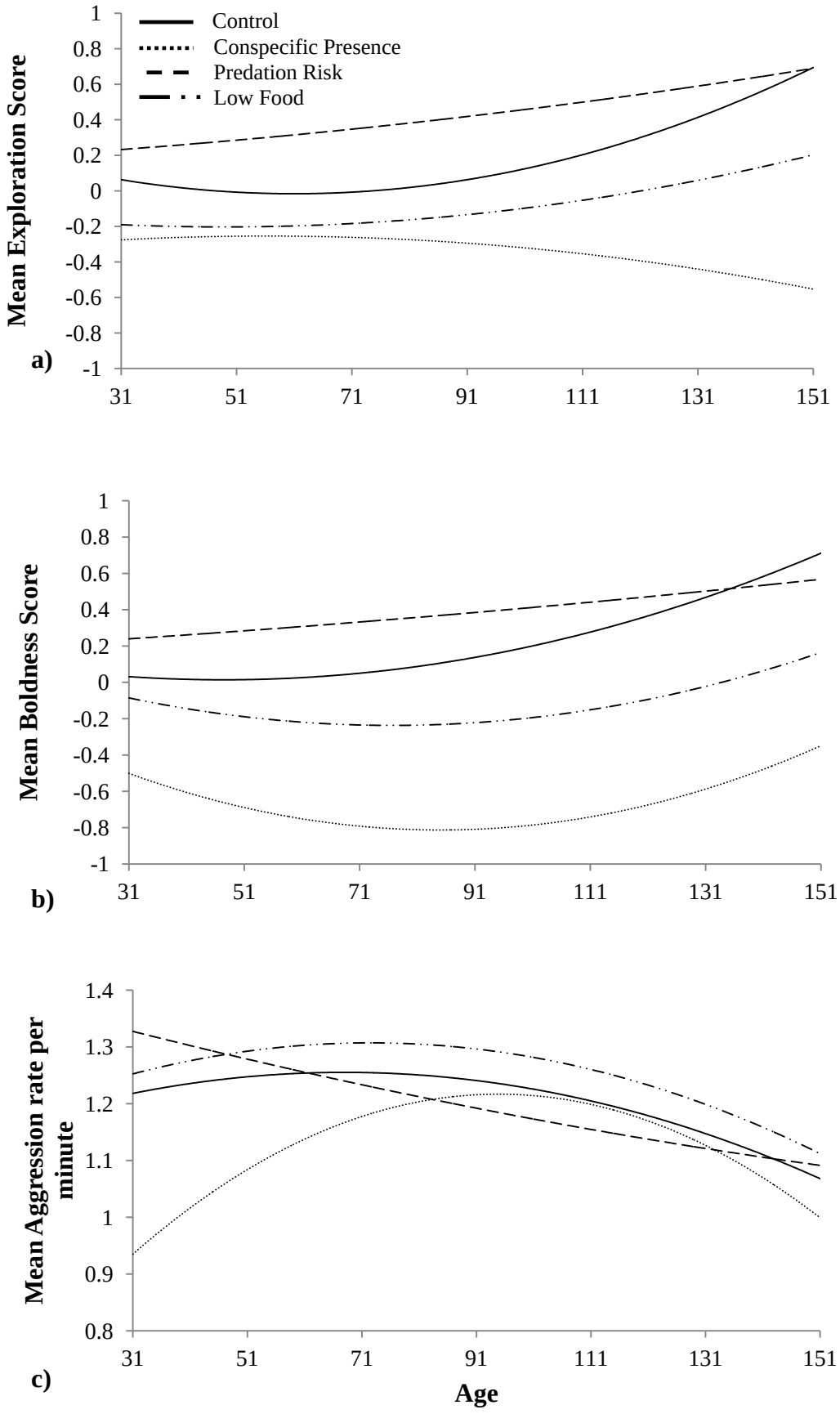


Figure 5.3. Treatment level developmental trajectories (i.e. treatment level mean age related behavioural expression during ontogeny) over the course of 151 days of ontogeny for a) exploration, b) boldness and c) aggression BTs, $N_{treatment}$=4, N_{total} = 240.

Table 5.3. Model averaged exploration, boldness and aggression estimates for parameters of importance identified by the IT approach (effects and confidence intervals denoted in bold script represent non-zero CI's that are significant at the 0.05 α level), $N_{genotype}$ =5 and $N_{treatment}$=4 per sample point during ontogeny (days 31, 91 and 151 of age), N_{total} = 240.

	Exploration				**Boldness**				**Aggression**			
Parameter	**Estimate**	**SE**	**Lower CI**	**Upper CI**	**Estimate**	**SE**	**Lower CI**	**Upper CI**	**Estimate**	**SE**	**Lower CI**	**Upper CI**
Intercept	0.31	0.15	0.01	0.59	0.18	0.18	-0.17	0.53	1.13	0.04	1.05	1.21
Age	8.31	2.42	3.57	13	4.83	2.24	0.43	9.22	-1.3	0.63	-2.52	-0.07
Genotype: 2	**-0.47**	**0.15**	**-0.76**	**-0.18**	-0.13	0.29	-0.69	0.44	0.05	0.04	-0.02	0.13
Genotype: 3	0.03	0.14	-0.25	0.31	**0.59**	**0.27**	**0.06**	**1.12**	**0.24**	**0.04**	0.17	0.32
Genotype: 4	-0.25	0.14	-0.54	0.02	0.02	0.23	-0.44	0.47	0.06	0.04	-0.02	0.13
Genotype: 5	-0.20	0.14	-0.49	0.07	-0.1	0.23	-0.54	0.34	**-0.09**	**0.04**	**-0.17**	**-0.02**
CPT	**-0.64**	**0.12**	**-0.89**	**-0.39**	-0.4	0.31	-1	0.2	**-0.13**	**0.03**	**-0.19**	**-0.06**
PST	0.17	0.13	-0.09	0.43	-0.14	0.26	-0.65	0.37	0.03	0.03	-0.04	0.09
LFT	**-0.26**	**0.13**	**-0.52**	**-0.003**	0.13	0.33	-0.51	0.76	0.05	0.03	-0.01	0.12
Age x CPT	**-7.66**	**2.29**	**-12.1**	**-3.18**	**-5.37**	**2.36**	**-9.99**	**-0.75**	**1.86**	**0.71**	**0.46**	**3.26**
Age x PST	-0.80	2.32	-5.34	3.74	-2.45	2.39	-7.13	2.23	-0.75	0.72	-2.17	0.66
Age x LFT	-2.8	2.33	-7.36	1.76	-4.24	2.39	-8.94	0.449	0.35	0.72	-1.07	1.77
Genotype: 2 x CPT	-	-	-	-	**-0.83**	**0.36**	**-1.53**	**-0.12**	-	-	-	-
Genotype: 2 x PST	-	-	-	-	0.17	0.37	-0.54	0.89	-	-	-	-
Genotype: 2 x LFT	-	-	-	-	**-0.86**	**0.37**	**-1.59**	**-0.13**	-	-	-	-
Genotype: 3 x CPT	-	-	-	-	**-0.82**	**0.35**	**-1.5**	**-0.14**	-	-	-	-
Genotype: 3 x PST	-	-	-	-	0.07	0.35	-0.61	0.75	-	-	-	-
Genotype: 3 x LFT	-	-	-	-	**-0.73**	**0.35**	**-1.42**	**-0.04**	-	-	-	-
Genotype: 4 x CPT	-	-	-	-	-0.53	0.35	-1.2	0.15	-	-	-	-
Genotype: 4 x PST	-	-	-	-	**0.8**	**0.35**	**0.11**	**1.49**	-	-	-	-
Genotype: 4 x LFT	-	-	-	-	**-0.84**	**0.35**	**-1.54**	**-0.15**	-	-	-	-
Genotype: 5 x CPT	-	-	-	-	-0.47	0.35	-1.15	0.2	-	-	-	-
Genotype: 5 x PST	-	-	-	-	0.62	0.35	-0.06	1.3	-	-	-	-
Genotype: 5 x LFT	-	-	-	-	-0.38	0.35	-1.07	0.31	-	-	-	-

5.3.4 Social/Asocial Boldness Experiment

The largest percentage of the total variance was explained by between individual (62%) and between aquaria (38%) random effect parameters, indicating that individuals and aquaria differed in their boldness scores. I found that the variance explained between genotypes and residual variance were both < 0.01% of the total variance suggesting that the genotype and unaccounted (residual) variation was low i.e. no between genotype differences or variation that was not explained by the model. Furthermore, variance explained between assaying contexts (asocial and social) accounted for < 0.01% of the total variance. These components indicate that genotypes exhibited similar boldness scores, and individual responses did not vary between social and asocial assaying protocols.

5.3.5 Life-history Responses to the Rearing Environment

Growth rates were explained by a single best model and this model contained fixed effects of age; treatment and age x treatment interactions (Appendix A5.1). When I consider this model, average growth rate exhibited a significant reduction throughout ontogeny (table 5.4). Furthermore, on average LFT exhibited significantly lower growth rates compared to CT fish, whereas CPT and PST did not (table 5.4). However, when I consider age by treatment effects, LFT fish exhibited significantly lower growth rates during development compared other treatment groups (table 5.4, also see Appendix A5.5 for mean growth rates during ontogeny). Age at first oviposition was also explained by a single best model containing fixed effect parameters of genotype and treatment (Appendix A5.1), being 22 AIC points better than any other model within the candidate set. CPT fish took on average 12.88 days longer to reach sexual maturity (table 5.4). Furthermore genotypes varied significantly in their age at first oviposition (table 5.4).

Table 5.4. Model averaged specific growth rate, age at first oviposition and total eggs laid estimates for parameters of importance identified by the IT approach (effects and confidence intervals denoted in bold script represent non-zero CI's that are significant at the 0.05 α level). $N_{genotype}$ =5 and $N_{treatment}$=4 per sample point during ontogeny (days 31, 91 and 151 of age), N_{total} = 240.

	%Specific Growth Rate				Age at first oviposition				Total Eggs Laid			
Parameter	**Estimate**	**SE**	**Lower CI**	**Upper CI**	**Estimate**	**SE**	**Lower CI**	**Upper CI**	**Estimate**	**SE**	**Lower CI**	**Upper CI**
Intercept	1.32	0.03			114.75	2.52	109.77	119.73	21.30	2.98	16.40	26.20
Age	**-22.40**	**0.85**	**-24.07**	**-20.73**	-	-	-	-				
Genotype: 2	-	-	-	-	-0.25	3.85	-7.85	7.35	-2.44	3.25	-7.78	2.91
Genotype: 3	-	-	-	-	**7.13**	**3.56**	**0.09**	**14.16**	1.46	3.10	-6.56	3.64
Genotype: 4	-	-	-	-	**9.50**	**3.56**	**2.46**	**16.54**	-3.56	3.14	-8.72	1.59
Genotype: 5	-	-	-	-	1.25	3.56	-5.79	8.29	**-9.50**	**3.10**	**-14.60**	**-4.40**
CPT	0.02	0.04	-0.06	0.11	**12.88**	**3.56**	**5.84**	**19.91**	-	-	-	-
PST	-0.02	0.04	-0.10	0.07	-5.63	3.56	-12.66	1.41	**5.32**	**2.44**	**1.30**	**9.34**
LFT	**-0.16**	**0.04**	**-0.25**	**-0.08**	2.39	3.69	-4.89	9.68	1.58	2.44	-2.44	5.60
Age x CPT	-0.55	0.94	-2.39	1.29	-	-	-	-	-	-	-	-
Age x PST	1.07	0.95	-0.79	2.94	-	-	-	-	-	-	-	-
Age x LFT	**3.29**	**0.95**	**1.41**	**5.16**	-	-	-	-	-	-	-	-

In contrast, all potential candidate models for reproductive output were equally good at explaining the data (Appendix A5.1). Model averaged coefficients indicate that genotypes differed in their number of eggs laid (table 5.4). Furthermore, treatment was also an important predictor of egg production with PST significantly increasing the number of eggs laid (table 5.4, Appendix A5.5)

5.4 Discussion

Whilst many studies have documented the occurrence of animal personality, current understanding of the mechanisms generating and maintaining BT variation remains limited. My results show that the rearing environment experienced during ontogeny can have a significant effect on behavioural expression. Interestingly I found that whilst predation risk did not have a significant effect on mean level behavioural scores, this treatment generated repeatability in boldness at the individual level and aggression at both the individual and genotype levels. This result suggests that risk perception as an important factor shaping behavioural consistency and thus the expression of personality. Importantly the presence of conspecifics resulted in lower mean behavioural scores during ontogeny. This finding highlight that the social environment during ontogeny has an important, influence upon the ontogeny of behaviour. Furthermore, I highlight the importance of genetic influences upon behavioural expression, showing that genotypes differed in their average behavioural scores and varied in the extent of environment-specific boldness plasticity i.e. genotype x environment effects. Surprisingly, although specific rearing environments influenced some life-history measures; these patterns were not congruent with those observed for behavioural plasticity. While predictions originating from theoretical research have suggested that labile states are likely to be important in BT expression, the results

reported here suggest that these candidate states (growth and reproductive measures) may be insufficient underpinnings of BT variation during ontogeny in this species.

5.4.1 Conspecific Presence treatment

Individuals reared in the CPT, were less bold, aggressive and exploratory when compared to CT fish. In addition, developmental trajectories for each of the behaviours assayed were on average lower throughout ontogeny when compared to CT developmental trajectories. This species is known to be highly aggressive (Earley and Hsu 2008; Earley et al. 2000a; Hsu et al. 2009; Hsu and Wolf 1999) and individuals are known to modulate aggression when aggregating in fossorial niches (Taylor 2000). In addition, Arnold and Tarborsky (2010) have shown that cooperatively breeding cichlids (*Neolamprologus pulcher)* raised with conspecifics expressed more appropriate social behaviour. In particular, when focal fish were reared with adults, they expressed more threat displays than fish reared with siblings. These findings suggest that during development, the social environment can differentially influence the behaviour expressed by individuals (Arnold and Taborsky 2010). The observed reduction in aggression in the mangrove killifish when reared with conspecifics may thus be adaptive, for example reduced aggression may limit potential injuries and reduce energy expenditure that would otherwise be high when engaging in repeated intense aggressive interactions (Arnold and Taborsky 2010; Earley et al. 2006). It is also particularly interesting that there is a similar reduction in both exploration and boldness scores in the CPT treatment. One potential mechanism that may drive this pattern are pleiotropic effects (Ketterson and Nolan 1999). Previous research has documented that the social environment modulates the serotonergic system via the hypothalamic-pituitary-interrenal-axis (HPI) (see Schjolden et al. 2006 for a discussion). For example, elevated stress responses result in increased serotonin activity which has an inhibitory effect on

aggression (see Nelson and Trainor 2007; Schjolden et al. 2006; Summers et al. 2005). Furthermore, increased serotonin activity has been shown, in multiple vertebrates, to decrease risk taking (Westergaard et al. 2003), and exploration (Winberg et al. 1993) among others (see Reale et al. 2007 for additional examples) in synchrony. The social environment experienced during development may therefore result in a general increase in serotonin activity in response to repeated dominance interactions or social defeat (Hoglund and Winberg 2001; Overli et al. 2004), resulting in reduced average BT expression within this treatment group. Further work investigating conspecific mediated serotonin activity during ontogeny would therefore be rewarding to fully elucidate this mechanism.

While developmental trajectories for each of my measured behaviours were on average lower throughout ontogeny in CPT fish compared CT fish (i.e. lower average age related behavioural expression during ontogeny), the shape of this developmental trajectory differed from other treatment groups. In particular, aggression rate/minute in CPT fish increased during early development reaching a maximum level around sexual maturity, followed by a subsequent reduction following reproductive onset (approx days 100-120). It is important to note that although an increase was observed, aggression rate/minute remained lower on average than CT fish throughout the course of the study. The observed initial increase in aggression prior to sexual maturity may relate to elevated energetic demands in response to the competitive environment experienced, as resources are partitioned between growth and reproductive development (Reznick 1983; Stearns 1992). Scramble competition for a finite resource could favour large body size in relation to within tank conspecifics to maximise resource acquisition (Ward et al. 2006) i.e. increased swimming speed (Beamish 1978), supporting the observed delay in reproductive onset in this treatment group. The observed increase in aggression,

directed towards an unfamiliar, intruding conspecific (mirror image) prior to maturity, could therefore be a mechanism by which additional competitors are excluded from accessing a limited resource. In contrast, reduced aggression following sexual maturity suggests that the finalisation of reproductive development influences the expression of this behaviour, potentially driven by changes in resource allocation and growth-reproduction trade-offs. Future work exploring the mechanisms underpinning conspecific mediated aggression developmental trajectories will therefore be fruitful in the coming years. In addition, exploring the role of food availability within a social rearing environment would also be an interesting future study; to explore how interactive effects within the rearing environments influence behavioural expression.

Importantly, the observed decrease in behavioural scores in response to the CPT, when compared to CT fish, is unlikely to be related to my asocial assaying protocol. This is because a later experiment revealed that boldness responses were not influenced by the social environment within this treatment group. This result is particularly surprising because research documents that boldness is influenced by conspecific presence (Magnhagen and Bunnefeld 2009) and group composition (Magnhagen and Staffan 2005) potentially via social facilitation of risk perception (Webster et al. 2007). In addition, studies investigating cooperative breeding cichlids (*Neolamprologus pulcher*) have also identified that increased group size increases aggression towards a invading conspecific, suggesting that social support positively influences the propensity for aggression towards an intruder (Witsenburg et al. 2010). While these studies used shoaling/schooling or cooperative breeding species of fish (Magnhagen and Bunnefeld 2009; Magnhagen and Staffan 2005; Webster et al. 2007; Witsenburg et al. 2010), *K.marmoratus* is primarily solitary with occasional refuging within fossorial habitats (i.e. land crab burrows) in response to adverse environmental conditions (Taylor 2000;

Taylor et al. 2008b). Further comparative studies investigating plasticity in socially obligate, facultative and solitary species would therefore be an interesting avenue of further research, that may permit understanding of how and when social environments influence behavioural plasticity.

Previous work exploring environmental effects on the expression of personality has tended to focus on predation risk (Bell and Sih 2007; Brown et al. 2007a; Dingemanse et al. 2007; Magnhagen and Borcherding 2008). My results suggest that the social environment may be a major factor, shaping behaviour during ontogeny. Research indicates that ontogeny permits a period where behaviour is flexible (Bell and Stamps 2004; Sinn et al. 2008) and during which social skills are acquired (see Del Guidice et al. 2009 for a review). Social cues during ontogeny therefore have the potential to modulate behaviour, which may be an adaptive response under high population densities. While mean aquaria level behavioural expression was not repeatable, it was not possible to determine individual behavioural consistency in this treatment group (i.e. individuals were not identifiable and thus mean level aquaria scores were used as response variables). Further work exploring how the social environment influences the development of individual consistency within a social context would therefore be rewarding and may provide insight into the potential role of social influences upon personality expression.

Although developmental aspects of personality in non-human animals still remain limited, researchers are beginning to highlight the potential importance of individual experiential factors during early life and their role in the formation of individual differences in BTs (e.g. Arnold and Tarborsky 2010, Stamps & Groothuis 2010a; b). Future work is therefore likely to elucidate the importance of not only the

ontogeny of personality expression, but also how variation in the social environment experienced by individuals influences consistent individual differences in BTs (Bergmuller and Taborsky 2010).

5.4.2 Predation Risk Treatment

Whilst experience of predation risk did not affect average behavioural scores this treatment did influence repeatability. In particular this rearing environment generated BTs i.e. personality. Individuals were for example found to be consistent in both boldness and aggression expressed throughout development, whereas all other treatment showed no consistency. In a meta-analysis of repeatability estimates, Bell et al. (2009) document that repeatability estimates are determined by multiple factors. Here I document exposure to risk during ontogeny as an additional driver of BT repeatability; to my knowledge this study is the first to identify risk perception as a potential developmental determinant of BT repeatability. I suggest exposure to my predator simulation and resulting risk perception during ontogeny may canalise the expression of these BTs. In particular, positive feedback loops (i.e. learning which improves predator escape ability) have the potential to reinforce and stabilise BT differences between individuals, leading to temporal consistency (McElreath et al. 2007; Wolf et al. 2008).

In contrast to my predictions, experience of predation risk during development did not affect average behavioural scores. This is somewhat surprising, because research has implicated predation risk as an important factor in average personality expression (Bell and Sih 2007; Brown et al. 2007a). My results do, however, indicate that this treatment influenced risk perception because individuals laid more eggs on average when compared to CT fish. These responses may be the result of individuals investing

heavily in reproduction, in a fitness trade-off against elevated perceived risk (Reznick and Endler 1982). When taken together these results suggest that current theoretical predictions for the maintenance of personality traits regarding current vs. future reproductive potential (Wolf et al. (Wolf et al. 2007b) may not drive BT variation in certain environmental conditions during ontogeny.

5.4.3 Low food treatment

In the LFT, individuals explored less and had slower growth rates. It is generally well documented that, in some species, smaller individuals can suffer high rates of predation due to size selective mortality (Sogard 1997). We may therefore expect smaller fish to trade-off increased mortality risk in a novel environment against potential recourse location payoffs and thus show reduced exploration. If size selective mortality was a major driver of reduced exploration in LFT fish, however, I would also predict that smaller individual would be shyer within the boldness assay, yet this was not observed. LFT fish did, however, exhibit boldness developmental trajectories that were on average lower than the CT (i.e. lower average age related behavioural expression during ontogeny), providing some evidence that size may influence risk perception in this treatment group. A second possibility that may drive lower exploration scores in LFT fish, relates to reduced expendable energy reserves (Montgomery 1953). Fish may reduce activity when resource location probabilities and potential energetic returns are unknown (Montgomery 1953). Alternatively, LFT fish may explore the arena rapidly followed by reduced activity when resources are found to be absent (Halliday 1968). While LFT fish were smaller during ontogeny, this treatment group did not differ in their age at sexual maturity or reproductive output when compared to CT fish. A reduction in activity may therefore have minimised energy expenditure, while permitting resources to be allocated to reproduction.

5.4.4 Genotype effects

Each genotype exhibited similar exploration and aggression responses (i.e. plasticity) within each respective rearing environment. In contrast, genotypes varied in their boldness response depending upon the rearing environment experienced. These results suggest that while genotypes exhibited similar levels of plasticity in exploration and aggression scores, boldness plasticity was dependent on genotype x environment interactions. Recent work has documented that genotypes differ in the extent of their BT plasticity following specific environmental challenges (Dingemanse et al 2009; Cohen et al 2008; Zhou et al.2008) which is potentially driven by genotype differences in stress responses (Cohen et al 2008). In addition, epigenetic effects may also play a role; generating genotype specific variation in gene expression and the resulting phenotypes expressed, in response to the developmental environment experienced i.e. DNA methylation (Bossdorf et al. 2008). Furthermore, regulatory and/or control regions of serotonin transporter genes have been shown to be characteristically polymorphic in humans (Veenstra-VanderWeele et al. 2000). Polymorphic genotypes have also been shown to be sensitive to the rearing environment (i.e. genotype x environment interactions) in rhesus macaques, *Macaca mulatta* (Champoux et al. 2002). Further work investigating potential underlying genetic mechanisms influencing genotype x environmental interactions in relation to plasticity are therefore set to provide exciting insights in to personality in the coming years in a variety of non-human organisms.

In conclusion, the patterns of BT expression and life-history differed from those predicted by theory; in particular I found few similarities between life-history plasticity and behavioural plasticity. Instead my results indicate that conspecific presence during ontogeny may be a key mechanism contributing to average behavioural expression and flexibility. Contrary to my expectations elevated risk had minimal effect upon average

behavioural scores; however, exposure to risk generated personality (i.e. repeatability), indicating positive feedback loops or stress responsiveness as possible underlying mechanisms. I found genotype x environmental interactions for boldness, further highlighting that genotypes show varied responses to different environmental conditions experienced in the early stages of life. Until recently research investigating developmental aspects of personality have been limited and future work investigating how environmental variability during ontogeny influences the development of BTs will provide further insight in to the factors shaping personality variation and maintenance within populations.

Maternal effects are a mechanism by which the maternal phenotype and/or the maternal environment influence the phenotype of the next generation (Mousseau and Fox 1998). Recent work within the animal personality framework has begun to document that maternal effects have the potential to act upon personality trait expression at several stages of development (Arnold et al. 2007b; Caldji et al. 1998; Groothuis et al. 2008; Ryan and Vandenbergh 2002). The next chapter therefore focuses upon how the parental rearing environments used in this study, influence the expression of personality traits across a single generation.

Parental Rearing Environments do not Influence Transgenerational Expression of Personality Traits

Abstract

While maternal effects have a long history of study within life-history research, maternal effects have only recently been shown to influence animal personality. Here I investigate maternal environmental effects across a single generation. I reared one offspring from each hermaphrodite (reared in each of the four rearing environments utilised in chapter 5) within a standardised environment (N=155). These maternal environmental conditions were maintained until after egg collection was complete, permitting transgenerational environmental effects upon both life-history traits (standard length and growth rate) and behaviour (exploration, boldness and aggression) to be investigated. Results indicate that offspring hatched from hermaphrodites reared within low food and conspecific presence treatments exhibited smaller hatching size in comparison to control treatment derived offspring. This smaller size was further maintained until 30 days of age in low food, but not conspecific presence offspring. In contrast, growth rate during the first 30 days of life was unaffected by the parental rearing environment. Results also suggest that genetics are an important factor shaping behavioural expression across a single generation in this species. These results highlight that life-history, but not behaviour, is directly affected by maternal environmental effects mediated via egg size or egg nutrition. I discuss these results in relation to current theoretical proposals and mechanisms maintaining behavioural variation within populations.

6.1 Introduction

Animal personality research focuses upon individual differences in behavioural types (BTs) that are consistent across time and contexts. For example, some individuals are relatively bold whilst others are shy (Sih et al. 2004b). Research indicates that these consistent individual differences in behaviour, instead of being noise surrounding an adaptive mean, are ecologically relevant, and have fitness implications (Dingemanse and Reale 2005; Smith and Blumstein 2008). While BT consistency is a ubiquitous phenomenon in a diverse array of species, representing multiple taxonomic groups, our understanding of the processes generating and maintaining individual differences in behaviour still remains limited. Currently, research suggests that BTs are genetically encoded, being heritable (Brown et al. 2007a; Dingemanse et al. 2002; Dingemanse et al. 2009; Funder 2001; Kendler and Greenspan 2006; Penke et al. 2007; Suomi et al. 1996; van Oers et al. 2004c; van Oers et al. 2004b) and influenced by natural selection in wild populations (Dingemanse et al. 2004; Reale and Festa-Bianchet 2003). The presence of genetic underpinnings of personality presents several possible processes that may generate and maintain this variation within populations. For example, frequency dependent selection (Roff 1998), sexual selection (Schuett et al. 2010), environmental heterogeneity (Mangel 1991) and a balance between weak selection and mutation (Santiago 1998), are all likely candidates. Although selection acts directly on gene frequencies and gene combinations underpinning personality traits, additional non-genetic mechanisms also have the potential to influence the expression of consistent individual variation. Maternal effects are one example of these non-genetically inherited transgenerational effects that have profound influence upon behaviour and life-history expression.

Maternal effects are a mechanism by which the maternal phenotype and/or the maternal environment influence the phenotype of the next generation (Mousseau and Fox 1998). Maternal effects are favoured when maternal environmental cues are reliable and thus offspring environment can be predicted with some certainty. Adjustment of offspring phenotypes in response to the maternal environment may therefore be a form of 'adaptive transgenerational plasticity' that has the potential to override the direct effects of transmitted genes (Marshall and Uller 2007). Although maternal effects are commonly viewed as adaptive responses permitting offspring to buffer local environmental stressors (Mousseau and Fox 1998), examples of non-adaptive maternal effects are also documented (see Marshall and Uller 2007 for a discussion). Maternal effects have received extensive interest in relation to life-history, and it is widely documented that they can have a long term influence upon morphology and behaviour (Strasser and Schwabl 2004) in some instances being identifiable in grand-offspring (Hafer et al. 2011).

Recent work within the animal personality framework has begun to document that maternal effects have the potential to act upon personality trait expression at several stages of development. For example, maternal variation in androgen concentrations within avian yolk, have been related to boldness (Groothuis et al. 2008) and aggression (Eising et al. 2006). Furthermore, female embryo position, in relation to male embryos in-utero, have been related to aggressiveness, exploration, activity and other behaviours (reviewed by Ryan and Vandenbergh 2002). Organisms which exhibit no parental care can also influence behaviour in the next generation by adjusting oviposition site decisions in relation to environmental cues. Kolbe and Janzen (2001) for example found that neonatal turtles differed in their dispersal patterns and survival depending on the density of vegetation at the nest site. In addition to pre-natal maternal effects, BTs may

also be modulated by mothers during neonatal stages of development. It has been shown, for example that variations in maternal care (Caldji et al. 1998) and parental mediated neonatal nutrition (Arnold et al. 2007b) can influence offspring BTs.

While maternal effects have recently been identified as factors influencing BT variation, researchers have recognised their importance in life-history strategy variation for decades. Differences in maternal resource provisioning to eggs or neonates for example, influence propogule size (Mousseau and Fox 1998), growth rate (Einum and Flemming 1999; also see Mousseau and Fox 1998 for examples) and age at sexual maturity (Lindholm et al. 2006). Within the animal personality literature, current theoretical proposals implicate life-history strategies (growth and reproduction) as potential underpinnings for BT variation and the maintenance of BT's within populations (Stamps 2007; Wolf et al. 2007b). Intuitively we may therefore predict that maternal effects which influence life-history strategies will also influence BTs. Exploring relationships between maternal environments, animal personality and life-history strategies across generations may therefore provide insight in to mechanistic aspects of behavioural variation within populations.

In this study I investigate whether the maternal rearing environment influences personality trait expression and growth rate across a single generation in a controlled laboratory setting. I harvest offspring originating from five homozygous genotypes replicated within and across each of four ecologically relevant rearing environments, which I have shown to influence within-generation behavioural expression during development (chapter 5). Offspring are reared in a standard environment which permits genotype and parental environmental effects to be directly investigated in relation to transgenerational effects upon BTs. I predict that transgenerational environmentally

induced effects upon life-history in the next generation will influence the expression of BTs indicating maternal determination of offspring behaviour. This species has no maternal care and thus I predict that these transgenerational effects will be mediated via environmentally induced differences in egg size or embryonic nutrition generating trade-offs that shape personality.

6.2 Methods

6.2.1 General protocols

All fish were housed in 20ppt synthetic salt water (Instant Ocean^{TM}) at 25˚C ± 0.5˚C in a 12hr light 12hr dark cycle. From each hermaphrodite reared within each ecologically relevant rearing environment presented in chapter 5, five freshly laid eggs were collected between days 230-235 of development (maternal environments were maintained until 240 days of age in the parental generation). For all treatment groups, each parental fish's five eggs were placed in separate 9cm petridishes containing 50ml salt water (20ppt) until hatching (see Edenbrow and Croft 2011 for details). Upon hatching one individual per parental fish was randomly selected and housed singularly in standard rearing aquaria (28 x 17.5 x 16cm) containing 2 litres of salt water and a sparse layer of gravel. (see chapter 4 for details of the standard rearing environment). These individual offspring were maintained within these rearing tanks for 33 days. Complete water changes were carried out on days 15 and 30 and fish were fed between 13.00-15.00 on a daily basis. On days 1-3 fish were fed 0.2ml of micro-worms (*Panagrellus redivivus)* (1mg/100ml suspension (average number of worms per 0.1ml: 23.3±2.6)) and for the remainder of the study fish received 0.2ml (days 4-15) and 0.4ml (days 16-33) artemia naupili (*Artemia salinas*) (average: 35.9±3.9 artemia/0.1ml)).

From each parental treatment; the following number of fish were generated for use in this experiment: control: N= 40, predation risk simulation: N=38 (1 fish failed to produce viable eggs and 1 mortality prior to assaying) and low food: N=37 (1 fish failed to produce viable eggs and 2 mortalities prior to assaying). For conspecific presence fish that were reared in groups of three, one median sized individual per aquaria was selected as the parental fish (N=40) and housed singularly while maintaining visual contact with their rearing partners. The median sized fish was selected as a parental individual to minimise standard length variation among aquaria within this treatment. This was completed because median sized fish exhibited the lowest variation in standard length among aquaria ((mean (mm) ± S.D): 27.1±0.9) in comparison to the largest (30.6±2.9) or smallest (25.9±2.1) fish. From these median sized conspecific presence fish, 5 eggs were collected and 1 hatchling per parental fish used during experiments, N=40 (see above for details). A total sample size of 155 offspring was used in this study representing each of five genotypes/parental treatment (see table 6.1 for genotype designations and number of offspring per genotype/parental treatment).

Table 6.1 Table showing total eggs hatched and successfully reared from each parental genotypes housed in each environmental treatment. All fish (N=155) were successfully assayed for each BT (exploration, boldness and aggression) between days 31 -33 of age.

Genotype	Parental Treatment Group				
	Control	Conspecific Presence	Predation Risk Simulation	Low Food	Total
1: BP11	8	8	8	7	31
2: LK15	8	8	7	8	31
3: LK2	8	8	8	8	32
5: LK6	8	8	7	7	30
6: NNKN1	8	8	8	7	31
N	40	40	38	37	**155**

6.2.2 Standard length measurements

Upon hatching (day 0), and again at day 30, individuals were transferred to a photographing chamber and photographed laterally using a Nikon D60 SLR (18-50mm zoom lens). Photographs were used to measure each individual's standard length (tip of the lower jaw to the posterior margin of the hypleural plate) using ImageJ photo analysis software v1.42.

6.2.3 Behavioural Assays

Behavioural trials were conducted on days 31-33 and each trial was recorded using a Sony ExwaveHad black and white video camera fitted with a Computar Vari Focal 5-50mm F1.3 lens. Ethovision XT version 6.0 (Noldus Information Technology) was used to record each fishes movements within each trial. I assayed three commonly studied behavioural types with assays separated by 24 hours to ensure independence of data points, namely exploration (day 31), boldness (day 32) and aggression (day 33). All 155 fish successfully completed all three behavioural assays between days 31 and 33 of development.

Exploration was assayed using a white novel maze (15 x 15 x 10cm) which was divided in to 24 equal zones (excluding the acclimatisation enclosure) (see Edenbrow and Croft 2011 and chapters 3-5 for arena details). Fish were introduced to the test tank via the acclimatisation enclosure (containing a sparse layer of white gravel) which was surrounded by a white opaque barrier. Following five minutes acclimatisation, individuals were released by remotely raising the opaque barrier. Behaviour was observed for a 10 minute period, during which time I recorded the distance travelled (mm), mean velocity (mm/second), duration of mobility (seconds) and the number of unique squares entered. While fish were exploring the novel maze, gravel was removed

from home tanks, to which identical structural components of the boldness trial arena were added. Fish were housed in these 'mock' arenas for a period of 24 hours following the exploration assay to limit any element of structural novelty during the boldness trials.

The boldness test arena was divided in to two sections along the length (see Edenbrow and Croft 2011 and chapters 3-5 for arena details). Following introduction to the boldness arena all individuals were given a 5 minute acclimatisation period after which time a weight suspended 13cm above the drop zone was released in to the arena, breaking the water's surface. Following the simulated aerial predation event I recorded time (seconds) to first movement (TTM) (categorised as movement of >1 body length), distance travelled (mm) and mean velocity (mm/second) during a 5 minute observation period following the predation simulation. During these trials 154 fish recovered from the drop test within the 5 minute period, one fish did not move during the trial and was given a maximum TTM score of 300 seconds. During the boldness trial I removed 'mock' boldness structures from the test fish's home tank and replaced these with structural components of the aggression arena. Individuals were then housed in these 'mock' aggression arenas for a period of 24 hours prior to aggression assays.

The aggression test arena was divided in to two sections along the length using a clear Perspex partition. 1cm behind this partition, a mirror was fixed. A remotely removable opaque partition, allowing the mirror to be revealed to the test fish, was positioned in front of the mirror and behind the Perspex partition. Following introduction to the aggression arena all individuals were given a 5 minute acclimatisation period after which we ensured fish were in the furthest half of the arena from the mirror zone, before raising the opaque barrier. An interaction zone was

predefined in front of the mirror (2 times the mean standard length of control fish (2cm)). A 10 minute trial then began when fish entered the interaction zone. During each trial I recorded total time spent within the interaction zone together with the frequency of bites and tail flicks directed towards the mirror image. Bites and tail flicks were summed to generate a measure of total aggression. Total aggression was then used to calculate aggression rate/minute based upon the duration of time spent within the interaction zone. Aggression rate was square root normalised and used as a response variable in subsequent analysis.

6.2.4 Statistical Analysis

6.2.4.1 Growth Rate

Standard length measurements were then used to calculate specific growth rate during the study using the following equation (Priestley et al. 2006).

$$\text{Specific Growth Rate [\%]} = 100 \times ((\ln SL_2 - \ln SL_1)/ t)$$

Where SL_2 the standard length at sample 2, SL_1 is the standard length at sample 1, and t is the time in days between measurements.

6.2.4.2 Exploratory principal component analysis

Principal component analysis (PCA) was conducted for exploration and boldness measures, and I found that measures for each assay loaded strongly on to single components that explained a high percentage of total variance (see table 6.2). PCA scores were extracted from this analysis, for each individual, and these scores were used in subsequent analyses. Bartlett sphericity and Kaiser-Meyer-Olkin (KMO) tests (see Budaev 2010 for a discussion) indicated that both exploration and boldness

matrices were suitable for use in PCA (exploration: KMO= 0.811, Bartlett's test $p<0.001$ and boldness: KMO = 0.647, Bartlett's test $p<0.001$). PCA analysis was completed using SPSS v 16.0.

Table 6.2. PCA component loadings and % variance explained for boldness and exploration measures on day 30 of development.

Behavioural Assay	Behaviours Included	Component Loading
Exploration	Distance Travelled (mm)	0.986
	Unique Zones	0.843
	Mean Velocity (mm/s)	0.986
	Mobility Duration (s)	0.956
	% Variance Explained	89.3
Boldness		
	TTM (s)	-0.817
	Distance Travelled (mm)	0.970
	Mean Velocity (mm/s)	0.970
	% Variance Explained	84.9

6.2.4.3 Linear mixed modelling

Linear mixed modelling was completed using R for statistical computing v2.12.0 (R development core team 2010) using the lmer function within the package lme4 (Bates and Maechler, 2009). Using maximum likelihood estimation I investigated the influence of parental treatment and genotype on BT expression and growth rate. BT scores for exploration, boldness, aggression, and life-history measures for growth rate and standard length were inputted as response variables in each respective model. Parental rearing environment and genotype and their interaction were included as fixed categorical effects. I additionally included random effect covariates for individual nested within genotype to control for replication within genotypes. All random and fixed effect covariates were included in the global model; I then refitted all possible reduced models by sequentially removing fixed effects, from which the best explanatory

model for the data was assessed (see Appendix A6.1 for model structures). Normality of model residuals was confirmed by visually inspecting normal probability plots, while homogeneity of variances was confirmed by plotting residuals versus fitted values (Faraway 2006). Models were fitted with Gaussian error and identity link functions (refer to supplementary material for each model structure).

6.2.4.4 Mixed model selection and inference

I assess model goodness of fit for each of our candidate models using the information-theoretic (I-T) approach as described by Burnham and Anderson (2002; 2004). Models were compared based upon AICc (for small sample sizes), by calculating delta AICc (Δ_i) and Akaike weights (w_i) for each respective model (Burnham and Anderson 2002; Burnham and Anderson 2004). If model weightings revealed no single best candidate model (w_i <0.9) I implemented model averaging of estimates and standard error across models <4 Δ_i of AIC_{min}, as described by Burnham and Anderson (2002; 2004) using the MuMln package (Bartoń 2009).In addition I calculate unconditional 95% confidence intervals around estimates, with confidence intervals excluding zero being considered as significant at the 0.05 α level (Nakagawa and Cuthill 2007).

6.3 Results

6.3.1 Behavioural expression

Exploration and boldness expression were both explained by two competing models (<4 Δ_i from the $AICc_{min}$ model) and the average model included first order fixed effects of genotype and the population intercept (i.e. no fixed effect parameters) (see Appendix A6.1). Results indicate an overall effect of genotype upon exploration and boldness; with model averaged estimates indicating that two genotypes were

significantly bolder and more exploratory (table 6.3, figure 6.1a and b). The fixed effect of the parental rearing environment was excluded from both exploration and boldness average models (Appendix A6.1) indicating that there were no transgenerational influences of parental rearing environments upon exploration and boldness BTs (table 6.3, figures 6.2a, b and c).

Table 6.3. Model averaged exploration, boldness and aggression estimates for parameters of importance identified by the IT approach (Effects and confidence intervals denoted in bold script represent non-zero CI's and thus significant at the 0.05 α level).

	Exploration				**Boldness**				**Aggression**			
	Estimate	**SE**	**Lower CI**	**Upper CI**	**Estimate**	**SE**	**Lower CI**	**Upper CI**	**Estimate**	**SE**	**Lower CI**	**Upper CI**
Intercept	-0.09	0.17	-0.43	0.23	-0.26	0.19	-0.64	0.12	0.93	0.07	0.79	1.08
Genotype												
2	-0.04	0.24	-0.52	0.43	0.30	0.23	-0.16	0.76	-0.06	0.1	-0.25	0.13
3	**0.58**	**0.23**	**0.12**	**1.03**	**0.88**	**0.22**	**0.43**	**1.33**	**0.18**	**0.09**	**0.003**	**0.37**
4	-0.36	0.23	-0.82	0.10	-0.08	0.23	-0.53	0.37	-0.05	0.09	-0.24	0.13
5	**0.44**	**0.24**	**-0.02**	**0.91**	**0.54**	**0.23**	**0.08**	**0.99**	**0.13**	**0.09**	**0.05**	**0.32**
Parental Environment												
Conspecific Presence	-	-	-	-	-	-	-	-	0.02	0.08	-0.15	0.19
Predation Risk	-	-	-	-	-	-	-	-	-0.06	0.08	-0.24	0.10
Low food	-	-	-	-	-	-	-	-	0.02	0.08	-0.14	0.20

Aggression rate was explained by two competing models containing genotype and genotype plus treatment effects respectively (see Appendix A6.1). Average model estimates revealed that, although the parental rearing environment in part explained the expression of aggression, confidence intervals surrounding these estimates span zero suggesting that these effects were minimal (table 6.3, see figures 6.2a, b and c). Genotype effects were, however, found to be important, with two genotypes being more aggressive (table 6.3, figure 6.1c).

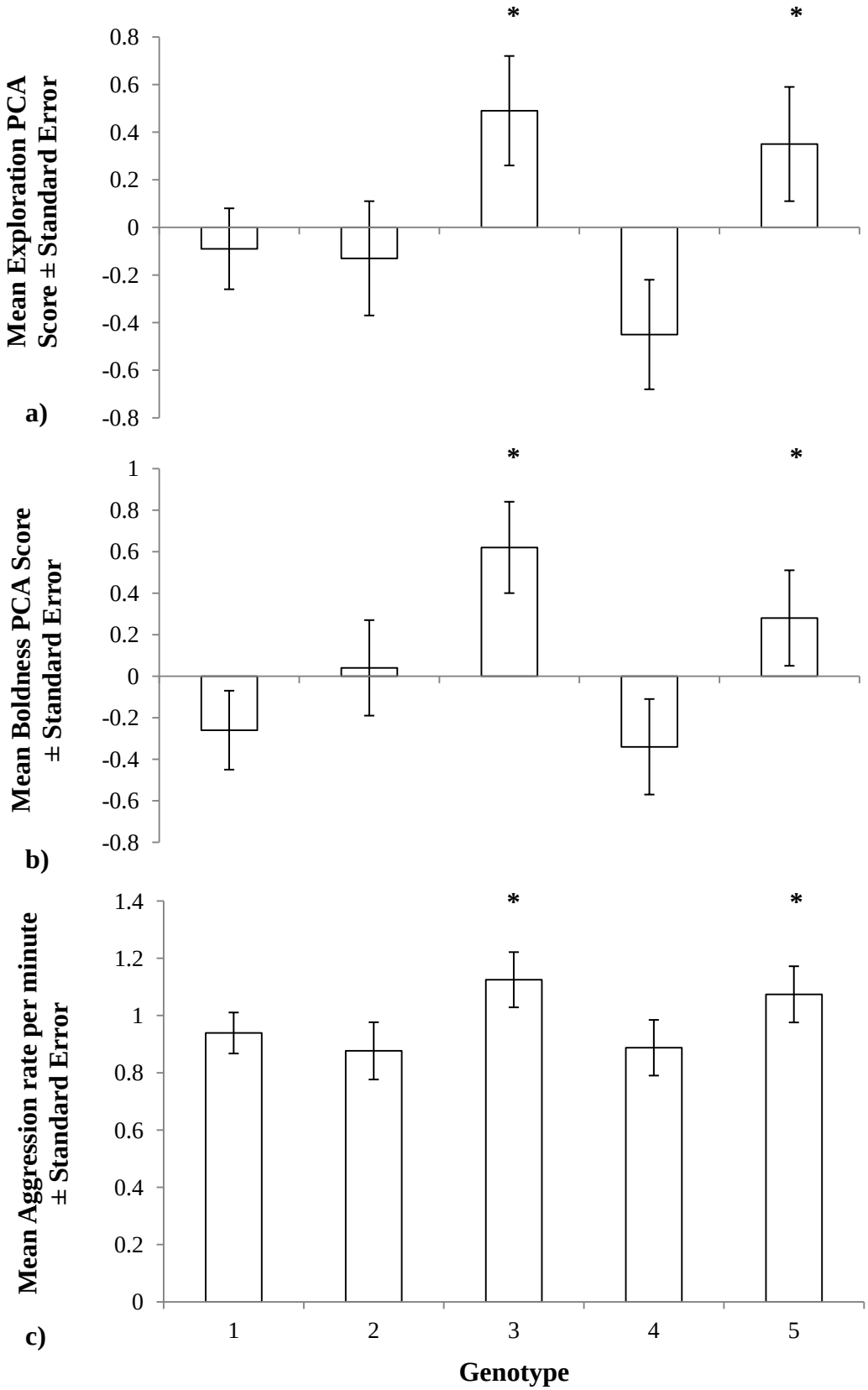


Figure 6.1. Bar charts presenting a) mean exploration PCA scores b) mean boldness PCA scores and c) mean aggression rate per minute for each of the sampled genotypes. Error bars represent standard error, * = genotypes that are significantly different from the reference genotype (genotype 1), $N_{individual}$ =155, $N_{genotype}$ = 5.

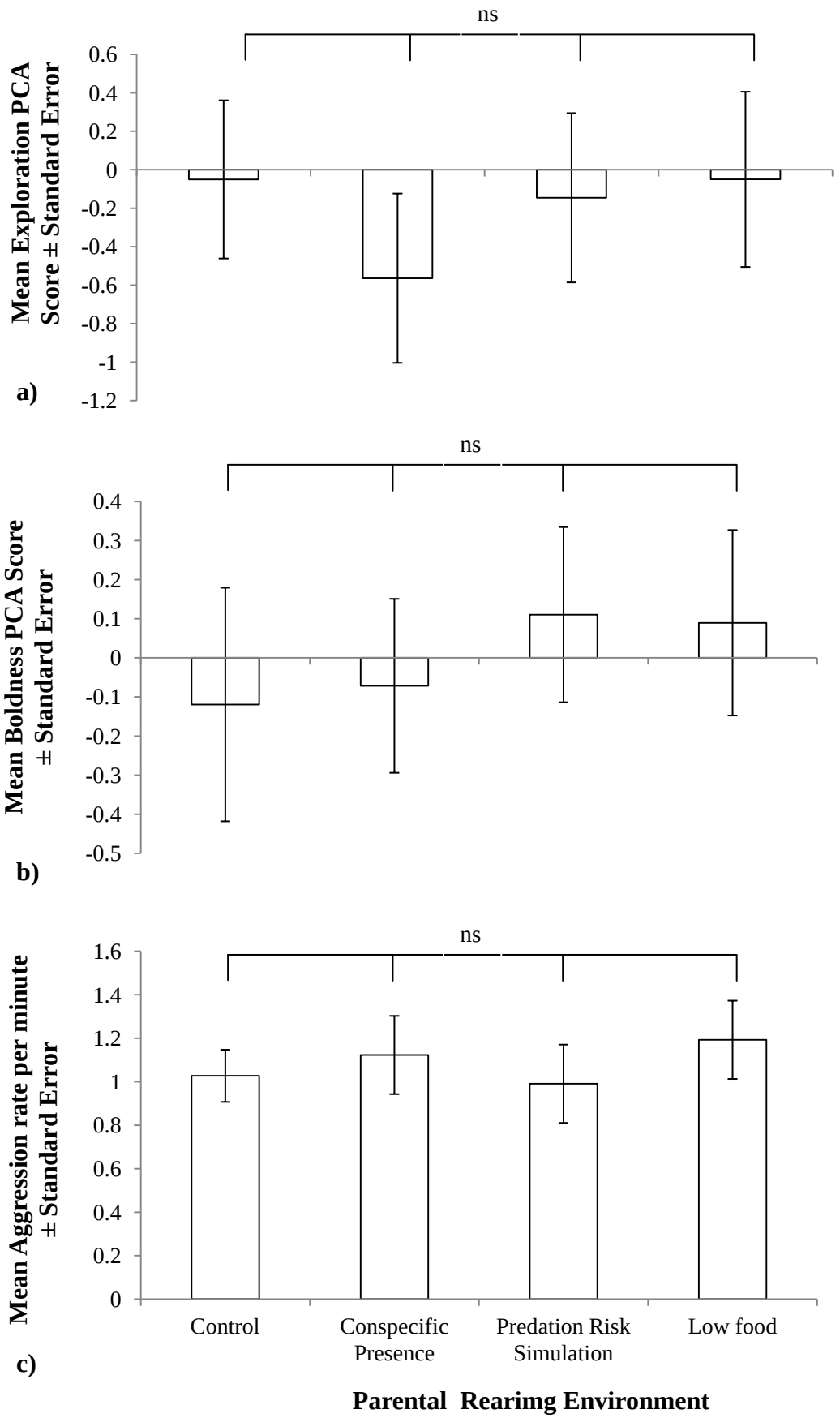


Figure 6.2. Bar charts presenting a) mean exploration PCA scores b) mean boldness PCA scores and c) mean aggression rate per minute for offspring reared from each parental rearing environment. Error bars represent standard error, ns = non significant in comparison to the control group, $N_{individual}$ =155, $N_{genotype}$ = 5.

6.3.1 Life-history

Standard length at hatching (day 2) was explained by three competing models containing the effect of parental treatment, genotype plus treatment, and the null model respectively (Appendix A6.1). Average model estimates indicate that offspring originating from the low food and conspecific presence treatments were significantly smaller than the control group on day 2 post hatching (table 6.4, figure 6.3a), while one genotype was significantly smaller than the other genotypes sampled (table 6.4, figure 6.4a). Two models explained standard length at day 30 and these models contained the fixed effects of genotype plus treatment and treatment effects respectively (Appendix A6.1). The average model revealed that offspring originating from the low food treatment group remained significantly smaller at day 30 compared to other treatment groups (table 6.4, figure 6.3b). This average model also revealed that two genotypes were significantly smaller at this age (table 6.4, figure 6.4b). Furthermore, growth rate was explained by three competing models containing fixed effects for genotype plus parental rearing treatment, genotype, and treatment effects (see Appendix A6.1). Although these effects were included, the average model revealed that all model estimates were surrounded by confidence intervals spanning zero for treatment effects. This suggests that fixed effect parameters for parental environmental rearing conditions had minimal influence upon offspring growth rates throughout the first 30 days of development (table 6.4, figure 6.3c). In contrast the same genotype that was the smallest at day 30 was also found to grow at a significantly slower rate (table 6.4, figure 6.4c).

Table 6.4. Model averaged standard length (day 2 and 30) and growth rate estimates for parameters of importance identified by the IT approach (effects and confidence intervals denoted in bold script represent non-zero CI's and thus significant at the 0.05 α level).

	Standard Length Day 2				**Standard Length Day 30**				**Growth Rate**			
	Estimate	**SE**	**Lower CI**	**Upper CI**	**Estimate**	**SE**	**Lower CI**	**Upper CI**	**Estimate**	**SE**	**Lower CI**	**Upper CI**
Intercept	4.99	0.06	4.87	5.12	10.4	0.15	10.1	10.7	2.4	0.06	2.27	2.52
Genotype												
2	**-0.15**	**0.06**	**-0.28**	**-0.02**	0.006	0.18	-0.34	0.35	0.10	0.07	-0.04	0.25
3	-0.09	0.06	-0.21	0.04	**-0.56**	**0.17**	**-0.89**	**-0.22**	**-0.14**	**0.07**	**-0.28**	**-0.001**
4	-0.09	0.06	-0.22	0.03	0.15	0.17	-0.19	0.49	0.11	0.07	-0.03	0.25
5	-0.10	0.06	-0.23	0.02	0.16	0.17	-0.18	0.50	0.12	0.07	-0.02	0.26
Parental Environment												
Conspecific Presence	**-0.12**	**0.06**	**-0.24**	**-0.005**	0.09	0.15	-0.21	0.39	0.11	0.06	-0.01	0.23
Predation Risk	-0.01	0.06	-0.13	0.10	-0.07	0.16	-0.39	0.23	-0.02	0.07	-0.15	0.11
Low food	**-0.15**	**0.06**	**-0.27**	**-0.04**	**-0.53**	**0.16**	**-0.84**	**-0.22**	-0.08	0.06	-0.21	0.05

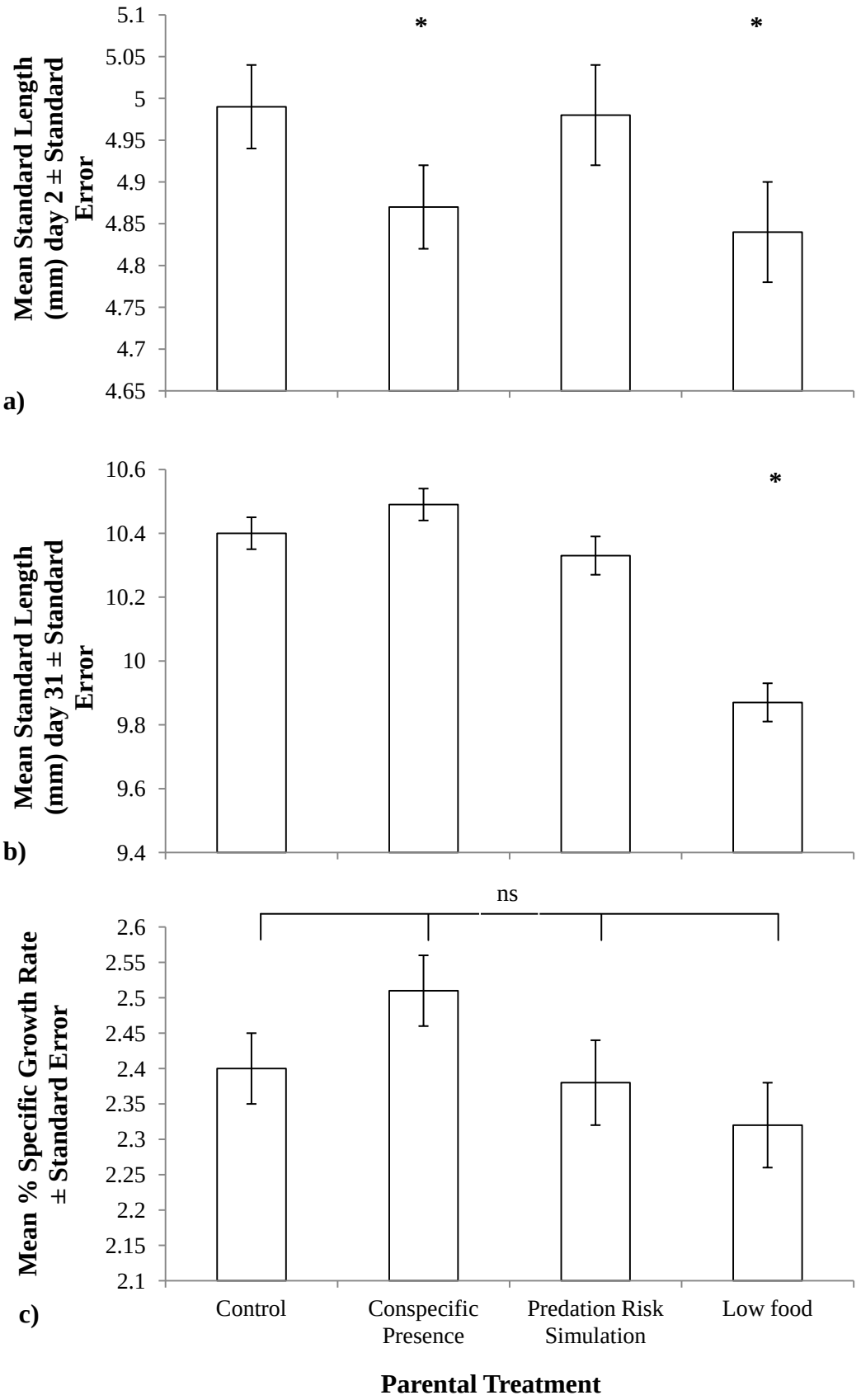


Figure 6.3. Bar charts presenting a) mean standard length (mm) day 2 b) mean standard length (mm) day 31 and c) mean %specific growth rate between days 2 and 31 of ontogeny for offspring reared from each parental rearing environment. Error bars represent standard error, ns = non significant in comparison to the control group, * = significant differences compared to the control group, $N_{individual}$ =155, $N_{genotype}$ = 5.

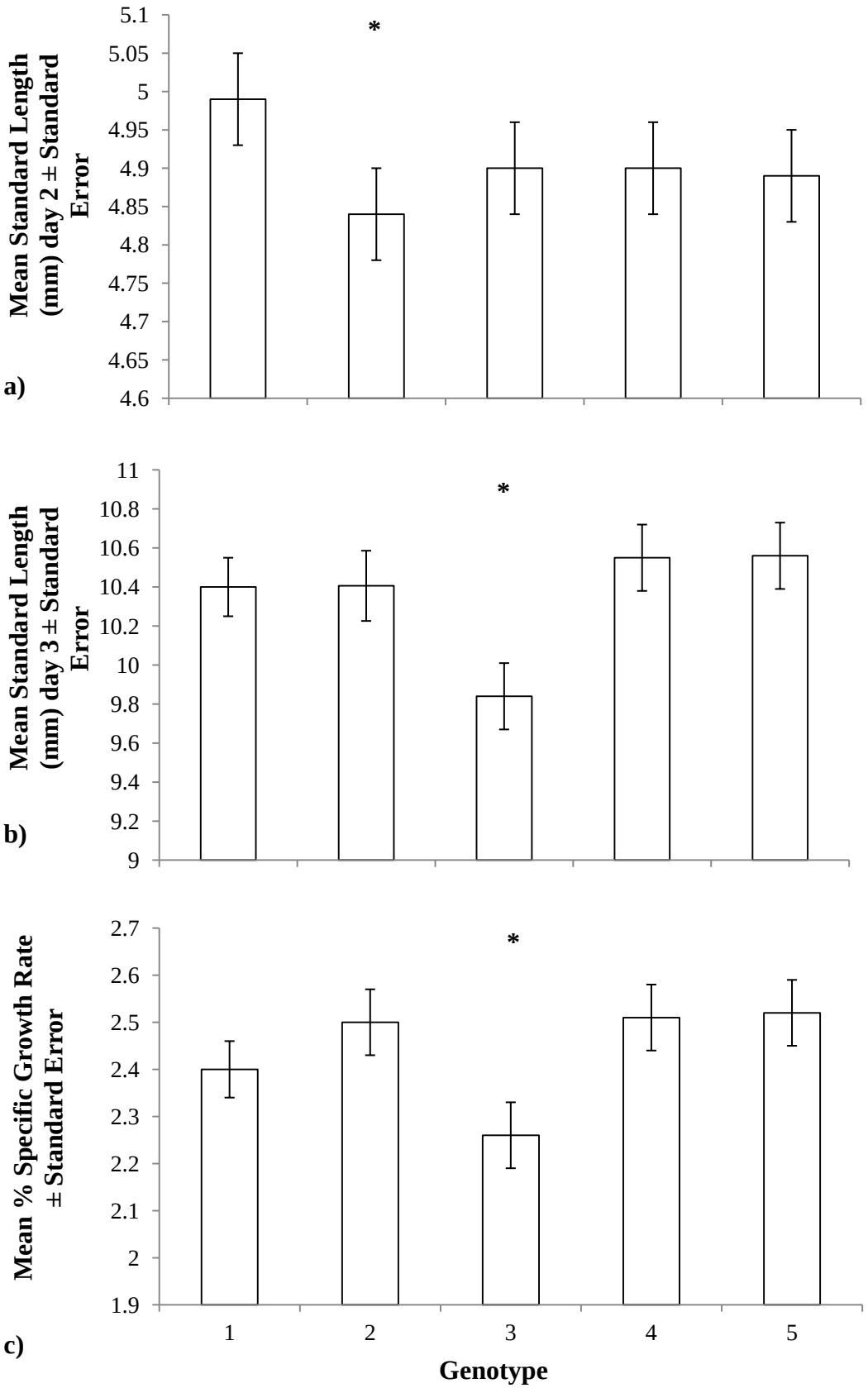


Figure 6.4. Bar charts presenting a) mean standard length (mm) on day 2 b) mean standard length (mm) on day 31 and c) mean %specific growth rate between days 2 and 31 of ontogeny for each of the sampled genotypes. Error bars represent standard error, * = significant differences compared to the control group, $N_{individual}$ =155, $N_{genotype}$ = 5.

6.4 Discussion

Exposure to low food and conspecific presence rearing environments in the maternal generation generated reduced offspring hatching size but had no significant effect upon growth rate during the first 30 days of life. This resulted in low food derived fish maintaining their smaller size until 30 days of age. In contrast, conspecific presence derived offspring did not differ from control fish in their standard length at 30 days of age, suggesting some compensatory growth occurred. In addition, although behavioural expression was plastic within the parental generation in response to the rearing environment experienced (chapter 5), these effects upon behavioural expression did not carry over to the next generation. These results therefore suggest that environmentally induced behavioural plasticity was reversible across a single generation. Interestingly genotype was the sole parameter of importance for behavioural expression, while life-history was only partially influenced by genotype. In particular results indicate that two genotypes were bolder, more exploratory and more aggressive compared to the reference genotype (genotype 1), suggesting a strong genetic component of behavioural expression at 30 days of age. This supports previous research documenting the importance of genetic components in determining behavioural trait variation and expression (Drent et al. 2003; van Oers et al. 2005a; van Oers et al. 2004b). Furthermore, this result corroborates the general importance of genotype that has been highlighted throughout this thesis.

Multiple studies have documented environmentally induced differential resource allocation to eggs as a mechanism by which the maternal phenotype influences the phenotype of the next generation (Mousseau and Fox 1998). For example, lower parental resource availability and intermediate levels of competition select for larger eggs/offspring which provides fitness advantages in the next generation (Bernardo

1996; Brockelman 1975). In contrast, the result reported in this study indicates that these parental environments generated smaller hatching sizes. In this instance parental fish within the low food environment were smaller and thus small eggs/offspring are likely to have been the result of physical constraints upon the maximum sized eggs that could be laid (Bernardo 1996). In relation to conspecific presence, models and empirical studies suggest that intermediate levels of competition may select for few, large offspring, while minimal and high competition select for many, small offspring (Allen et al. 2008; Brockelman 1975). It is therefore possible that the conspecific presence rearing treatment generated a high competition environment and thus small egg size was the optimal strategy in this instance. Although I did not measure egg size variation within and between treatments, hatchling size is likely to be a strong indicator of egg size. To fully determine this potential mechanism, however, exploring egg size variation between treatment groups would be beneficial in the future.

I predicted that growth rates would be highest in fish originating from treatment groups that generated small individuals at hatching i.e. compensatory/catch up growth, even though food availability was standardised. This prediction, in general, is not supported because results revealed no significant differences in growth rates as a function of maternal rearing environment. Although growth rates were similar, it is important to note that conspecific presence fish exhibited the highest growth rates during early development and these growth rates removed hatching size differences in this rearing treatment by day 30. It therefore appears that offspring originating from this treatment group exhibited some compensatory growth. The occurrence of compensatory growth in this treatment group is interesting; and suggests that maternal competition influenced early offspring developmental trajectories. Parental competition may for example, increase competitive ability via elevated growth rate in offspring (Bashey

2007). This maternal effect may therefore be adaptive if competitive environments are encountered in the next generation (Bashey 2007). While it is difficult to determine the precise mechanisms that permitted some compensatory growth in this treatment, it has been long suggested that elevated growth may be achieved by directing resources away from maintenance i.e. tissue repair, towards growth (Ardent 1997; Pendersen 1997). In contrast to the conspecific presence treatment offspring, there was no compensatory growth observed in low food offspring, which maintained their smaller hatching size throughout the first 30 days of development. This result suggests that food limitation in the maternal generation had a large impact upon life-history during early development. These results mirror observations in rats, where maternal calorie restriction generated smaller birth size that was maintained until 12 weeks of age (Woodall et al. 1996). In rats however, catch up growth was delayed and size variation was later eliminated between 12 and 30 weeks of age (Woodall et al. 1996). It is therefore possible that low food derived *K.marmoratus* offspring may exhibit catch up growth at a later stage of development and thus further long term experimentation is necessary to elucidate this possibility.

An additional explanation for the absence of compensatory growth in low food offspring may relate to the 'thrifty phenotype hypotheses' (Hales and Barker 1992; Hales and Barker 2001). This hypothesis suggests that foetuses exposed to low nutrition environments in-utero are subjected to growth and developmental constraints resulting in adaptive growth retardation. In particular, decreased growth rate is thought to be accomplished via metabolic thrift where resources are diverted from less important functions in favour of essential organs, resulting in metabolic rate reduction (see Desai and Hales 1997; Wells 2007 for reviews). In addition, thrifty strategies are thought to present fitness advantages in low resource environments in later life stages in

comparison to non-thrift phenotypes (Desai and Hales 1997). In relation to the results reported in this study, limited nutrition in the parental generation is likely to result in reduced resource allocation to eggs, resulting in a low nutritional environment during embryonic development. If poor embryonic nutrition pre-hatching is driving depressed growth in this parental treatment group, a thrifty developmental trajectory may thus be an optimal strategy, especially if growth rate plasticity is costly during early development (Lindström 1999; Metcalf and Monaghan 2001). For example, if individuals are selected for thrift, compensatory growth may have long term consequences that reduce fitness (Mangel and Munch 2005). The most optimal strategy in low food derived offspring may therefore be to trade-off life-history events i.e. maturity, in order to maintain a lower, and potentially fitter thrifty growth rate (see Metcalf and Monaghan 2001 for a discussion).

In addition, the documentation of some compensatory growth within conspecific presence derived offspring may be expected to generate risk prone behaviour (exploratory, bold and aggressive). For example risk prone strategies may increase resource location and acquisition probability, whereby growth can be maximised and energy diversion from maintenance functions, minimised (Damsgard and Dill 1998; Gotceitas and Godin 1991; Nicieza and Metcalf 1999). In addition, if low food derived offspring are programmed for metabolic thrift during early embryonic development; we may expect that low food offspring would express comparable 'thrifty' behavioural expression i.e. reduced behavioural scores. Behavioural results in contrast do not confirm these expectations; instead offspring originating from each parental environment did not differ in their exploration, boldness or aggression. It is therefore possible that, although life-history was influenced via maternal effects, these exerted minimal or no influence upon offspring behavioural expression. These results confirm

those from chapters 4 and 5 of this thesis which suggest that life-history has limited effect upon the expression of behaviour during ontogeny in this species. These results are therefore additionally unable to support current theoretical predictions suggesting that life-history variation underpins BT variation.

The disassociation between maternally influenced life-history and behaviour expression in this species may be adaptive within the scope of this species natural environment. For example, *K marmoratus* has a long evolutionary history within mangrove habitats that are characteristically heterogeneous in time and space and have developed many adaptations to deal with these i.e. emersion behaviour/aerial respiration and ability to survive under low food conditions for extended time periods (Taylor 1990). An evolutionary history of environmental unpredictability between generations may therefore have selected against maternal effects upon the behaviour expressed because environmental cues may be unreliable across generations. Furthermore, dispersal can result in offspring experiencing heterogeneous environmental conditions and thus maternal effects may be maladaptive (Marshall and Uller 2007). The absence of maternal effects upon behaviour in this study therefore has the potential to be an adaptive response to environmental unpredictability in natural populations. Within this scenario it is likely that individual experience is more important in generating variation in behaviour than life-history strategy determination (Edenbrow and Croft 2011).

One limitation of this study is the use of a single offspring replicate per parental hermaphrodite; future work would therefore benefit from rearing multiple replicates per hermaphrodite per rearing environment. I suggest this as a future direction because research has shown that when environmental conditions are unpredictable, the adaptive maternal strategy may be to bet hedge i.e. parents may increase within-brood phenotype

variation (Crean and Marshall 2009; Marshall et al. 2008). This may, for example, manifest as increased offspring/egg size variation (Marshall et al. 2008; Olofsson et al. 2009) or developmental variation (Simons and Johnston 1997) which is suggested to maximise maternal fitness (Crean and Marshall 2009; Marshall et al. 2008). While maternal bet hedging has generally been related to life-history research, this may be particularly relevant in determining behavioural variation in the next generation. Exploring within-hermaphrodite variance in egg/offspring size and behaviour may therefore allow bet hedging to be investigated within the animal personality framework.

An additional limitation of this study is the relatively short developmental period investigated i.e. 33 days. While this study investigated the early stages of ontogeny, where I predicted that maternal effects would be most apparent (Heath et al. 1999), it is possible that some maternal effects manifest at later life stages. It would therefore be rewarding to explore the ontogeny of behaviour/life-history in offspring derived from differing maternal environments to investigate if and how maternal effects influence behaviour/life-history throughout development. Although I document maternal effects upon life-history and not behaviour in this study, it is clear that this approach was only a limited snap short of the potential transgenerational influences imposed by the maternal environment. Further work may therefore provide valuable and exciting insights, in the coming years that may fully elucidate the role of maternal effects in behaviour/personality expression during the life course.

In conclusion this study shows that the maternal rearing environment, specifically conspecific presence and low food rations, influence life-history traits at hatching (size) and that these maternal effects additionally influence body size in low food derived fish at 30 days of age. Contrary to current theoretical predictions,

behavioural expression was unaffected by the maternal rearing environment and thus environmentally induced behavioural expression in the maternal generation is reversible across a single generation. The absence of maternal effects upon behavioural expression and resulting transgenerational plasticity across generations has the potential to be adaptive within the natural environment from which this species originates, however, further long term developmental approaches are needed to explore this result further. When taken together with the results of chapter 5 this work suggests that individual experiences are an important determinant of personality expression.

While I observe that the social rearing environment is important in determining intra-generation behavioural expression and personality during development (chapter 5), social interactions are dynamic and are non-random. For example, active choice/avoidance of association partners (Croft et al. 2003b; Hoare et al. 2000b) is known to be driven by multiple factors, including phenotype (Croft et al. 2003a; Croft et al. 2003c) relatedness (see Brown and Brown 1996b and; Ward and Hart 2003 for reviews) and familiarity (see Griffiths 2003 for a review). Behavioural trait expression in response to conspecifics during ontogeny may therefore be dependent upon additional social factors. In the next chapter I investigate whether individuals modulate their behaviour based upon familiarity and kin structure of the immediate social environment.

Kin and Familiarity Influence Association Preferences and Aggression in the Mangrove Killifish

Abstract

Association preferences and aggression were investigated in relation to kin and familiarity in the self-fertilising, isogenic vertebrate; the mangrove killifish *K.marmoratus*. My results indicate that fish preferentially associated with, and exhibited reduced aggression towards members of their own genotype (kin), compared to members of a different genotype (non-kin). Furthermore, when fish were presented with stimulus shoals of the same genotype that were familiar or unfamiliar, fish preferentially associated with and exhibited reduced aggression towards familiar shoals. These results indicate that this species prefer to associate with both kin and familiar individuals and modulate aggression accordingly. I discus these results with reference to the adaptive benefits of kin recognition and preferences for familiars, and place the results within the context of current knowledge of the ecology of *K.marmoratus*.

7.1 Introduction

Group living is common amongst Teleost fish (Shaw 1978) and confers multiple benefits relating to foraging (Pitcher et al. 1982), access to mates (Nordell and Valone 1998) and predation risk (i.e. predator sensory confusion (Landeau and Terborgh 1986) and dilution effects (Foster and Treherne 1981), among others (see Godin 1997; Krause and Ruxton 2002 for reviews)). However, grouping may also generate costs including increased competition (Pitcher and Parrish 1993) and elevated disease/parasite transmission (Loehle 1995). Individuals must therefore assess the relative cost and benefits of grouping, in terms of fitness, and make appropriate decisions of when to leave (fission) or join (fusion) a group (Alexander, 1974). These fission-fusion dynamics are complex and have the potential to generate non-random group structures via active choice/avoidance of association partners (Croft et al. 2003b; Hoare et al. 2000b). For example; fish groups tend to be assorted by phenotype i.e. body size (Croft et al. 2003a; Croft et al. 2003c) which can reduce predation risk via the oddity effect (Hoare et al. 2000a; McRobert and Bradner 1998; Ward and Krause 2001).

In addition to assortment by morphological traits (e.g. size), there may also be adaptive benefits associated with assortment by relatedness or familiarity. Multiple species have, for example, been documented to discriminate kin from non-kin, and laboratory studies indicate relatedness as an important mechanism shaping social associations (see Brown and Brown 1996b and; Ward and Hart 2003 for reviews). Reduced aggression towards kin for example, is widely documented to minimise potential injury (Brown and Brown 1996b) while maximising inclusive fitness (Hamilton 1964; Hamilton 1972). Reduced territorial aggression within kin groups has also been found to facilitate increased feeding and growth rates in laboratory studies (Brown and Brown 1996a; Brown and Brown 1996b). Kin discrimination additionally

facilitates inbreeding avoidance (Arnold 2000) and can reduce the likelihood of cannibalism (Pfennig et al. 1993; Pfennig et al. 1994). While some species exhibit innate kin discrimination via self-referent phenotype matching (Holmes and Sherman 1982), others require a period of learning, during which a kin template is developed (Olsén et al. 2002). Learned recognition via phenotype matching is therefore potentially confounded by familiarity.

Familiarity has been widely documented in multiple fish species (see Griffiths 2003 for a review) and can occur irrespective of relatedness. Familiarity has been suggested to confer multiple fitness benefits, for example Chivers et al (1995) document that shoal cohesion and anti-predator responses in fathead minnows (*Pimephales promelas*) are increased in groups of non-related familiar individuals. Moreover, familiarity can increase survival (Seppä et al. 2001), the potential for social learning (Swaney et al. 2001) and reduce aggression (Utne-Palm and Hart 2000) which is likely to result from dominance hierarchy stabilisation (Höjesjö et al. 1998). Context-independent familiarity can take time to develop (Griffiths and Magurran 1997a) and can be constrained by group size (Griffiths and Magurran 1997b). However once established, context-independent familiarity can persist for extended periods of time (Bhat and Magurran 2006; Brown and Smith 1994).

Previous work on social partner preferences based on kinship and familiarity have tended to focus on shoaling (e.g. Griffiths and Magurran 1999) and territorial species (e.g. Brown and Brown 1993). For some fish species social aggregations occur as a result of refuging behaviour in response to adverse environmental conditions (Abel et al. 1987; Taylor 2000; Taylor et al. 2008b) or social conditions, for example sexual harassment (Sims 2005). However, very little is known about the role of familiarity and

kinship in the decisions of species of fish that predominately form aggregations during refuging behaviour.

In this study the role of kinship and familiarity in structuring social interactions in a refuging species; mangrove killifish *K.marmoratus* was examined. *K. marmoratus* is a synchronous hermaphroditic fish that reproduces via self-fertilisation (Harrington 1963). Populations are comprised of individuals that are homozygous across most, if not all of their genome (Kallman and Harrington 1964). In addition, rare males (<1% in some populations) have been suggested to outcross with hermaphrodites, generating heterozygous offspring, which revert to homozygosity following multiple generations of selfing (Mackiewicz et al. 2006a; Mackiewicz et al. 2006b; Mackiewicz et al. 2006c). Homozygosity, coupled with selfing therefore generates multiple distinct genotypes that are each genetically identical within their respective lineage. This reproductive system therefore presents a naturally occurring extreme form of kin in comparison to sexually reproducing species.

Very little is known about the social behaviour of *K.marmoratus* (Martin 2007), however, this species is widely documented to be aggressive towards conspecifics (Earley and Hsu 2008; Earley et al. 2000b; Hsu et al. 2009; Hsu and Wolf 2001; Hsu et al. 2006) and generally considered as solitary (Mackiewicz et al. 2006c). Furthermore, this species regularly leaves standing water (owing to their ability to survive for extended periods of time in moist conditions via respiration across the epidermis (Grizzle and Thiyagarajah 1987; Taylor 1990)), during which time it often refuges in high density aggregations within fossorial habitats such as land crab burrows and emergent logs (Taylor 1990; Taylor 2000; Taylor et al. 2008b). In this study I address two questions; firstly, do individuals that have been reared in social isolation modulate

their association and aggression behaviour based upon a conspecifics genotype (i.e. kin and non-kin) in a free swimming dyadic interaction context? Secondly, do individuals reared in kin groups adjust association preferences and aggression towards within-genotype familiars over within-genotype non-familiars?

7.2 Methods

7.2.1 General protocols

For all housing and experimental protocols fish were maintained in 20ppt synthetic salt water (Instant Ocean^{TM}) and at 25˚C ± 0.5˚C in a 12hr light 12hr dark cycle and fed ad libitum. All trials were video recorded using a Sony ExwaveHad black and white video camera fitted with a Computar Vari Focal 5-50mm F1.3 lens. In experiment 2, I recorded fish movements using Ethovision XT version 6.0 (Noldus Information Technology). Genotypes (BP11, LK2, LK6, LK15 and NNKN1) used in this study were descendents of founder individuals collected from Florida in 2007 which have been maintained in the laboratory since collection. All fish were propagated from a single parental fish and individuals used were from the F3 generation. All fish were fed at 15.00 hours the day prior to behavioural assays to standardise hunger levels.

7.2.2 Experiment 1: Kin

A total of 40 individuals, representing five homozygous genotypes were used in this experiment with eight fish per genotype housed singularly in standard aquaria (280 x 175 x 160mm) containing a sparse layer of 2-4mm gravel and two litres of salt water. All fish (adults) were housed under these conditions from hatching until 300 days of age (± 8 days across replicated tanks) prior to the experiment and thus all fish were unfamiliar (opaque barriers separated tanks).

A test arena (see figure 7.1a for arena structure and dimensions) was constructed using a standard aquarium divided in to two equal sized sections along the length using a removable opaque barrier (as described by Earley and Hsu 2008). All but one side of the arena were surrounded by brown paper to limit disruptions to fish during experiments. The fourth side was left clear to allow each experiment to be video recorded. Tanks contained a sparse layer of gravel and three litres of salt water, which was replaced between experiments.

I tested each fish twice, once with a randomly assigned fish from within their own genotype (kin), and once with a randomly assigned fish from a different genotype (non-kin). The kin and non-kin treatments (separated by one week) and all genotype pairings were balanced so that each genotype was exposed to all of the available non-kin genotypes. I tested half of the test fish in the kin treatment first, followed by the non-kin treatment and vice versa to control for order effects, and all individuals were similar in size (standard length: 28.5mm ± 1.9mm). For identification purposes I marked all individuals with visible implant Elastomer (VIE) for identification (see Croft et al. 2003a for details), one week prior to the behavioural trials. No mortality occurred as a result of the marking procedure.

Twenty-four hours prior to each assay, test fish were placed singularly in to each of the two separated compartments (see figure 7.1a) to allow individuals to acclimate to their new surroundings. In addition, I counterbalanced acclimatisation sections across experiments to control for any differences between these sections. At the time of experimentation the removable partition was remotely raised allowing test fish to interact freely in a dyadic contest (as described by Earley and Hsu 2008). Data were scored from each video and the observer (ME) was blind to the identity of fish or

kin/familiarity treatment to avoid any bias during data collection. From these videos a 10 minute trial began when test fish approached within 60mm (2 body lengths) and the total time (seconds) that fish associated was scored. Associations were defined when individuals were within 2 body lengths of their testing partner. During each 10 minute trial I additionally recorded total aggression frequency (bites and tail flicks) directed towards stimulus fish, for both individuals within each dyad. Each individual's total aggression was then standardised as aggression rate per minute based upon the time spent associating with their test partner.

7.2.3 Experiment 2: Familiarity

One hundred and twenty individuals representing the same 5 homozygous genotypes used in experiment 1 were used for experiments (housed in 40 tanks: 8 replicated tanks per genotype). Fish were housed in groups of three individuals from hatching (± 1 day hatching difference within each tank and ± 14 days across replicated tanks). All fish were 300 days of age at the time of experimentation (± 2 days across replicated tanks) and maintained in standard aquaria (280 x 175 x 160mm) containing a sparse layer of 2-4mm gravel and 2 litres of salt water. All fish within a tank thus had the opportunity to develop social familiarity.

Fish were tested in a binary choice experimental tank (see figure 7.1b for arena structure and dimensions), containing 4 litres of salt water. The experimental tank was divided in to three sections along the length using clear perforated Perspex dividers, permitting olfactory and visual cue transmission. Directly in front of each plastic partition an association zone was defined measuring 60mm (two body lengths) to quantify associations with stimulus fish. Water was replaced with fresh salt water between trials to remove olfactory cues from the testing arena.

From each replicated tank of three individuals, the median sized individual was selected as a focal fish (standard length: 25mm ± 1.5mm) generating a sample size of 37 individuals (3 replicated tanks were not used in experiments due to a single mortality during development). I marked the median-sized individual with visible implant Elastomer (VIE) for identification purposes (see Croft et al. 2003a for details) 1 week prior to behavioural tests. No mortality was observed as a result of the marking process. The remaining two individuals per replicated tank were used as stimulus fish.

Within this experiment, focal fish were presented with two stimulus shoals simultaneously, 1 representing their tank mates (familiar shoal) and the other representing a randomly designated shoal from within the same genotype (unfamiliar shoal) (see figure 7.1b for arena structure and dimensions). Stimulus shoals were counter balanced between stimulus sections of the arena to control for any side preferences. At the start of each experiment the focal test fish was placed in to the centre of the test arena and permitted to enter each association zone to ensure equal information of each stimulus before commencement of a trial (average time to visit both sides and re-cross the centre line = 2.14 ± 1.01 minutes). During a 10 minute trial, beginning when fish re-crossed the centre line of the test arena, I recorded focal fish movements using Ethovision. During each trial I recorded the duration of association (seconds) with each stimulus shoal based upon time spent within each association zone. I additionally quantified total aggression frequency (bites and tail flicks) directed towards each stimulus shoal. Total aggression was then standardised as aggression rate per minute based upon time within each association zone.

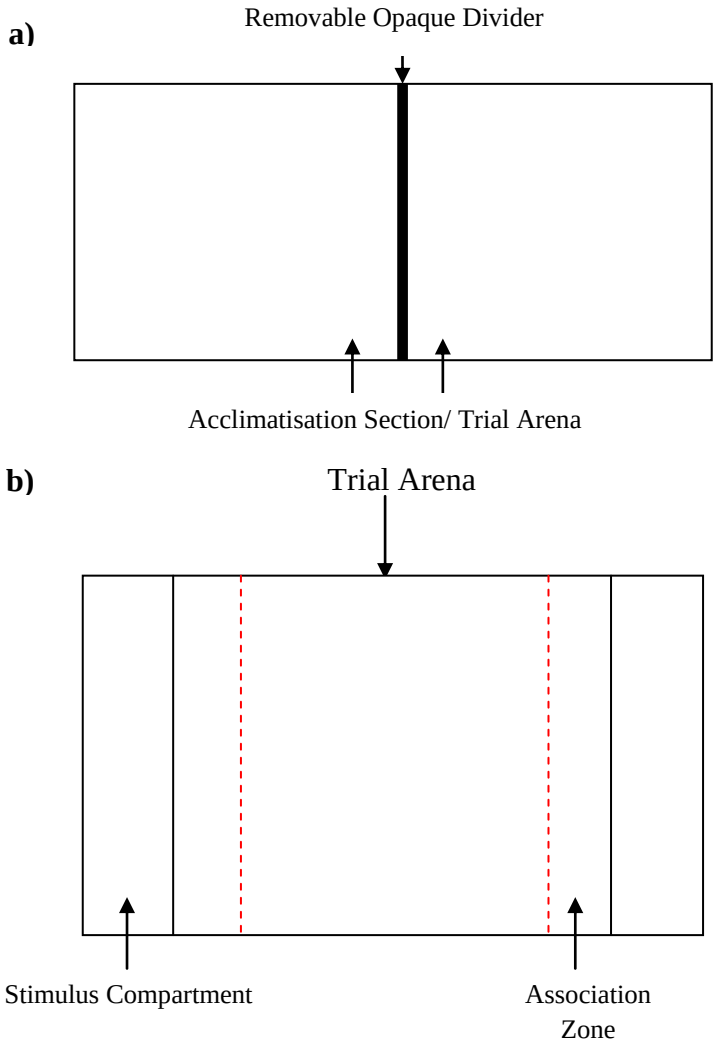


Figure 7.1. Trial arena schematic diagrams for a) Experiment 1: Kin, free swimming dyadic interaction experiment arena measuring: trial arena: 280 x 175 x 160mm, divided in to two acclimatisation sections each measuring: 140 x 175 x 160mm. b) Experiment 2: Familiarity, binary choice arena measuring: Trial arena: 394 x 250 x 140mm, stimulus compartments: 80 x 250 x 140mm and association zone: 60 x 250 x 140 mm, and

7.2.4 Statistical analysis

7.2.4.1 Linear mixed model structure

Linear mixed modelling was completed with R for statistical computing v2.12.0 (R development core team 2010) using the lmer function within the package lme4 (Bates and Maechler, 2009). Residuals for association data exhibited approximate

normal distributions and equal variances; however, aggression rate data did not. Aggression rate was therefore square-root transformed to meet mixed modelling assumptions. I implemented full factorial models, with association duration and aggression rate/min as response variables. Stimulus (familiar/unfamiliar or kin/non-kin) and genotype were also included as fixed categorical effects using maximum likelihood estimation. In addition to fixed categorical effects, I included a hierarchical random effect for individual nested within genotype and an additional random effect for trial. These random effects permitted statistical control for both replication within genotypes and non-independence of measures within trials. All random and fixed effect covariates were included in global models and models were refitted with all possible fixed effect parameter combinations (random effect structure maintained in each refitting), generating a global and a reduced set of candidate models, from which the best explanatory model for the data was assessed (see Appendix A7.1 for model structures).

7.2.4.2 Mixed model selection and inference

I assess model goodness of fit for each of our candidate models using the information-theoretic (I-T) approach as described by Burnham and Anderson (2002; 2004). Models were compared based upon AICc (for small sample sizes), by calculating delta AICc (Δ_i) and Akaike weights (w_i) for each respective model (Burnham and Anderson 2002; Burnham and Anderson 2004). If model weightings revealed no single best candidate model (w_i <0.9) I implemented model averaging of estimates and standard error across models <4 Δ_i of AIC_{min}, as described by Burnham and Anderson (2002; 2004) using the MuMln package (Bartoń 2009). In addition I calculate unconditional 95% confidence intervals around estimates, with confidence intervals excluding zero being considered as significant at the 0.05 α level (Nakagawa and Cuthill 2007).

7.3 Results

7.3.1 Experiment 1: Kin

A single best explanatory model containing stimulus fish (kin/non-kin) as a fixed effect explained association data (see Appendix A7.1). The exclusion of genotype indicates that there were no differences between genotypes in their response to stimulus shoals. Individuals associated for significantly longer with unfamiliar kin compared to unfamiliar non-kin individuals during free swimming dyadic interactions (table 7.1). Model estimates indicate that fish spent on average 83 seconds longer associating with unfamiliar kin over unfamiliar non-kin (table 7.1, figure 7.2a).

Similarly to association preferences, a single best explanatory model was identified for aggression rate which again identified stimulus as the only fixed effect of importance (see Appendix A7.1). This model indicates a significant difference in the rate of aggression directed towards unfamiliar kin and non-kin. In particular, the rate of aggression per minute directed towards kin was significantly lower than aggression towards an individual from a different genetic background (table 7.1). Model estimates identify a reduction of 0.57 aggressive interactions per minute during interactions with kin (table 7.1, figure 7.2b).

7.3.2 Experiment 2: Familiarity

Two competing models were found to explain association preference towards familiar vs. unfamiliar stimuli. These two models contained the fixed effect of stimulus and the population intercept, respectively, but the model containing stimulus scored highest for Δ_i and w_i estimates (Appendix A7.1). The average model estimates identified that focal fish exhibited a significant association preference for familiar fish (table 7.1).

In particular focal fish spent on average 72 seconds longer associating with familiars compared to unfamiliar (table 7.1, figure 7.3a).

Table 7.1. Estimated linear mixed model estimates for Experiment 1: kin ($N_{individual}$=40, $N_{genotype}$=5) (single best explanatory model) and model averaged estimates for Experiment 1: familiarity ($N_{individual}$=37, $N_{genotype}$=5) (averaged estimates across competing best explanatory models) for the association duration and aggression rate differences between familiar/unfamiliar stimulus shoals and non-kin/kin individuals. Table includes estimated standard error and unconditional 95% confidence intervals (CI). Estimates, SE and CI's denoted in bold script identify significant directional effects.

Experiment	**Parameter**	**Estimate**	**SE**	**Lower CI**	**Upper CI**
1: Association duration	Intercept	241.06	20.30	200.56	281.56
	Non-Kin	**-83.23**	**28.71**	**-140.50**	**-25.96**
1: Aggression rate	Intercept	0.39	0.19	0.01	0.77
	Non-Kin	**0.60**	**0.15**	**0.30**	**0.89**
2: Association duration	Intercept	297.00	24.30	249.00	345.00
	Unfamiliar	**-71.70**	**29.80**	**-130.00**	**-13.30**
2: Aggression rate	Intercept	0.59	0.11	0.37	0.81
	Genotype:				
	LK15	-0.16	0.17	-0.49	0.17
	LK2	-0.26	0.17	-0.59	0.08
	LK6	0.15	0.17	-0.17	0.48
	NNKN1	-0.31	0.17	-0.63	0.02
	Unfamiliar	**0.35**	**0.07**	**0.22**	**0.49**

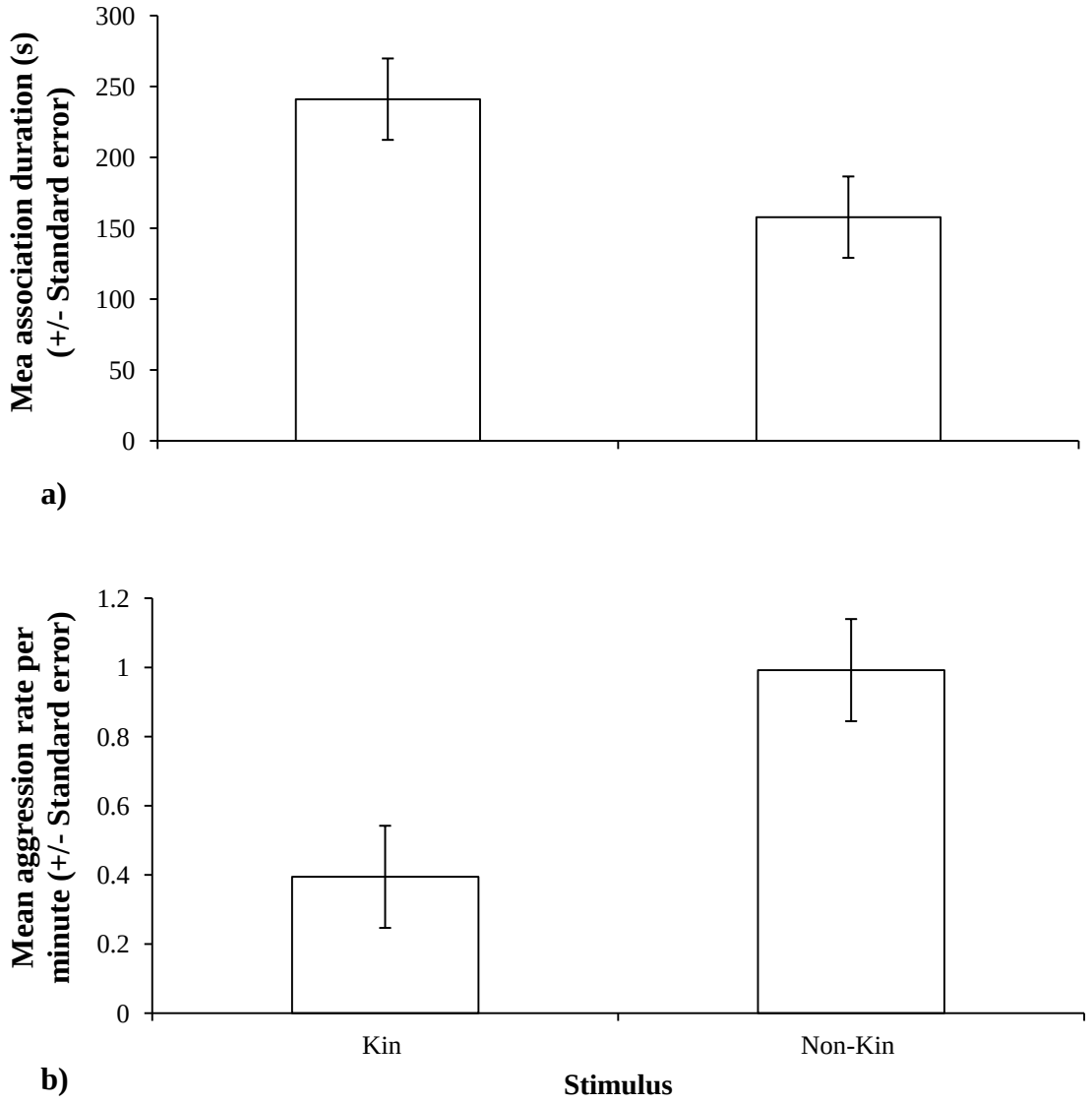


Figure 7.2. Bar charts showing results of a) mean duration of association (seconds) with both kin and non kin individuals ($N_{individual}$=40, $N_{genotype}$=5 and b) mean aggression rate/minute directed towards kin and non-kin individuals $N_{individual}$=40, $N_{genotype}$=5. Error bars represent standard error.

Two competing models were also found to explain aggression rate directed towards familiar and unfamiliar shoals (see Appendix A7.1). These two models contained the fixed effect of stimulus and stimulus + genotype, respectively. The model containing only the fixed effect of stimulus, however, scored highest for Δ_i and w_i estimates. Furthermore, average model estimates identified non-significant differences between genotypes i.e. confidence intervals spanned zero (table 7.1). The rate of aggressive behaviour directed towards unfamiliar shoals was, however, found to be

significantly higher than that of familiar shoals. In particular, focal fish expressed an average increase of 0.35 aggressive interactions per minute towards unfamiliar compared to familiar stimulus shoals (table 7.1, figure 7.3b)

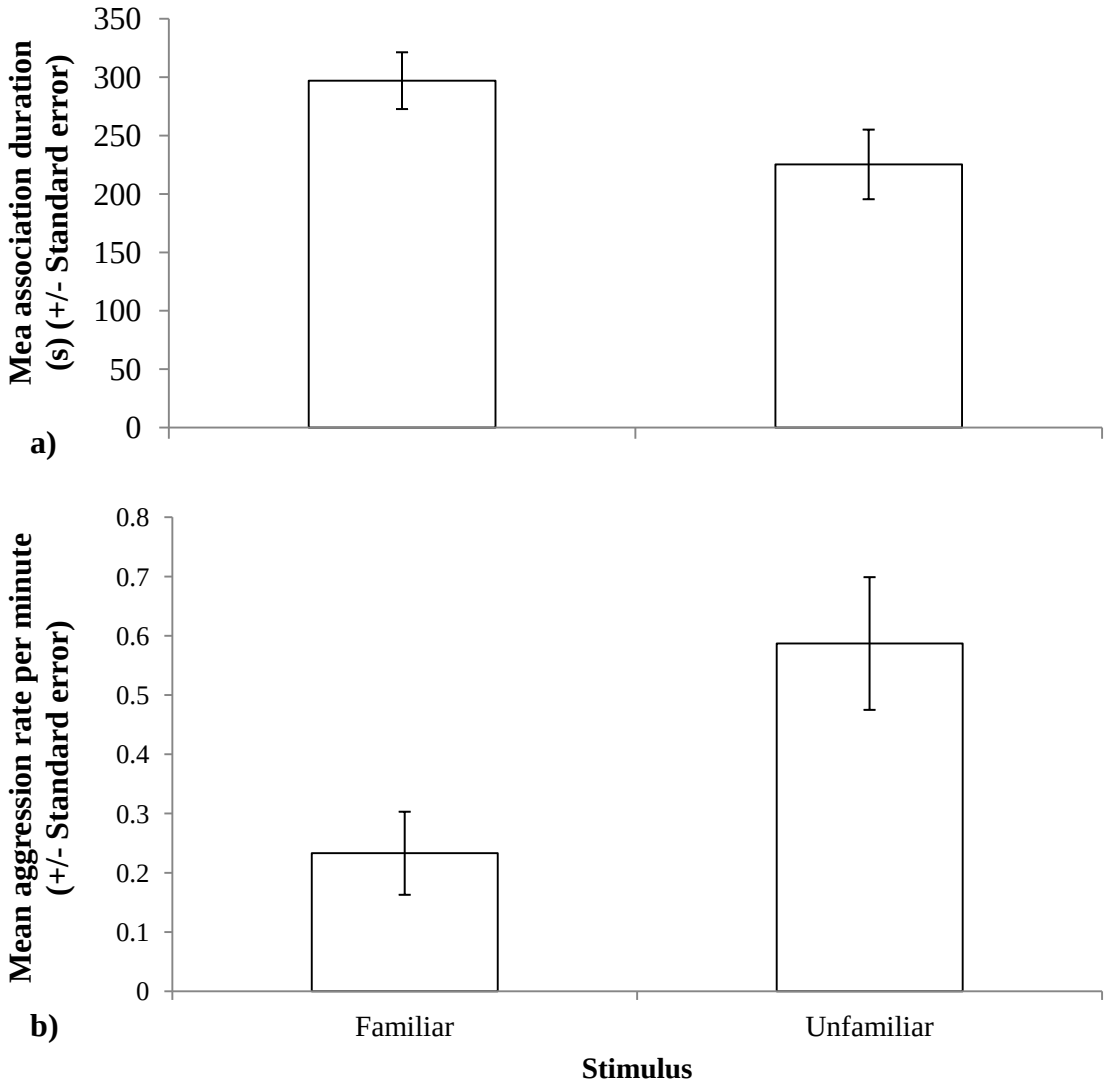


Figure 7.3. Bar charts showing a) mean duration of association (seconds) with both familiar and unfamiliar stimulus shoals ($N_{individual}$=37, $N_{genotype}$=5) and b) mean aggression rate/minute directed towards familiar and unfamiliar individuals ($N_{individual}$=37, $N_{genotype}$=5). Error bars represent standard error.

7.4 Discussion

I found that both kinship and familiarity had a significant effect on social partner preferences and aggression within this species. Results show that individuals who were

socially isolated from hatching preferred to associate with and reduced aggression directed towards kin compared to non-kin. Furthermore, individuals that were reared as part of a kin social group of three individuals preferred to associate with and reduced aggression directed towards familiar over unfamiliar kin.

My results show that fish which were reared in social isolation from hatching showed a significant preference to associate with a member of the same genotype to which they also showed reduced levels of aggression. This result supports the widely documented influence of kin upon association preferences (Arnold 2000; Frommen and Bakker 2004; Van Havre and Fitzgerald 1988) and aggression modulation (see Brown and Brown 1996b for a review in fishes; Walls and Roudebush 1991). It is not surprising that individuals of this species have the ability to discriminate kin from non-kin and thus modulate behaviour accordingly. This is because, self-fertilisation and the resulting homozygosity represents an extreme form of relatedness i.e. within a genotype individuals are essentially monozygotic twins. Kin discrimination ability is therefore likely to have strong effects upon inclusive fitness (Hamilton 1964; Hamilton 1972). Reduced aggression towards kin for example, has been suggested to provide benefits in terms of increased feeding, higher growth rate and reduced competition (Brown and Brown 1996a), while reducing potentially fatal injuries (Brown and Brown 1996b).

An additional, potential benefit of kin discrimination in *K.marmoratus* is in the context of mate choice (Arnold 2000). Within most populations of *K.marmoratus*, males are extremely rare (<1%) and populations are mostly homozygous. In some populations, however, primary gonochoristic males can comprise ~25% of the population (Davis et al. 1990; Turner et al. 1992) which is suggested to facilitate out-crossing, resulting in high levels of heterozygosity (Mackiewicz et al. 2006a;

Mackiewicz et al. 2006b; Mackiewicz et al. 2006c; Taylor et al. 2001). These results may therefore implicate kin discrimination as a mechanism by which out-crossing with non-kin males is achieved. Within androdioecious species, however, selection tends to favour hermaphrodite propagation via self-fertilisation, and thus generates conflict between the sexes (Chasnov 2010). Understanding selection, conflict and kin discrimination in relation to mating decisions in this species and the environmental influences generating population specific heterozygosity patterns, would therefore be an interesting direction for future research.

Although it is widely reported that organisms have the ability to discriminate kin from non-kin, research exploring kin assortment in natural environments has reported equivocal results (Avise and Shapiro 1986; Naish et al. 1993; Peuhkuri and Seppa 1998) while others find support for kin assortment (Buston et al. 2009; Fraser et al. 2005; Gerlach et al. 2001; Piyapong et al. 2011). There is also evidence suggesting that in natural conditions fitness benefits may not be as important as suggested by theory. For example, Greenberg et al. (2002) document that individuals within kin groups grow at slower rates when compared to individuals within groups containing mixtures of siblings and non-siblings and this may be related to heterogeneous advantage outweighing kin selection in free ranging conditions (Griffiths and Armstrong 2001). Future work with this species in both the laboratory and field would therefore be rewarding to determine if aggregation within fossorial niches is structured by kin and if assortment influences fitness within these habitats.

Results suggest an innate kin-recognition mechanism via self referent phenotype matching (Holmes and Sherman 1982) because individuals were isolated from hatching until the time of testing. Studies have identified that olfactory cues are important

components of phenotype matching (see Brown and Brown 1996b for a review), however, the exact molecules that are used for self referent phenotype matching still remain unclear. One possibility is the major histocompatibility complex and associated variation in urinary and pheromone composition (Olsén et al. 2002; Olsén et al. 1998; Penn 2002). Although innate recognition is a likely candidate for kin discrimination in this study, learned template formation during embryonic development (pre-hatching) cannot be ruled out, because eggs were housed in clutches until hatching. Evidence does, however, suggests that within coho salmon *Oncorhynchus kisutch* learned templates are formed post hatching and not during the embryonic stage (Courtenay et al. 2001). Courtenay *et al*., (2001) suggested that odours generated by conspecific embryos may not reach sufficient concentrations or be of a sufficient quality to allow the development of a learned template. In addition, Gerlach *et al*., (2008) documented a 24 hour widow for kin discrimination template formation at 6 days post hatching in zebrafish (*Danio rerio*). This study attributed the absence of olfactory template formation prior to day 6 of development as either delayed expression of olfactory receptors or the absence of olfactory cues. Moreover Harden *et al*., (2006) report that zebrafish exposed to an olfactory compound during early development results in up-regulation of genes associated with olfactory apparatus around days 2-3 post hatching. During propagation of each genotype within this study, clutches of eggs were subjected to water changes every 48 hours during embryonic development. It is therefore unlikely that genotype specific olfactory cues reached sufficient concentrations during embryonic development or that apparatus essential for template formation was not sufficiently developed at hatching. An innate recognition mechanism therefore seems likely in this study, however, future work exploring the potential for embryonic olfactory cues and template formation prior to hatching would be necessary to completely address this possibility.

My results additionally indicate that *K. marmoratus* reared in kin groups developed social familiarity and preferred to associate with familiar individuals. Furthermore aggression rate was reduced during interaction with familiar fish. These results are complimentary to the widely documented familiarity influences upon association preferences (Brown and Smith 1994; Brown and Colgan 1986; Croft et al. 2004; Dugatkin and Wilson 1992; Griffiths and Magurran 1999; Magurran et al. 1994; Miklosi et al. 1992; Van Havre and Fitzgerald 1988) and aggression (Beaugrand 1997; Höjesjö et al. 1998; Johnsson 1997; Morris et al. 1995; Utne-Palm and Hart 2000).

K. marmoratus are known to be highly aggressive (Earley and Hsu 2008; Earley et al. 2000b; Hsu et al. 2009; Hsu and Wolf 2001; Hsu et al. 2006) and generally considered as solitary (Mackiewicz et al. 2006c). However, the species is well documented to congregate at high densities within land crab burrows (Taylor 1990), suggesting that grouping may be either beneficial, or crab burrow availability limited. Either way, refuging is likely to present a platform for social interaction within this species and thus familiarity may facilitate the development and stabilisation of dominance hierarchies (Höjesjö et al. 1998). This in turn may be a potential driver of reduced aggression which is commonly documented within these fossorial habitats (Taylor 1990). Whilst very little is generally known about this behaviour in the species, fish can remain within a refuge for up to 9 months (Taylor 1990) therefore, environmental conditions are likely to vary between emersion within the fossorial niche and re-emergence. If fish remain in groups during initial stages of re-emergence, familiarity may provide a number of adaptive benefits including increased shoal cohesion and anti-predator responses (Chivers et al. 1995), as well as increased cooperative tendencies (Dugatkin 1997) which may influence survival (Seppä et al. 2001). Exploring these possibilities in carefully constructed laboratory and field studies

would therefore be extremely rewarding and thus future work addressing these predictions will be beneficial.

Although this study did not directly investigate the exact sensory modalities used in familiar conspecific discrimination, both visual and olfactory cues were available and both have received support in the literature (Brown and Smith 1994; Brown and Colgan 1986). The use of different sensory modalities have, however, been found to be species specific and thus further work exploring the importance of both visual and/or olfactory cues and their role in familiar individual discrimination would be beneficial in this species.

In conclusion, I highlight that *K.marmoratus* has the ability to discriminate kin from non-kin and familiar from un-familiar individuals, potentially due to self referent phenotype matching and individual/olfactory recognition respectively. Preferential association with, and reduced aggression towards kin and familiars therefore has the potential to influence fitness in natural environments. Furthermore, this species' propensity for refuging provides an interesting social situation in a predominantly solitary species which may lay a foundation for the development of familiarity. Furthermore, kin discrimination may also facilitate kin assortment during refuging. Further research in both the laboratory and field exploring kin and familiarity in relation to decision making, population differences in reproduction and fitness benefits are therefore likely to yield exciting insights in to association and population dynamics of this elusive and enigmatic species.

General Discussion

The aim of this thesis was to examine the influence of genotype and the biotic environment, and how these interact to shape the expression of personality, from a developmental perspective. In this chapter, I summarise the findings presented and discuss the implications for this research area. I consider directions for future research by reviewing the unanswered questions that have arisen during this thesis and discuss the potential for mangrove killifish as a model for investigating phenotypic plasticity. Specifically, I discuss microsatellite data confirming high levels of homozygosity and genetic independence of founder individuals, from which I later propagated a laboratory population for experimental work. I then move on to focus upon the developmental perspective linking results from several chapters throughout this study to explore possible trends and patterns. Within the developmental perspective I focus upon three main sections, namely; the ontogeny of personality, environmental effects during ontogeny and life-history strategy as a mechanism underpinning BT expression during development. I also discuss how immediate social environments relating to kin and familiarity influence behaviour.

8.1 The mangrove killifish: a powerful model organism

In chapter 2, I confirm the occurrence of high levels of homozygosity within founder mangrove killifish, collected from both natural populations and supplied from other research institutions. Furthermore, I identify 20 genetically independent genotypes from which I founded and propagated a laboratory population for use within experiments. The documentation of high within individual homozygosity supports the documentation of self-fertilisation and resulting formation of isogenic genotypes in this species (Harrington 1963; Harrington and Kallman 1968; Kallman and Harrington 1964; Mackiewicz et al. 2006c; Tatarenkov et al. 2009). The mangrove killifish is therefore an excellent model organism that is particularly suited to several disciplines.

The use of isogenic genotypes is, however, by no means a new approach within science, with several hundred isogenic mouse models generated to date in the medical sciences (Beck et al. 2000). Isogenic genotypes present a valuable tool because research can benefit from low variation both between measurements, and between replicates (Tatarenkov et al. 2010). While many of the model organisms used within this approach are generated via artificial selection, the occurrence of naturally occurring isogenic organisms, such as the mangrove killifish, present an exciting opportunity. In particular, isogenic genotypes in the mangrove killifish are a product of natural selection making this species useful within ecology and evolution research. The mangrove killifish, however, has rarely been adopted as the organism of choice, outside of carcinogenesis and ecotoxicogenomics research (Lee et al. 2008). This is particularly surprising considering this species' small size, ease of maintenance and simplicity of propagating high numbers of offspring within the laboratory. This being said, within behavioural ecology a small number of researchers have noticed its potential (e.g. Earley and Hsu 2008; Hsu et al. 2009).

One of the most relevant approaches to which, the mangrove killifish is suited is the study of phenotypic plasticity. In the strictest sense, phenotypic plasticity describes the ability of a genotype/clone to produce multiple phenotypes in response to environmental gradients (Pigliucci 2001). In recent years phenotypic plasticity has been proposed on several occasions as a highly useful framework within the burgeoning study of animal personality i.e. the reaction norm approach (Dingemanse et al. 2010; Nussey et al. 2007; Stamps and Groothuis 2010a). This approach permits us to explore environmental determination of behaviour i.e. genotype differences and genotype specific plasticity, simultaneously. For example ecological factors, response to selection, or experiential effects may all be investigated using the mangrove killifish,

while controlling for genetic variation. This approach is therefore set to permit us to begin to tease apart how genetic and environmental influences, as well as interactions between the two, generate the complexities of personality we see in the natural word.

8.2 Developmental perspectives in animal personality

In a variety of taxa, the documentation of consistent individual differences in BTs and suites of functionally different BTs, suggests a general phenomenon in the animal kingdom (Bell 2007; Reale et al. 2007; Sih et al. 2004a). While this is accepted, researchers have only recently begun to address the proximate and ultimate drivers of consistent individual differences in non-human personality (see Dingemanse and Wolf 2010; Stamps and Groothuis 2010a for discussions). While the current change of focus from categorising BTs and syndromes in different species, to understanding the whys of personality is a particularly exciting time, there are currently some major gaps in the literature. Personally I, and others (e.g. Groothuis and Trillmich 2011; Stamps and Groothuis 2010a; Stamps and Groothuis 2010b), believe that one important but overlooked aspect of personality, is the developmental perspective.

For example, consistency is a key ingredient for the documentation of personality that has implications for our interpretation of the patterns we observe. However, the vast majority of research has investigated consistency in the short term and assumed that this relates to long term consistency, potentially for the entire life of the organism (Smith and Blumstein 2008). Unsurprisingly, meta-analysis has revealed that increased test-retest intervals, decreases repeatability estimates observed (Bell et al. 2009) suggesting individuals are at least partly plastic over longer time periods. This in turn has quite large implications for research, for example; changes in the selective forces between years have been shown to differentially effect BTs (e.g. Dingemanse et

al. 2004; Reale and Festa-Bianchet 2003). However, these studies have assumed individuals to be repeatable across years and thus either subjected to higher or lower selection pressures accordingly. While this assumption may not be problematic, long term repeatability over the life course still needs to be confirmed to have unwavering confidence in these interpretations.

Interestingly, in a recent study Bell and Sih (2007) show that plasticity can occur in response to selection, suggesting that some individuals in a population may adjust their behaviour to new environmental conditions in an adaptive manner. Furthermore, research suggests that social aspects can influence the expression of BTs (Magnhagen 2007; Magnhagen and Bunnefeld 2009; Magnhagen and Staffan 2005), while Cohen et al, (2008) show that exposure to a single predator odour cue can generate plastic responses in mice. These studies therefore suggest that not only can personality be plastic and have implications for studies that assume long term consistency within their interpretation, but different experiences are likely to influence individual BT expression. Experiential effects upon personality therefore appear to be important in BT expression, and are beginning to be discussed, and empirically addressed within the literature (Cohen et al. 2008; Stamps and Groothuis 2010a). In contrast, developmental perspectives, have received limited attention (but see Bell and Stamps 2004; Edenbrow and Croft 2011; Sinn et al. 2008), even though they permit different aspects of both ontogeny and experiential/environmental influences to be investigated simultaneously (see Groothuis and Trillmich 2011; Stamps and Groothuis 2010a; Stamps and Groothuis 2010b for detailed discussion). At the current time there are several unanswered questions in the literature regarding BT development (see Groothuis and Trillmich 2011; Stamps and Groothuis 2010a; Stamps and Groothuis 2010b for detailed discussion). In this thesis I aimed to address three specific questions

relating to BT development; namely; i) Is BT expression plastic during ontogeny? ii) Are correlations between BTs age specific? and iii) Do specific ecologically relevant environments shape the ontogeny of personality?

8.3 The ontogeny of personality

During the past decade there have been some, although limited, studies that have addressed BTs and behavioural syndrome development (Bell and Stamps 2004; Edenbrow and Croft 2011; Sinn et al. 2008). The results reported in chapter 4 reveal interesting results, in particular, I find that individuals within each genotype were not repeatable i.e. did not exhibit personality during development. In addition, I found that behavioural developmental plasticity varied considerably between genotypes potentially indicating genetic constraints (DeWitt et al. 1998; Pigliucci 2005). Genotype variation and individual flexibility may therefore permit developmentally flexible genotypes to respond adaptively to differing environmental conditions (Scheiner 1993; Schlichting 1986), while less flexible genotypes may be limited to fewer suitable habitats or face environment-BT mismatch (DeWitt et al. 1998). However, if a less flexible genotype remains within, or disperses to a favourable environment fitness may be maximised. Investigating genotype specific constraints upon commonly studied personality traits and their fitness consequences in the field would therefore be rewarding to understand the ecological consequences of this pattern. In addition, further work investigating the potential for niche picking i.e. dispersal into optimal habitats, and/or niche construction (Stamps and Groothuis 2010a), may indicate a potentially adaptive response that offsets genotypic constraints.

During ontogeny, strong correlations between behaviours additionally emerged at day 61 and were maintained throughout the rest of the study i.e. bold genotypes were

more exploratory, indicating these behaviours are expressed in concert from this age onwards suggesting some aspects of personality are present. The non-independence of these behaviours suggests an underlying mechanism that limits the potential for independent behavioural expression. In this instance, I suggest that pleiotropic effects of sex hormones as a likely mechanism generating these patterns (Ketterson and Nolan 1999). I suggest hormones here because *K.marmoratus* begin to secrete oestrogen, androgen and progestin between 34 and 46 days (Minamimoto et al. 2006) which falls in the middle of the inter-test interval in this study (i.e. sampled at day 31 and 61). The emergence and maintenance of behavioural correlations therefore appears to mirror sexual development. It is however, important to note that hormonal effects will be species-specific for example, Bell and Stamps (2004), documented that correlations between behaviours were unstable during hormonal restructuring at sexual maturity in sticklebacks.

As mentioned in chapter 4, the emergence of behavioural correlations appears to mirror sexual development suggesting reproductive hormonal influences may be a driver of behavioural covariance in this instance. Additional hormones may, however, also play a role in behavioural syndrome emergence during ontogeny. In particular, hormones associated with the hypothalamic-pituitary-adrenal axis (HPA), or the analogous system in fish: the hypothalamic-pituitary-interrenal axis (HPI), are possible alternative underlying mechanisms in this instance. For example many of the hormones associated with these pathways, including corticosteroids (stress hormones), serotonin and vasopressin etc, have been shown to vary by age and be influenced by experience (Deville et al. 2003; Wada et al. 2009). Moreover, these hormonal pathways have been shown to influence BT and behavioural syndromes expression (see Carere et al. 2003; Deville et al. 2003; Hoglund et al. 2008; Koolhaas et al. 1999; Martin and Reale 2008;

Martins et al. 2007; Schjolden et al. 2006; Veenema et al. 2003 for examples). Further longitudinal research characterising multiple hormonal profiles and investigating links with behavioural expression during development would therefore be an interesting future direction. This may then permit us to determine how specific hormones secreted at key stages of development influence the expression of behaviour in this and other species.

The simultaneous documentation of behavioural correlations and behavioural plasticity/individual behaviural inconsistency in chapter 4 additionally supports the work of Bell and Stamps (2004) that also highlighted plasticity in behaviour even though syndrome structure was maintained. The presence of behavioural syndromes are therefore not reliant upon behavioural consistency to be present in individuals within a population and thus greater understanding of why traits are mutually expressed is needed. For example, if behavioural covariance is determined by pleiotropic effects (Ketterson and Nolan 1999; Sih et al. 2004b), identifying candidate genes and/or hormones will provide valuable insights in to when and how behavioural covariance is likely to occur. Moreover, candidate gene/hormonal approaches may provide a greater understanding of why behaviours often correlate in some but not all species/populations (Bell 2005; Brydges et al. 2008; Dingemanse et al. 2007) as well as how and why repeatability is present in some instances and not others. Moreover these approaches will provide insight in to how and when behavioural plasticity and behavioural syndromes can be mutually expressed during ontogeny, as shown in this thesis and also by Bell and Stamps (2004). Interestingly, I show in chapter 3, that adults of this species (males and hermaphrodites) exhibit short term individual level repeatability (i.e. personality) for all three of my measured behaviours. While my inter-test intervals in both infant/juvenile (chapter 4) and adult individuals were different (i.e. 30 and 7 days

respectively), the documentation of both developmental flexibility and adult personality may indicate a canalising effect of age, similarly to that observed in humans (see McCrae et al. 2000; and Roberts and DelVecchio 2000 for discussions). Further controlled research is, however, necessary to fully explore this possibility. In particular, utilising equal inter-assay intervals throughout all life stages is needed to gain insight in to life-time behavioural expression and the potential for age-dependent personality.

At the present time, there is a major under-representation of developmental approaches within the animal personality literature using non-model organisms. While general patterns have been documented in adults across multiple species, developmental perspectives are set to provide a greater understanding of if, why and when these patterns are expressed in the natural world. Currently, further studies would benefit from focusing upon how behavioural plasticity, individual flexibility and behavioural syndrome structure varies by species during development. Moreover, investigating physiological and molecular underpinnings of developmental plasticity, individual flexibility and the formation and maintenance of behavioural syndromes would be particularly rewarding.

8.4 Environmental determination of behavioural expression during ontogeny

Phenotypic plasticity, where a single genotype can produce different morphological, life-history and behavioural responses depending upon the environment experienced, is widely documented in the life-history literature (DeWitt et al. 1998; West-Eberhard 1989). In recent years personality studies have begun to investigate the propensity for plasticity, with reports revealing that consistency (i.e. individual rank order) can be maintained even though plasticity occurs. This has been shown for example, in response to temperature (Biro et al. 2010), food availability/competition

(Carere et al. 2005a), group composition (Magnhagen and Bunnefeld 2009; Magnhagen and Staffan 2005), and predation pressure (Bell and Sih 2007). In a developmental sense, plasticity has the potential for experiences and environmental effects to be incorporated in to personality expression at later life stages (Stamps and Groothuis 2010a; Stamps and Groothuis 2010b) and thus experiential influences may generate adaptive personality expression (e.g. Bell and Sih 2007). In chapter 5, I investigated behavioural and life-history during ontogeny in response to 4 rearing environments, namely; predation risk, low food, conspecific presence and a controlled standard environment to determine how these ecologically relevant environments influence behavioural expression and the development of BTs. My results show that conspecific presence during ontogeny had a large influence upon average behavioural expression and average behavioural developmental trajectories, while low food and predation risk has minimal effects.

One of the most widely documented environmental influences thought to determine BT expression and syndrome structure is predation risk (Bell 2005; Bell and Sih 2007; Brown and Braithwaite 2004; Dingemanse et al. 2009; Herczeg et al. 2009; Magnhagen and Borcherding 2008). Surprisingly, and contrary to expectations, I document that exposure to the predation risk simulation during ontogeny had minimal effect upon behavioural plasticity i.e. there was no average difference between this behavioural expression in this rearing environment and the control group. It is possible that this was the result of my simulation protocol not generating the expected response, however, I observed that this rearing environment influenced reproduction in the direction predicted for elevated risk perception e.g. increased number of eggs laid (Reznick and Endler 1982). Interestingly, this treatment also generated behavioural consistency (BTs) suggesting risk perception may have a canalising effect upon

behavioural expression within-individuals during development. The presence of repeatability in this treatment group additionally suggests that risk perception during early development may reinforce and stabilise behavioural variation within a population, potentially via positive feedback loops (i.e. learning which improves predator escape ability) (McElreath et al. 2007; Wolf et al. 2008). It therefore seems that while average behavioural expression was unaffected, risk perception is likely to be a key component driving the expression of personality during development. Further comparative research between populations differing in predation regime and the resulting influences upon BT development and population specific repeatability in relation to learning and endocrine responses may elucidate potential mechanisms driving these patterns.

In contrast to risk perception, I document that the presence of conspecifics has the largest effect upon behaviour. In particular this rearing environment resulted in a reduction in average behavioural expression throughout ontogeny. This result implicates importance of social environments and their effect upon behaviour which are beginning to be discussed in the literature (e.g. Bergmuller and Taborsky 2010). In this instance I suggested aggression modulation, which has been documented within high density fossorial niche aggregations (Taylor 2000), as an adaptive response to the social environment resulting in coordinated reductions in boldness and exploration via pleiotropy (Ketterson and Nolan 1999). One potential hormonal influence may be the hypothalamic-pituitary-interrenal-axis reactivity and associated feedback upon the serotonergic system resulting in behaviour inhibition (see Schjolden et al. 2006 for a discussion). The developmental social environment may therefore result in a general increase in serotonin activity and subsequently inhibit behavioural expression. Further work investigating conspecific mediated serotonin activity during ontogeny would

therefore be rewarding to fully elucidate this mechanism. While average behaviour was markedly affected, there was no evidence that this treatment generated personality i.e. repeatability. It is, however, important to highlight that these repeatability estimates were assessed at the aquaria level, because individuals could not be identified within each aquaria. Further work exploring within aquaria repeatability may additionally provide insight in to the potential for social interactions, dominance hierarchy formation and social niche partitioning as a driver of consistent individual differences in behaviour during ontogeny (Bergmuller and Taborsky 2010).

8.5 Kin and familiarity

While I observed that the presence of conspecifics is important in determining behavioural expression during development, social interactions are extremely complex with individuals adjusting their behaviour and association preferences based upon specific characteristics of their social partners. For example, the structure of social groups tend to be non-random, with active choice/avoidance of association partners (Croft et al. 2003b; Hoare et al. 2000b) being driven by multiple factors, including phenotypic assortment (Croft et al. 2003a; Croft et al. 2003c) relatedness (see Brown and Brown 1996b and; Ward and Hart 2003 for reviews) and familiarity (see Griffiths 2003 for a review). The ability to discriminate kin (see Brown and Brown 1996b and; Ward and Hart 2003 for reviews) and familiar individuals (see Griffiths 2003 for a review) in turn permits behaviours such as aggression (Brown and Brown 1996b) to be modulated and confers multiple benefits, relating to predation risk (Chivers et al. 1995) survival (Seppä et al. 2001), and social learning (Swaney et al. 2001).

In chapter 7, I show that *K.marmoratus*, a facultatively social species that congregates in high densities in the fossorial niche (Taylor 1990), has the ability to

discriminate conspecifics based upon both kin (unfamiliar) and familiarity (controlling for kin) and express appropriate, potentially adaptive plastic responses to the immediate social situation. In particular fish associated for longer periods and reduced aggression (a commonly studied BT) directed towards both kin and familiars. This result supports the widely documented discrimination abilities and subsequent behavioural modulation in social species (see Brown and Brown 1996b and; Griffiths 2003; Ward and Hart 2003 for reviews). Future work investigating the potential for kin and familiarity discrimination in the wild and how this influences population dynamics i.e. dispersal, aggregation and behavioural interaction, would be interesting to see how these patterns influence natural populations.

These results together with results presented in chapters 3 through 5, indicate that differences in genetics, rearing environment and experience are likely to be important in shaping animal personality and behavioural expression. In natural habitats for example, gene-environment interactions, risk and sociality, and resulting phenotypic expression, is dynamic. Slight variation in experiences and stochastic effects within a single population during ontogeny may therefore differentially influence the resulting individual phenotype expressed. The resulting accumulation of individual specific experiences during early life may therefore be an important mechanism influencing inter-individual variation in BTs. The ability to replicate within and between genotypes of *K.marmoratus* presents an exciting opportunity to build upon the results presented within this thesis. For example it would be rewarding to investigate how specific experiences accumulate and shape intra-individual variation, how rearing environment variability and experience intensity i.e. risk, influence the development of BTs. Furthermore, understanding how specific experiences influence different life stages (i.e. sensitive developmental periods) would additionally be an interesting avenue to explore

in the coming years. Obviously the first step in addressing these possibilities will focus upon laboratory research and thus the mangrove killifish presents a highly suitable model organism that is set to be a central component in developing and addressing these concepts empirically in the coming years.

8.6 Maternal effects

In addition to behavioural and life-history plasticity in response to the rearing environment during ontogeny, I postulated that life-history plasticity would influence offspring behaviour in the next generation. Maternal effects are long recognised to be important mechanisms by which the maternal environment/phenotype can influence the phenotype of the next generation (Mousseau and Fox 1998). For example life-history research suggests that offspring size is influenced by the maternal phenotype, resulting in increased fitness in the next generation (Rossiter 1991). In contrast, relatively little work has explored maternal effects upon personality or behavioural traits commonly studied within the animal personality literature (but see Arnold et al. 2007b; Caldji et al. 1998; Groothuis et al. 2008; Ryan and Vandenbergh 2002).

In Chapter 6, I present results revealing that, although behaviours were influenced within the parental generation in response to the rearing environment experienced (chapter 5), these behaviour patterns were not expressed the next generation. This result is somewhat surprising considering that offspring derived from low food and conspecific presence mothers were smaller at hatching and this size dissimilarity was maintained for the first 30 days in low food derived young. These results suggest that while life-history traits were influenced via maternal effects, behavioural expression was not. While this result is interesting I stress that this does not mean that maternal effects are unimportant in behavioural or personality expression, far

from it, these results instead suggest that these environmental treatments had little effect upon behaviour in this instance. Further work exploring additional rearing environmental conditions may, however, generate large effects upon BT and behavioural expression across generations.

Interestingly, I also report that behaviour and life-history showed dissimilar patterns i.e. life-history was effected but behaviours were not, and this seems to corroborate the findings reported in chapters 4 and 5, which indicate that life-history is unlikely to explain inter-individual differences in behaviour (see below for detailed discussion). In contrast I instead found that behavioural data were primarily explained by genetic influences (genotype) suggesting these commonly studied personality traits were not determined via non-genetic maternal effects in this instance. In the future I would, however, like to investigate these maternal effects using a larger sample size which would permit relationships between behaviour and life-history to be statistically investigated (e.g. correlations) which was not possible in this instance Moreover I would additionally like to investigate maternal effects over a longer period of development to assess maternal effects upon repeatability to explore if these environmental conditions in the maternal generation resulted in personality within the next generation.

8.7 Mechanisms underpinning BT variation: do life –history strategies matter?

Empiricists highlight two ubiquitous phenomena in the animal personality literature; i) individuals consistently differ in their behaviour and ii) functionally different behaviours co-vary. In recent years theoreticians have made significant advances in addressing potential explanations for the evolution and maintenance of these consistent individual differences in personality (see Dingemanse and Wolf 2010

for a discussion). This has given rise to multiple theoretical and verbal models, each of which generates testable predictions that feedback in to the empirical literature. Unfortunately while significant theoretical advances are apparent, relatively little empirical testing has proceeded these advances (but see Adriaenssens and Johnsson 2011; Bell et al. 2011; Careau et al. 2009; Conrad and Sih 2009; Kobler et al. 2009). Two of the first proposed models focused primarily upon labile states relating to life-history tradeoffs. Particularly, predictions from Stamps (2007), suggest fast growth, while Wolf et al (2007b) predicts that earlier reproduction (i.e. asset protection (Clark 1994)), could both result in bolder, more aggressive and exploratory BTs. The predictions of these two models were addressed within my experimental protocols outlined in chapter 4. Surprisingly, within a standard rearing environment, I documented no significant differences between genotypes in growth rates during development which directly contrasts with a study by Gregada et al, (2005). However, the small number of genotypes sampled (i.e. only 2) within Gregada et al, (2005) study is likely to be driving the inconsistencies with the results reported here (using 20 genotypes). In addition, a recent genetic study (Tatarenkov et al. 2010) has identified that one of the genotypes used by Gregada et al, (2005) namely, the PAN-RS genotype, aligned more closely to sister species, *K.ocellatus* than to *K.marmoratus.* It is therefore likely that this comparison may represent an inter-species comparison.

During development I also documented no relationship between growth rate and either exploration or boldness, even though I observed considerable variation in the developmental trajectories of these behaviours. The observed general absence of a relationship reported in this thesis also supports recent work that documents growth as an unlikely driver of behavioural and BT variation (Adriaenssens and Johnsson 2011; Bell et al. 2011; Conrad and Sih 2009). When I considered the predictions of Wolf et al,

(2007) I document some trends between reproduction and behaviour, however, these were not significant after correction for multiple tests. Although these trends were not significant they suggest an opposite relationship to those predicted. Greater replication at the genotype level is therefore necessary to fully elucidate this hint of reproduction as a possible underpinning of exploration tendency.

In chapter 5, I additionally explored the potential for growth rate and reproduction as drivers of behavioural variation when fish were reared within differing environmental treatments. Unfortunately, during this study I was unable to analyse potential relationships directly (i.e. correlation analysis) because, owing to the number and time consuming nature of these experiments, only 5 genotypes were utilised. I did, however, observe differential trends for both life-history and behavioural expression i.e. low measures for life-history traits did not result in low measures of behaviour, suggesting that the predicted patterns were not supported in this instance. This was particularly surprising because these environments were chosen to differentially influence either life-history (low food) or BTs (predation risk and conspecific presence). I therefore expected that if BTs and life-history traits are related, plasticity in one trait would result in coordinated plasticity in the other and that repeatability would be generated. While this was generally not observed, half food rations had a significant negative effect upon both growth rate and exploration suggesting a relationship. However, I observed no effect upon boldness or aggression which does not support this prediction. It is possible that reduced exploration in this instance was related to limited energy reserves and thus reduced activity, instead of an underlying driving relationship. Furthermore, while the presence of conspecifics generated the largest reduction in behavioural scores compared to control groups, life-history measures were not affected

in the same direction by this rearing environment. These results therefore suggest that labile states are unlikely to be direct underpinnings for the expression of BTs.

One interesting result I observed related to genotype-specific plasticity in boldness in response to the rearing environment. While all genotypes exhibited a reduction in response to conspecific presence, genotypes differed in the magnitude of their response. This result may potentially indicate genotype specific differences in stress responses (Cohen et al 2008), epigenetic effects i.e. DNA methylation (Bossdorf et al. 2008), or genotype specific serotonin transporter gene polymorphisms (Champoux et al. 2002; Veenstra-VanderWeele et al. 2000). Further work exploring potential underlying genetic mechanisms that determine genotype x environmental interactions are set to provide exciting insights in to personality in the coming years in a variety of non-human organisms.

In summary, life-history states are unlikely to be directly related to behavioural expression during ontogeny regardless of the environmental conditions experienced, whereas genetic influences and gene x environment interactions differentially effect behavioural expression and plasticity in the mangrove killifish. The ontogeny of behaviour is important, and the presence of genotype-specific developmental plasticity has consequences for research especially when BT consistency is assumed. Furthermore, experiential effects during development provide a possible explanation for the ubiquity of behavioural variation observed in the natural world. I also highlight that while risk perception influences repeatability and is likely to contribute to consistent individual differences in personality, experience with conspecifics during ontogeny, which has largely been neglected in the literature, has a large effect upon the expression of behaviour.

Chapter 1

A1.1: Confirmation letters from the Home office regarding project licence 30/2713 awarded to D.P Croft and The University of Exeter ethics approval confirmation letter under which all experimental protocols were approved.

Home Office

No. PPL 30/2713

ANIMALS (SCIENTIFIC PROCEDURES) ACT 1986

PROJECT LICENCE

to carry out a programme of scientific procedures
on living animals

In pursuance of the powers vested in him by the above Act, the
Secretary of State hereby licences

Dr D P Croft
The University of Exeter
School of Psychology
Washington Singer Laboratories
Perry Road Exeter
EX4 4QG

to carry out the project specified in paragraphs 1, 18 and 19 of the Schedule subject to the restrictions and provisions contained in the Act and subject also to the limitations and conditions contained in this licence and to such other conditions as the Secretary of State may from time to time prescribe.

Under this project licence number PPL 30/2713 the Secretary of State grants authority only for the work specified on the Schedule. The deputy project licence holders, the procedures and animal types which may be used, the permitted purposes and the place or places at which the work may be carried out are specified in the Schedule. Authority is granted only for the severity limits attached to individual procedures specified in paragraph 19 of the schedule.

This licence, unless earlier revoked, shall be in force until
12 April 2015.

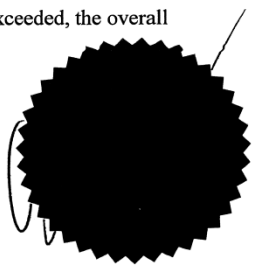
Home Office
2 Marsham Street
London SW1P 4DF

For the Secretary
of State

12 April 2010

NB. This licence does not authorise the project licence holder or any other person to carry out procedures on any animals unless he/she holds a personal licence issued under the Act which authorises him/her to carry out those procedures on the animals of those types.

The overall severity band attached to this licence is **Mild**.
If in practice the severity limits of the individual procedures are significantly exceeded, the overall severity band may require adjustment after discussion with the Inspector.



WORKING TOGETHER TO PROTECT THE PUBLIC

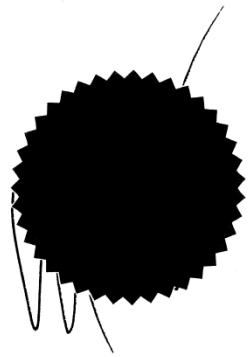
Home Office

No. PPL 30/2713
12 April 2010

PROJECT LICENCE - ADDITIONAL CONDITIONS

This Licence is subject to the following additional conditions -

No additional conditions.



WORKING TOGETHER TO PROTECT THE PUBLIC

UNIVERSITY OF EXETER

SCHOOL of PSYCHOLOGY
ETHICS COMMITTEE

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Telephone +44 (0)1392 264626
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To: **Mathew Edenbrow**
From: **Cris Burgess**
CC: **Darren Croft**
Re: **Application 2008/137 Ethics Committee**
Date: **April 7, 2009**

The School of Psychology Ethics Committee has now discussed your proposal. The project has been approved in principle, for the duration of your study, on the condition that a Home Office license is issued.

The agreement of the Committee is subject to your compliance with the British Psychological Society Code of Conduct and the University of Exeter procedures for data protection (http://www.ex.ac.uk/admin/academic/datapro/). In any correspondence with the Ethics Committee about this application, please quote the reference number above.

I wish you every success with your research.

Cris Burgess
(Acting) Chair of School Ethics Committee

Chapter 3

A3.1 Assessing the most appropriate measure of exploratory behaviour

Statistical methods

I used PCA and extracted a composite score which included exploration (unique zones entered) and activity measures (distance travelled (mm), mean velocity (mm/s) and duration of mobility (s)). Each of these variables within the PCA loaded strongly on to a single component that explained a high percentage of the total variance, as described in the main text of chapter three. In addition, I isolated a single pure measure of exploration, namely the number of unique zones entered during the exploration assay. To test each of these behavioural scores, and to explore whether using PCA scores or a pure exploration score explained the same patterns in the data, I implemented mixed modelling statistical approaches in the same manner as described in chapter three. Briefly, I implemented Linear mixed modelling for exploration/activity PCA scores (Gaussian error and identity link (residuals were normally distributed)) and Generalised linear mixed models for pure exploration (Poisson error structure and log link function (count data is non-normally distributed)). Using multi-model selection and inference techniques within the Information-Theoretic approach (IT) (Burnham and Anderson 2002; Burnham and Anderson 2004, also see main text of chapter three for details), I identified the best fitting models for each variable separately. In addition, using multi-model inference approaches within the Information-Theoretic framework (I-T) I assess the results obtained from modelling each response variable to determine whether or not results differed qualitatively. I also explored sex specific and overall repeatability estimates for each of the response variables. If results were different using differing response variables this would suggest that activity may be a contaminating component within my PCA measure of exploration, and thus indicate that unique zones entered (exploration) should be analysed separately from activity measures. If on the

other hand exploration PCA scores and pure exploration yielded comparable results, this would indicate that activity did not contaminate the behavioural score for exploration suggesting that PCA approaches are suitable for use throughout this thesis.

Results

Using both PCA scores (exploration + activity) and unique zones entered (pure exploration) revealed comparable results. In particular, both analyses highlight that the null model was the best fitting model for the data in both instances (table A3.1a). The second best explanatory model (<4 DeltaAICc points of the best fitting model (null)), for both response variables, reveals that the inclusion of sex as a fixed effect parameter at least in part explained some of the patterns in the data (table A3.1a). All of the additional models within the candidate sets (containing fixed effects of genotype, sex x genotype, sex + genotype + sex x genotype), were not supported by the data in either of the analyses employed (table A3.1a). Average model estimates of these two best fitting explanatory models, for both PCA and pure exploration scores, reveal that although some difference was observed between the sexes, sex was non-significant highlighted by average model estimates surrounded by 95%CIs that spanned zero (table A3.1b). This shows that modelling either PCA scores or pure exploration response variables yielded qualitatively similar results.

In addition, analysis of overall and sex specific repeatability reveals that PCA scores and the pure exploration measure additionally yielded comparable results (table A3.1c). In particular all repeatability analyses show that only individuals, and not genotypes, were repeatable in exploration measures at each level of analysis (overall, male and hermaphrodite, respectively) (table A3.1c). While there is some difference in

the estimates of repeatability between measures there results were not qualitatively different and thus had no effect upon interpretation of repeatability estimates.

Table A3.1a, All potential model structures for measures of pure exploration (unique zones entered only) and Exploration + Activity (PCA scores), and their respective I-T approach AICc, Δ_i and w_i values. K represents the number of parameters in each respective model. Models denoted in bold indicate those models within 4 Δ_i of the best fitting model. **Note that the ranking of models is identical for each measure of exploration and the best fitting models in both instances are the null model (βi) and models containing the fixed effect of sex (βSex).**

	Model Number	Model Structure	k	AICc	Δ_i	w_i
Unique zone entered (pure exploration)	**1**	**Bi**	**7**	**394.4**	**0**	**0.63**
	2	**βSex**	**8**	**395.5**	**1.06**	**0.37**
	3	βGenotype	16	407.3	12.84	0
	4	βSex + βGenotype	17	408.7	14.26	0
	5	βSex + βGenotype + βSex x βGenotype	26	430.4	35.98	0
Unique Zones Entered + Activity (PCA)	**1**	**Bi**	**8**	**340.6**	**0**	**0.5**
	2	**βSex**	**9**	**340.7**	**0.037**	**0.49**
	3	βGenotype	17	351.8	11.17	0
	4	βSex + βGenotype	18	351.9	11.25	0
	5	βSex + βGenotype + βSex x βGenotype	27	374.1	33.43	0

Table A3.1b Model averaged estimates for measures of pure exploration (unique zones entered only) and exploration + activity (PCA scores) for parameters of importance identified by the IT approach (estimates = effect size in comparison to the reference sex i.e. males compared to hermaphrodites. Effects and confidence intervals denoted in bold script represent non-zero CI's and thus significant at the 0.05 α level, $N_{individual}$ =60, $N_{genotype}$ = 10. **Note that both exploration response variables reveal comparable results that are qualitatively identical.**

Average Model Estimates					
		Estimate	**SE**	**Lower CI**	**Upper CI**
Unique zone entered (pure exploration)	Intercept	2.39	0.14	2.1	2.67
	Male	-0.22	0.19	-0.62	0.17
Unique zone entered + Activity (PCA)	Intercept	0.04	0.17	-0.28	0.35
	Male	-0.31	0.2	-0.7	0.08

Table A3.1c. Repeatability estimates (R), p values and 95% confidence intervals estimates for measures of pure exploration (unique zones entered only) and exploration + activity (PCA scores). Estimates for all data combined (overall) and each sex separately are presented for both types of response variable at the individual and genotype levels. Estimates denoted in bold script indicate significant repeatabilities, $N_{individual}$ =60, $N_{genotype}$ = 10. **Note that although estimates are slightly different between response variables modelled, qualitatively repeatability estimates exhibit qualitatively comparable results i.e. both exploration measures are repeatable at the individual level but not the genotype level.**

		Level	R	*p*	95%CI
Unique zone entered (pure exploration)	Overall	ID	**0.581**	**0.001**	**0.424-0.734**
		Genotype	0.055	0.153	0.00-0.185
	Male	ID	**0.503**	**0.002**	**0.356-0.796**
		Genotype	0.034	0.196	0.00-0.245
	Hermaphrodite	ID	**0.544**	**0.001**	**0.222-0.753**
		Genotype	0.066	0.122	0.00-0.301
Unique zone entered + Activity (PCA)	Overall	ID	**0.438**	**0.001**	**0.208-0.627**
		Genotype	0.07	0.105	0.000-0.234
	Male	ID	**0.392**	**0.007**	**0.061-0.659**
		Genotype	0.13	0.063	0.000-0.396
	Hermaphrodite	ID	**0.488**	**0.002**	**0.147-0.725**
		Genotype	0.016	0.333	0.000-0.124

A3.2. All potential model structures for exploration, boldness and aggression, and their respective I-T approach AICc, Δ_i and w_i values. K represents the number of parameters in each respective model. Models denoted in bold indicate those models within 4 Δ_i of the best fitting model.

Model Number	Model Structure	k	AICc	Δ_i	w_i
	Exploration				
1	**βi**	**8**	**340.6**	**0**	**0.50**
3	**βSex**	**9**	**340.7**	**0.037**	**0.49**
2	βGenotype	17	351.8	11.17	0
4	βSex + βGenotype	18	351.9	11.25	0
5	βSex + βGenotype + βSex x βGenotype	27	374.1	33.43	0
	Boldness				
1	**βi**	**8**	**1293**	**0**	**0.76**
3	**βSex**	**9**	**1296**	**2.34**	**0.24**
2	βGenotype	17	1308	15.15	0
4	βSex + βGenotype	18	1311	17.92	0
5	βSex + βGenotype + βSex x βGenotype	27	1321	28.11	0
	Aggression				
2	**βGenotype**	**17**	**69.05**	**0**	**0.49**
1	**βi**	**8**	**70.76**	**1.71**	**0.21**
4	**βSex + βGenotype**	**18**	**70.79**	**1.74**	**0.20**
3	**βSex**	**9**	**72.25**	**3.2**	**0.10**
5	βSex + βGenotype + βSex x βGenotype	27	93.74	24.69	0

Chapter 4

A4.1 Assessing the most appropriate measure of boldness behaviour

Statistical methods

Firstly, I used PCA to extract a composite score which included time to recover post disturbance (TTM) and activity measures (distance travelled (mm), and mean velocity (mm/s)). All behavioural measures within this composite score loaded on to a single component that explained a high %variance, as described in the main text of chapter 4. In addition, I utilised a single measure of boldness (TTM) as a separate pure boldness variable. To statistically test these each of these behavioural scores and to determine whether either PCA or pure TTM explained the same patterns in the data, I implemented mixed modelling approaches as described in chapter four (i.e. random regression techniques to quantify variation in random effects). Briefly, I implemented linear mixed models for both PCA scores and TTM (residuals were normally distributed in both cases) and modelled random effects of individual and genotype (intercepts) and permitted slopes to vary by age for each random effect. In addition, I modelled age as a fixed effect covariate to explore overall age effects upon behavioural expression. Using multi-model selection and inference techniques within the Information-Theoretic approach (IT) (Burnham and Anderson 2002; Burnham and Anderson 2004, also see main text of chapter four for details), I identified the best fitting models for each variable separately and calculated the percentage of variance explained by each random effect within the best fitting model to determine whether or not results differed qualitatively. If results were different using each of the response variables this would suggest that activity may be a contaminating component within my PCA measure of boldness and thus indicate that TTM (boldness) should be analysed separately from activity measures. If on the other hand boldness PCA scores and TTM yielded comparable results, this would indicate that activity did not contaminate the behavioural

score for boldness, suggesting that PCA approaches are suitable for use throughout this thesis.

Results

Fitting either a PCA scores or a pure measure of boldness (TTM only) as response variables revealed comparable results (table A4.1a). The best explanatory model from the candidate set of models, identified using the I-T framework, reveals that both response variables (PCA or TTM respectively) were best explained by the global model (table A4.1a). These global models have 99% probability of being the best fitting to the data in both instances, while all other candidate models had <1% probability of explaining the data (i.e. >4 deltaAIC points lower than the global model) (table A4.1a). This indicates that both response variables (boldness PCA scores or TTM) yielded qualitatively similar results.

The qualitatively similar results using each response variable is further highlighted when I calculate the percentage of the total variance explained by each of the random effect parameter within these global models. For example, fitting each of the response variables (PCA scores and TTM respectively), highlights that the majority of the variance in each global model is explained by genotype variation in developmental trajectories i.e. 92% for PCA scores and 80% for TTM (table A4.1b). In addition, it is important to note that each of the additional random effect parameters was found to represent similar and minimal percentages of the total variance explained. More importantly, the only difference in variance explained that was documented between these two response variables is represented by the residual variation (variation unaccounted for in the model). For example, modelling TTM alone in comparison to PCA scores increased the residual variation from 2% (PCA score) to 19.3% (TTM)

(table A4.1b). This suggests that modelling PCA scores more accurately explain more of the variation in the data when compared to TTM alone which exhibits higher unaccounted variation.

Table A4.1a. Linear mixed model structures for all *a priori* models constructed for boldness PCA scores and pure boldness scores (TTM only). Table includes each models respective AIC value, Relative AIC difference (Delta AIC), and Akaike weight (w_i). Model structures are written in R code format, for reference to structure a model example is provided above the table (1 denotes the population average i.e. removal of a fixed or random effect covariate). Models denoted in bold type represent the best fitting model with the highest likelihood and model weight) Note that the best explanatory model for both response variables (PCA score or pre boldness) is the global model containing all random effect parameters and the fixed effect of age. In addition note that both response variables have identical model weight estimates ($\boldsymbol{w_i}$). These results show that in using both response variables are explained by the global model having 99% chance of being the single best explanatory model from the candidate set and thus containing the parameters of importance.

Model Structure Example: Response Variable ~ Fixed effect covariate + (Random slope | Random intercept)

	Model N^o	**Mod Structure**	**AIC**	**Delta AIC**	$\boldsymbol{w_i}$
Boldness PCA Score (TTM + Activity)	**1 (Global)**	**PCA Score ~ Age + (Age | Genotype) + (Age | ID)**	**1468**	**0**	**0.99**
	3	PCA Score ~ Age + (1 | Genotype) + (Age | ID)	1486	18	0
	2	PCA Score ~ 1 + (Age | Genotype) + (Age | ID)	1492	24	0
	4	PCA Score ~ Boldness ~ Age + (Age | ID)	1504	36	0
Boldness (Time to move (TTM))	**1 (Global)**	**TTM~ Age + (Age | Genotype) + (Age | ID)**	**7695**	**0**	**0.99**
	4	TTM ~ Age + (Age | ID)	7707	12	0
	3	TTM ~ Age + (1 | Genotype) + (Age | ID)	7709	14	0
	2	TTM ~ 1 + (Age | Genotype) + (Age | ID)	7715	20	0

Table A4.1b. Percentage variance explained by each respective random effect parameters included in the global models fitted with boldness PCA scores and pure boldness measure response variables. Note that in both models the highest percentage of variance explained by the global model is the variation between genotype developmental trajectories. In addition TTR as a single measure of boldness is characterized by a higher residual variation (variance unaccounted for in the model) suggesting that TTM alone explains less of the variation when compared to the model fitted with the PCA score (i.e. PCA residual = 2% of the total variance, compared to TTM residual = 19.3% of the total variance).

	Random effect	**Percentage Variance Explained**
Boldness PCA Score (TTM + Activity)	ID Intercept (mean difference)	0.1
	ID Developmental Trajectory (slope)	4.9
	Genotype Intercept (mean difference)	0.3
	Genotype Developmental Trajectory (slope)	**92.6**
	Residual (unaccounted variation)	2.0
Boldness (TTM Only)	ID Intercept (mean difference)	0.1
	ID Developmental Trajectory (slope)	0.0
	Genotype Intercept (mean difference)	0.0
	Genotype Developmental Trajectory (slope)	**80.6**
	Residual (unaccounted variation)	19.3

Table A4.2. Mean genotype exploration measures during ontogeny: mean distance travelled (mm) ± standard error and R^2 values over the course of development. In addition exploration repeatability estimates, 95% Confidence intervals and p values for each genotype calculated using ANOVA based repeatabilities (Lessells and Boag 1986)

Genotype N°	Exploration						Repeatability	
	R^2	Mean Distance Travelled ± SE (mm)						
		Day 2	Day 31	Day 61	Day 91	Day 151	R (95% CI)	p
1	0.306	1732 ± 816	4540 ± 1244	6534 ± 1202	6221 ± 940	9533 ± 1193	0.203 (-0.03 to 0.44)	0.096
2	0.398	2335 ± 487	5559 ± 1245	8265 ± 589	7928 ± 1468	13125 ± 1490	-0.185 (-0.08 to 0.90)	0.905
3	0.597	1159 ± 503	4092 ± 1225	7598 ± 1133	6975 ± 1285	12719 ± 867	-0.169 (-0.58 to 0.14)	0.922
4	0.658	1199 ± 476	4621 ± 538	7617 ± 791	9776 ± 566	7518 ± 753	-0.125 (-0.43 to 0.18)	0.815
5	0.512	881 ± 397	3803 ± 707	9653 ± 1861	6130 ± 1866	11225 ±1130	-0.011 (-0.28 to 0.26)	0.417
6	0.334	499 ± 384	4675 ± 1269	5330 ± 767	3782 ± 822	9253 ± 833	-0.119 (-0.42 to 0.18)	0.797
7	0.296	1046 ± 460	5517 ± 565	6887 ± 1587	5597 ± 1115	6023 ± 958	0.013 (-0.08 to 0.10)	0.413
8	0.256	493 ± 272	4585 ± 586	4752 ± 566	3396 ± 663	4827 ± 675	-0.172 (-0.49 to 0.14)	0.929
9	0.224	1333 ± 530	4620 ± 979	7046 ± 907	5177 ± 670	5707 ± 1667	0.022 (-0.24 to 0.28)	0.379
10	0.452	1554 ± 498	5912 ± 638	9391 ± 1673	8281 ± 1944	14771 ± 367	0.253 (0.18 to 0.32)	0.055
11	0.717	499 ± 422	4028 ± 380	9075 ± 476	7320 ± 987	10658 ± 600	-0.216 (-0.54 to 0.11)	0.989
12	0.308	774 ± 434	5569 ± 784	8579 ± 1014	6280 ± 2223	12701 ± 2655	-0.127 (-0.54 to 0.17)	0.82
13	0.440	1921 ± 586	4298 ± 715	8530 ± 703	5630 ± 1407	12400 ± 1151	0.061 (-0.19 to 0.31)	0.278
14	0.276	1177 ± 462	5537 ± 586	8707 ± 1433	2909 ± 1203	10848 ± 1456	-0.168 (-0.47 to 0.14)	0.92
15	0.794	177 ± 69	4797 ± 403	8626 ± 419	9075 ± 826	11984 ± 1415	-0.228 (-0.56 to 0.10)	0.996
16	0.532	2090 ± 438	5264 ± 363	9093 ± 1112	9802 ± 1720	11329 ± 665	-0.049 (-0.33 to 0.23)	0.584
17	0.120	1294 ± 463	3900 ± 679	5145 ± 729	4007 ± 652	5244 ± 947	-0.038 (0.32 to 0.24)	0.552
18	0.412	1555 ± 536	5507 ± 918	13139 ± 953	9980 ± 1628	12716 ± 2701	0.061 (-0.19 to 0.31)	0.285
19	0.734	645 ± 410	4681 ± 589	9585 ± 1070	10099 ± 1853	9070 ± 1097	-0.071 (-0.36 to 0.22)	0.622
20	0.108	1975 ± 748	3926 ± 707	6710 ± 1484	4515 ± 1446	8036 ± 1292	0.136 (-0.09 to 0.37)	0.154

Table A4.3 Mean genotype boldness measures during ontogeny: time to recover (s) (TTM) and distance travelled (mm) ± standard error and R^2 values over the course of development. In addition boldness repeatability estimates, 95% Confidence intervals and p values for each genotype calculated using ANOVA based repeatabilities (Lessells and Boag 1986)

Genotype Nº	**Boldness**						**Repeatability**	
	R^2	**Mean TTM ±SE (s) /Distance Travelled ± SE (mm)**						
		Day 2	**Day 31**	**Day 61**	**Day 91**	**Day 151**	**R**	**p**
1	0.293	139 ± 66/ 947 ± 539	83 ± 16/ 770 ± 395	104 ± 28/ 945 ± 342	42 ± 10/ 2389 ± 330	42 ± 6/ 2210 ± 224	0.061 (-0.22 to 0.34)	0.096
2	0.368	30 ± 11/ 682.3 ± 314	17 ± 5/ 2466 ± 188	15 ± 4/ 2911 ± 125	47 ± 11/ 2285 ± 616	30 ± 7/ 2760 ± 348	-0.157 (-0.25 to -0.05)	0.905
3	0.379	52 ± 22/ 1250 ± 365	75 ± 29/ 1130 ± 312	49 ± 12/ 1930 ± 316	25 ± 6/ 3641 ± 458	57 ± 12/ 3293 ± 477	-0.063 (0.35 to 0.22)	0.922
4	0.081	55 ± 27/ 1290 ± 768	72 ± 31/ 1084 ± 282	39 ± 9/ 1613 ± 369	60 ± 11/ 2343 ± 326	69 ± 16/ 1032 ± 277	-0.073 (-0.36 to 0.21)	0.815
5	0.158	97 ± 28/ 562 ± 207	79 ± 28/ 1126 ± 466	72 ± 30/ 1603 ± 438	70 ± 17/ 1851 ± 436	85 ± 18/ 1975 ± 273	0.264 (0.06 to 0.46)	0.417
6	0.235	112 ± 39/ 534 ± 217	60 ± 23/ 1161 ± 244	63 ± 12/ 930 ± 90	94 ± 26/ 1433 ± 427	59 ± 10/ 2005 ± 583	-0.041 (-0.32 to 0.24)	0.797
7	0.117	56 ± 27/ 216 ± 70	40 ± 5/ 1696 ± 447	112 ± 21/ 902 ± 159	82 ± 27/ 1125 ± 241	61 ± 7/ 1666 ± 378	0.087 (0.001 to 0.17)	0.413
8	0.115	117 ± 20/ 557 ± 153	61 ± 27/ 1454 ± 472	131 ± 35/ 700 ± 142	47 ± 16/ 1843 ± 417	79 ± 11/ 1590 ± 295	-0.140 (-0.45 to 0.17)	0.929
9	0.328	32 ± 6/ 197 ± 89	82 ± 21/ 890 ± 173	57 ± 13/ 1387 ± 315	42 ± 7/ 1787 ± 277	68 ± 33/ 1852 ± 378	0.059 (-0.19 to 0.31)	0.379
10	0.399	53 ± 16/ 630 ± 243	52 ± 17/ 1581 ± 451	47 ± 25/ 1446 ± 366	40 ± 11/ 2892 ± 582	30 ± 7/ 3035 ± 533	0.120 (0.04 to 0.19)	0.055
11	0.519	182 ± 50/ 175 ± 54	46 ± 17/ 1460 ± 185	54 ± 18/ 1444 ± 279	66 ± 19/ 1967 ± 386	53 ± 9/ 2185 ± 230	-0.105 (-0.40 to 0.19)	0.989
12	0.710	177 ± 48/ 356 ± 114	123 ± 35/ 864 ± 301	59 ± 11/ 2205 ± 376	33 ± 7/ 3067 ± 336	27 ± 3/ 3474 ± 470	-0.094 -0.39 to 0.20)	0.82
13	0.528	135 ± 36/ 149 ± 71	84 ± 21/ 907 ± 215	47 ± 21/ 2045 ± 369	50 ± 25/ 2195 ± 709	46 ± 6/ 2604 ± 358	-0.093 (-0.39 to 0.20)	0.278
14	0.323	63 ± 23/ 379 ± 280	63 ± 12/ 877 ± 151	71 ± 14/ 1809 ± 315	51 ± 16/ 1726 ± 339	72 ± 21/ 1977 ± 508	-0.172 (-0.49 to 0.14)	0.92
15	0.659	154 ± 39/ 449 ± 326	52 ± 16/ 1371 ± 86	46 ± 13/ 2300 ± 328	42 ± 9/ 2833 ± 414	55 ± 12/ 2963 ± 490	-0.161 (-0.47 to 0.15)	0.996
16	0.621	55 ± 10/ 362 ± 121	25 ± 14/ 2027 ± 406	26 ± 9/ 3009 ± 219	34 ± 10/ 3423 ± 433	47 ± 18/ 2395 ± 310	-0.099 (-0.39 to 0.20)	0.584
17	0.117	96 ± 35/ 589 ± 211	31 ± 6/ 1611 ± 450	75 ± 26/ 1203 ± 324	81 ± 19/ 1097 ± 334	127 ± 21/ 893 ± 236	0.142 (-0.09 to 0.37)	0.552
18	0.350	93 ± 23/ 326 ± 120	33 ± 12/ 2476 ± 618	33 ± 8/ 2932 ± 279	81 ± 24/ 2087 ± 512	40 ± 6/ 3343 ± 536	-0.065 (-0.35 to 0.22)	0.285
19	0.487	78 ± 55/ 265 ± 166	89 ± 17/ 1421 ± 280	59 ± 9/ 2039 ± 349	47 ± 12/ 2550 ± 595	52 ± 16/ 2400 ± 354	0.013 (-0.25 to 0.28)	0.622
20	0.061	133 ± 37/ 396± 223	54 ± 13/ 1421 ± 675	93 ± 15/ 1397 ± 262	109 ± 28/ 739 ± 166	144 ± 43/ 1668 ± 611	-0.090 (-0.38 to 0.20)	0.154

Appendix

Table A4.4 Within- and between-individual variation observed for each genotype during 151 days of ontogeny from which repeatability estimates were calculated (Tables A4,1 and A4.1 above). Yellow highlighted estimates indicate higher variance either within- or between-individuals (note that the majority of exploration and boldness variance is explained within-individuals), Blue highlighted values indicate variance estimates that are similar both within and between-individuals.

	Exploration		Boldness	
Genotype N°	Between-Individual Variation	Within-Individual Variation	Between-Individual Variation	Within-Individual Variation
1	1.25	0.55	1.40	1.05
2	0.29	0.97	0.29	0.74
3	0.37	1.35	0.87	1.24
4	0.32	0.73	0.59	0.91
5	1.22	1.29	1.76	0.63
6	0.49	1.05	0.52	0.65
7	0.65	0.61	0.58	0.41
8	0.12	0.47	0.29	0.76
9	0.66	0.59	0.65	0.49
10	2.39	0.93	1.45	0.89
11	0.14	1.29	0.50	0.95
12	0.79	1.82	1.11	1.94
13	1.08	0.81	0.78	1.35
14	0.29	1.04	0.17	0.65
15	0.10	1.49	0.38	1.27
16	0.48	0.63	0.59	1.08
17	0.34	0.42	0.84	0.46
18	2.01	1.51	0.96	1.38
19	0.72	1.08	0.93	0.87
20	1.25	0.70	0.57	0.98

Table A4.5. Mean life-history measures ± standard error for each genotype during ontogeny

Genotype Designation	Genotype N^{o}	Mean Standard Length (mm) ± SE					Age at First lay ± SE (days)	Mean No Eggs Laid ± SE
		Day 2	Day 31	Day 61	Day 91	Day 151		
5091	1	4.6 ± 0.15	9 ± 0.18	12.4 ± 0.56	14.9 ± 0.41	18.6 ± 0.47	106 ± 4.6	26.2 ± 5.1
BP3	2	4.7 ± 0.08	9.5 ± 0.12	12.5 ± 0.30	15.3 ± 0.25	19.4 ± 0.28	122 ± 5.3	12.1 ± 3.6
BP11	3	4.9 ± 0.13	9.0 ± 0.14	12.6 ± 0.33	15.3 ± 0.20	18.8 ± 0.38	131 ± 1.1	8.8 ± 2.3
DAN	4	4.4 ± 0.11	8.5 ± 0.16	12.8 ± 0.13	15.3 ± 0.19	20.2 ± 0.30	130 ± 1.5	9.3 ± 1.5
ENP02-2	5	4.9 ± 0.11	9.3 ± 0.14	12.3 ± 0.29	15.4 ± 0.34	19.4 ± 0.33	136 ± 2.2	7.0 ± 1.2
HON2	6	4.7 ± 0.08	9.1 ± 0.15	12.7 ± 0.20	15.7 ± 0.21	18.8 ± 0.26	122 ± 3.0	14.1 ± 1.7
HON7	7	4.7 ± 0.18	9.1 ± 0.20	12.4 ± 0.54	15.7 ± 0.35	18.9 ± 0.47	110 ± 2.2	15.8 ± 3.6
HON9	8	4.7 ± 0.07	9.0 ± 0.10	12.9 ± 0.11	15.6 ± 0.39	19.5 ± 0.43	104 ± 1.5	20.3 ± 3.6
HON11	9	4.6 ± 0.11	9.4 ± 0.24	13.0 ± 0.44	16.2 ± 0.35	19.5 ± 0.45	114 ± 1.0	17.5 ± 2.0
LK2	10	4.9 ± 0.10	8.8 ± 0.26	12.1 ± 0.25	14.5 ± 0.16	19.0 ± 0.25	130 ± 5.5	7.1 ± 2.1
LK4	11	4.9 ± 0.15	8.8 ± 0.11	12.4 ± 0.29	14.7 ± 0.18	19.1 ± 0.18	128 ± 3.9	8.8 ± 1.4
LK6	12	4.9 ± 0.10	8.9 ± 0.17	12.2 ± 0.15	15.0 ± 0.22	19.3 ± 0.25	125 ± 2.3	13.6 ± 2.1
LK7	13	4.9 ± 0.05	9.1 ± 0.07	12.2 ± 0.26	14.2 ± 0.09	18.8 ± 0.39	131 ± 4.9	9.5 ± 2.5
LK13	14	5.1 ± 0.08	9.2 ± 0.16	12.5 ± 0.23	15.4 ± 0.12	19.7 ± 0.20	132 ± 0.9	7.3 ± 1.6
LK15	15	4.9 ± 0.09	9.0 ± 0.14	12.1 ± 0.21	14.5 ± 0.17	19.2 ± 0.28	126 ± 1.8	14.6 ± 1.8
NNKN1	16	4.8 ± 0.09	8.8 ± 0.16	11.8 ± 0.25	14.8 ± 0.19	19.1 ± 0.17	129 ± 3.8	12.3 ± 1.9
R2	17	4.7 ± 0.08	8.5 ± 0.23	12.3 ± 0.30	15.2 ± 0.22	19.4 ± 0.23	128 ± 3.0	11.6 ± 1.8
SLC8E	18	4.6 ± 0.08	9.1 ± 0.11	12.6 ± 0.13	14.8 ± 0.19	19.0 ± 0.33	125 ± 5.2	9.1 ± 2.1
SSLL	19	4.7 ± 0.04	8.5 ± 0.21	12.7 ± 0.36	15.4 ± 0.21	19.2 ± 0.40	135.6 ± 4.6	11.8 ± 3.1
VOL	20	4.5 ± 0.10	8.8 ± 0.18	12.7 ± 0.31	14.9 ± 0.25	20.0 ± 0.46	136 ± 2.4	7.5 ± 1.8

Table A4.6. Mixed effect model estimates for age at first laying and total eggs laid for each genotype. Intercept represents genotype 1 and each estimate is the difference (i.e. effect size and direction) compared to the intercept with lower and upper 95% confidence intervals. Estimates in bold script identify those genotypes that do not differ from genotype 1.

	Age First Lay			**Total Eggs Laid**		
Genotype Nº	**Estimate**	**Std. Error**	**95% CI**	**Estimate**	**SE**	**95% CI**
Intercept (1)	106	3.971	98.1 to 13.8	26.2	2.471	21.2 31.1
2	21.33	5.377	10.6 to 32	-14.033	3.345	-20.6 to -7.3
3	29	5.377	18.3 to 39.6	-17.367	3.345	-23.9 to -10.7
4	24.17	5.377	13.4 to 34.8	-16.867	3.345	-23.4 to -10.2
5	30.17	5.377	19.4 to 40.8	-19.2	3.345	-25.8 to -12.5
6	16.17	5.377	5.4 to 26.8	-12.033	3.345	-18.6 to -5.3
7	11.33	5.377	0.6 to 22	-10.367	3.345	-16.9 to -3.7
8	**-1.67**	**5.377**	**-12.3 to 9**	**-5.867**	**3.345**	**-12.5 to 0.7**
9	**8**	**5.377**	**-2.6 to 18.6**	-8.7	3.345	-15.3 to -2
10	31.5	5.377	20.8 to 42.1	-19.033	3.345	-25.6 to -12.3
11	22.5	5.377	11.8 to 33.1	-17.367	3.345	-23.9 to -10.7
12	19.5	5.377	8.8 to 30.1	-12.533	3.345	-19.1 to -5.8
13	25.67	5.377	14.9 to 36.3	-16.7	3.345	-23.3 to -10
14	29.5	5.377	18.8 to 40.1	-18.867	3.345	-25.4 to -12.2
15	20.17	5.377	9.4 to 30.8	-11.533	3.345	-18.1 to -4.8
16	23.5	5.377	12.8 to 34.1	-13.867	3.345	-20.4 to -7.2
17	22.67	5.377	11.9 to 33.3	-14.533	3.345	-21.1 to -7.8
18	19.17	5.377	8.4 to 29.8	-17.033	3.345	-23.6 to -10.3
19	29.6	5.616	18.9 to 40.2	-14.4	3.494	-21 to -7.7
20	32.67	5.377	21.9 to 43.3	-18.7	3.345	-25.3 to -12

Chapter 5

Table A5.1 Model structures of all candidate models for exploration, boldness, aggression, growth rate and reproductive measures and respective I-T approach model AICc, Δ_i and w_i values. Models denoted in bold represent those falling within 4 Δ_i of the best fitting model. K represents the number of parameters.

Model No	Model Structure	K	AICc	Δ_i	w_i
	Exploration				
12	**βi + βGenotype + βAge + βTreatment + βAge x Treatment**	**19**	**1177**	**0**	**0.7**
10	**βi + βAge + βTreatment + βAge x Treatment**	**15**	**1179**	**1.61**	**0.3**
8	βi + βGenotype + βAge + βTreatment	16	1183	6.49	0
7	βi + βAge + βTreatment	12	1185	8.30	0
6	βi+ βGenotype + βTreatment	15	1186	8.94	0
4	βi+ βTreatment	11	1188	10.7	0
13	βi + βGenotype + βAge + βTreatment + βGenotype X Treatment + βAge x Treatment	31	1191	14.4	0
9	βi+ βGenotype + βTreatment + βGenotype X Treatment	27	1200	22.9	0
11	βi+ βGenotype + βAge + βTreatment + βGenotype X Treatment	28	1203	26.3	0
5	βi+ βGenotype + βAge	13	1211	34.1	0
3	βi+ βAge	9	1212	35.2	0
2	βi+ βGenotype	12	1214	36.6	0
1	βi	8	1215	37.7	0
	Boldness				
11	**βi+ βGenotype + βAge + βTreatment + βGenotype x Treatment**	**28**	**1213**	**0**	**0.5**
13	**βi + βGenotype + βAge + βTreatment + βGenotype x Treatment + βAge x Treatment**	**31**	**1213**	**0.9**	**0.3**
12	**βi + βGenotype + βAge + βTreatment + βAge x Treatment**	**19**	**1216**	**3.6**	**0.08**
8	βi + βGenotype + βAge + βTreatment	16	1217	4.5	0
7	βi + βAge + βTreatment	12	12216	8.4	0
10	βi + βAge + βTreatment + βAge x Treatment	15	12215	8.9	0
9	βi+ βGenotype + βTreatment + βGenotype X Treatment	27	12289	15.5	0
6	βi+ βGenotype + βTreatment	15	1232	19.2	0
4	βi+ βTreatment	11	1236	23.8	0
5	βi+ βGenotype + βAge	13	1265	52.7	0
3	βi+ βAge	9	1269	56.6	0
2	βi+ βGenotype	12	1281	68.0	0
1	βi	8	1285	72.0	0
	Aggression				
12	**βi + βGenotype + βAge + βTreatment + βAge x Treatment**	**19**	**88.5**	**0**	**0.8**
10	**βi + βAge + βTreatment + βAge x Treatment**	**15**	**92.37**	**3.9**	**0.1**
6	βi+ βGenotype + βTreatment	15	94.19	5.7	0
8	βi + βGenotype + βAge + βTreatment	16	96.1	7.6	0

13	βi + βGenotype + βAge + βTreatment + βGenotype X Treatment + βAge x Treatment	31	96.2	7.7	0
7	βi + βAge + βTreatment	12	99.95	11.4	0
4	βi+ βTreatment	11	100.9	12.4	0
11	βi+ βGenotype + βAge + βTreatment + βGenotype X Treatment	28	103.2	14.7	0
9	βi+ βGenotype + βTreatment + βGenotype X Treatment	27	103.9	15.4	0
5	βi+ βGenotype + βAge	13	113.1	24.6	0
2	βi+ βGenotype	12	114	25.5	0
3	βi+ βAge	9	119.1	30.6	0
1	βi	8	120.1	31.6	0
	Growth Rate				
10	**βi + βAge + βTreatment + βAge x Treatment**	**15**	**335.4**	**0**	**0.9**
12	βi + βGenotype + βAge + βTreatment + βAge x Treatment	19	342.3	6.8	0
7	βi + βAge + βTreatment	12	352.1	16.7	0
8	βi + βGenotype + βAge + βTreatment	16	354.6	19.1	0
3	βi+ βAge	9	362.4	27.0	0
13	βi + βGenotype + βAge + βTreatment + βGenotype X Treatment + βAge x Treatment	31	367.3	31.9	0
5	βi+ βGenotype + βAge	13	369.1	33.6	0
4	βi+ βTreatment	11	377.7	42.2	0
11	βi+ βGenotype + βAge + βTreatment + βGenotype X Treatment	28	379.4	43.9	0
6	βi+ βGenotype + βTreatment	15	380.2	44.8	0
1	βi	8	392.1	56.7	0
2	βi+ βGenotype	12	394.7	59.3	0
9	βi+ βGenotype + βTreatment + βGenotype X Treatment	27	404.9	69.5	0
	Age first Lay				
5	**βi + βGenotype + βTreatment + βGenotype x Treatment**	**23**	**1074**	**0**	**1**
4	βi + βGenotype + βTreatment	11	1096	22.2	0
3	βi + βTreatment	7	1098	23.5	0
2	βi + βGenotype	8	1118	43.7	0
1	βi	4	1119	44.4	0
	Total Eggs Laid				
3	**βi + βTreatment**	**5**	**879.6**	**0**	**0.3**
4	**βi + βGenotype + βTreatment**	**9**	**879.8**	**0.2**	**0.2**
1	**βi**	**3**	**880.1**	**0.5**	**0.2**
2	**βi + βGenotype**	**7**	**880.2**	**0.6**	**0.2**
5	βi + βGenotype + βTreatment + βGenotype x Treatment	17	884.9	5.3	0

Table A5.2. Treatment and genotype mean values for exploration distance travelled and respective standard errors at each age sampled during ontogeny.

	Distance Travelled (mm) ± SE			
	Day 31	**Day 91**	**Day 151**	**Overall Mean**
Treatment				
Control	4914.4 ± 405.9	9325.2 ± 637.6	10275.3 ± 467.8	8171.6 ± 366.7
Conspecific Presence	4316.9 ± 226.6	7238.6 ± 430.6	5828.9 ± 318.8	5794.8 ± 221.2
Predation Risk	5381.4 ± 333.	10032.8 ± 531.6	10135.9 ± 567.6	8516.7 ± 348.7
Low food	4930.1 ± 386.2	7215.3 ± 604.1	8175.3 ± 545.2	6761.2 ± 323.9
Genotype N^{o}				
1	4232.7 ± 430.6	10146.8 ± 484.6	9371.3 ± 729.0	7916.9 ± 422.2
2	4166.5 ± 306.8	6508.3 ± 607.2	8238.1 ± 551.9	6304.3 ± 341.7
3	6088.1 ± 346.6	9851.0 ± 750.5	7625.1 ± 621.8	7854.7 ± 376.2
4	4032.8 ± 335.6	8516.3 ± 534.2	8994.1 ± 611.9	7161.4 ± 371.7
5	5736.0 ± 337.2	6978.2 ± 605.7	8633.1 ± 580.4	7115.8 ± 322.0

TableA5.3. Treatment and genotype mean values for boldness measures; time to recover (TTM) and distance travelled and their respective standard errors at each age sampled during ontogeny.

	Mean TTM (s) ±SE /Distance Travelled (mm) ± SE			
	Day 31	**Day 91**	**Day 151**	**Overall Mean**
Treatment				
Control	58.8 ± 8.2/2736.4 ± 198.8	49.8 ± 5.9/3055.5 ± 189.5	36.0 ± 3.0/3638.3 ± 217.5	48.2 ± 3.6/3143.4 ± 120.9
Conspecific Presence	66.9 ± 4.9/2152.5 ± 120.2	69.9 ± 7.0/1967.4 ± 128.5	58.7 ± 3.4/2287.3 ± 108.0	71.8 ± 3.3/2135.7 ± 69.3
Predation Risk	42.6 ± 4.8/2802.8 ± 202.3	49.5 ± 5.3/3421.9 ± 223.7	34.9 ± 3.0/3496.4 ± 179.7	42.3 ± 2.6/3240.4 ± 119.6
Low food	36.1 ± 5.1/2399.3 ± 172.6	69.5 ± 9.4/2628.0 ± 242.8	45.3 ± 7.3/2967.7 ± 193.3	50.3± 4.5/2662.3 ± 119.5
Genotype N^{o}				
1	53.9 ± 5.5/2428.9 ± 151.1	48.3 ± 4.8/2879.8 ± 184.2	41.9 ± 5.2/3201.5 ± 176.6	48.1 ± 3.0/2836.8 ± 103.2
2	56.2 ± 5.7/196.4 ± 196.4	75.7 ± 8.7/2158.6 ± 216.7	56.9 ± 7.1/2614.4 ± 240.7	62.9 ± 4.2/2297.9 ± 127.1
3	36.9 ± 5.6/3263.3 ± 207.8	49.4 ± 7.0/3317.3 ± 236.8	37.2 ± 4.4/3145.4 ± 180.6	41.1 ± 3.3/3242.0 ± 120.0
4	68.1 ± 8.0/2087.7 ± 182.3	49.4 ± 10.0/3031.7 ± 202.1	44.3 ± 4.3/3402.7 ± 260.2	61.5 ± 4.6/2834.6 ± 152.9
5	42.6 ± 8.1/2623.3 ± 189.7	81.1 ± 9.1/2339.1 ± 216.2	40.9 ± 4.9/3038.5 ± 224.6	54.9 ± 4.7/2667.0 ± 123.9

Table A5.4. Treatment and genotype mean values for Aggressin rate/minute and respective standard errors at each age sampled during ontogeny.

	Rate/min ± SE			
	Day 31	**Day 91**	**Day 151**	**Overall Mean**
Treatment				
Control	1.58 ± 0.12	1.63 ± 0.12	1.25 ± 0.11	1.49 ± 0.07
Conspecific Presence	0.90 ± 0.05	1.52 ± 0.08	1.03 ± 0.05	1.15 ± 0.04
Predation Risk	1.88 ± 0.15	1.48 ± 0.09	1.25 ± 0.09	1.54 ± 0.07
Low food	1.64 ± 0.11	1.76 ± 0.12	1.36 ± 0.09	1.59 ± 0.06
Genotype N^{o}				
1	1.39 ± 0.15	1.45 ± 0.13	1.13 ± 0.11	1.32 ± 0.07
2	1.36 ± 0.12	1.62 ± 0.09	1.22 ± 0.07	1.40 ± 0.05
3	1.95 ± 0.17	2.04 ± 0.10	1.77 ± 0.10	1.92 ± 0.07
4	1.30 ± 0.12	1.76 ± 0.09	1.20 ± 0.06	1.42 ± 0.06
5	1.45 ± 0.11	1.11 ± 0.09	0.77 ± 0.07	1.11± 0.06

Table A5.5 Treatment and genotype mean values for life-history measures and their respective standard errors at each age sampled during ontogeny.

	Life-history				Age at first lay	Total eggs laid
	Standard length (mm) ± SE					
	Day 31	**Day 91**	**Day 151**	**Overall Mean**		
Treatment						
Control	11.5 ± 0.13	19.2 ± 0.47	22.9 ± 0.17	17.9 ± 0.47	118.4 ± 1.37	18.6 ± 1.41
Conspecific Presence	11.5 ± 0.08	18.9 ± 0.44	22.1 ± 0.52	17.5 ± 0.46	124.0 ± 1.21	- -
Predation Risk	10.9 ± 0.13	18.5 ± 0.27	22.1 ± 0.24	17.2 ± 0.45	114.4 ± 1.65	23.97-±2.49
Low food	9.9 ± 0.07	15.9 ± 0.39	18.7 ± 0.09	14.8 ± 0.37	120.3 ± 1.10	20.1 ± 1.27
Genotype N^{o}						
1	11.3 ± 0.15	19.1 ± 0.30	21.9 ± 0.33	17.5 ± 0.49	117.1 ± 1.74	19.5 ± 19.5
2	10.7 ± 0.15	17.2 ± 0.85	20.5 ± 0.78	16.1 ± 0.59	115.7 ± 1.23	18.5 ± 1.70
3	10.3 ± 0.14	17.2 ± 0.55	20.9 ± 0.26	16.1 ± 0.49	122.4 ± 1.46	19.4 ± 1.68
4	11.1 ± 0.14	18.2 ± 0.26	21.6 ± 0.34	16.9 ± 0.48	120.5 ± 1.36	16.4 ± 2.29
5	11.3 ± 0.17	19.0 ± 0.30	22.3 ± 0.36	17.5 ± 0.50	120.5 ± 1.92	11.8 ± 1.31

Chapter 6

Table A6.1. Model structures of all candidate models (exploration, boldness, aggression, growth rate and Standard length (days 2 and 31)) and their respective I-T approach model AICc, Δ_i and w_i values. Models denoted in bold represent those falling within 4 Δ_i of the best fitting model. K = number of parameters.

Model Number	Model Structure	k	AICc	Δ_i	w_i
	Exploration				
2	**βi + βGenotype**	**8**	**414.7**	**0**	**0.807**
1	**βi**	**4**	**417.9**	**3.283**	**0.156**
4	βi + βGenotype + βTreatment	11	421.2	6.563	0.03
3	βi + βTreatment	7	424.2	9.501	0.007
5	βi + βGenotype + βTreatment +β Genotype x Treatment	23	439.6	24.94	0
	Boldness				
2	**βi + βGenotype**	**8**	**406.9**	**0**	0.831
1	**βi**	**4**	**410.8**	**3.859**	0.121
4	βi + βGenotype + βTreatment	11	413	6.009	0.041
3	βi + βTreatment	7	416.4	9.49	0.007
5	βi + βGenotype + βTreatment +β Genotype x Treatment	23	426.2	19.21	0
	Aggression				
2	**βi + βGenotype**	**8**	**132.6**	**0**	0.764
4	**βi + βGenotype + βTreatment**	**11**	**135.9**	**3.302**	0.147
1	βi	4	137	4.445	0.083
3	βi + βTreatment	7	141.9	9.347	0.007
5	βi + βGenotype + βTreatment +β Genotype x Treatment	23	155.4	22.85	0
	Standard Length Day 2				
3	**βi + βTreatment**	**7**	**30.73**	**0**	**0.654**
4	**βi + βGenotype + βTreatment**	**11**	**33.27**	**2.54**	**0.184**
1	**βi**	**4**	**34.27**	**3.543**	**0.111**
2	βi + βGenotype	8	36.51	5.782	0.036
5	βi + βGenotype + βTreatment +β Genotype x Treatment	23	38.34	7.61	0.015
	Standard Length Day 31				
2	**βi + βGenotype**	**8**	**338.7**	**0**	**0.615**
4	**βi + βGenotype + βTreatment**	**11**	**340.3**	**1.528**	**0.287**
1	βi	4	343.2	4.512	0.064
3	βi + βTreatment	7	344.5	5.807	0.034
5	βi + βGenotype + βTreatment +β Genotype x Treatment	23	361.1	22.41	0
	Growth Rate				
2	**βi + βGenotype**	**8**	**91.94**	**0**	**0.628**
1	**βi**	**4**	**94.36**	**2.413**	**0.188**
4	**βi + βGenotype + βTreatment**	**11**	**95.01**	**3.072**	**0.135**
3	βi + βTreatment	7	97.02	5.081	0.049
5	βi + βGenotype + βTreatment +β Genotype x Treatment	23	111.8	19.89	0

Chapter 7

Table A7.1. Mixed model structure fitted with association and aggression response variables and fixed effects of stimulus, genotype and stimulus x genotype and their respective model inference values: AICc, Δ_i, w_i *and* K represents the number of parameters in each respective model. Models denoted in bold script represent competing models within 4 Δ_i of the best explanatory model

Experiment	Model Number	Fixed Effect Parameters	K	AICc	Δ_i	w_i
Kin: Association duration	**3**	**βi + βStimulus**	**7**	**943.9**	**0.0**	**0.8**
	1	**βi**	**6**	**947.1**	**3.1**	**0.2**
	4	βi + βGenotype + βStimulus	11	954.4	10.5	0
	5	βi + βGenotype + βStimulus + βGenotype x Stimulus	15	954.9	11.0	0
	2	βi + Genotype	10	957.2	13.3	0
Kin: Aggression	**3**	**βi + βStimulus**	**7**	**83.5**	**0**	**0.7**
	4	**βi + βGenotype + βStimulus**	**11**	**85.4**	**1.9**	**0.3**
	5	βi + βGenotype + βStimulus + βGenotype x Stimulus	15	94.0	10.6	0
	1	βi	6	100.7	17.2	0
	2	βi + βGenotype	10	102.4	18.9	0
Familiarity: Association Duration	**3**	**βi + βStimulus**	**6**	**103.1**	**0**	**0.9**
	4	βi + βGenotype + βStimulus	10	107.7	4.5	0
	1	βi	5	112.0	8.9	0
	2	βi + βGenotype	9	136.4	33.2	0
	5	βi + βGenotype + βStimulus + βGenotype x Stimulus	14	179.5	76.4	0
Familiarity: Aggression	**3**	**βi + βStimulus**	**10**	**123.3**	**0**	**0.9**
	4	βi + βGenotype + βStimulus	6	127.9	4.6	0
	5	βi + βGenotype + βStimulus + βGenotype x Stimulus	14	133.9	10.5	0
	2	βi + βGenotype	9	135.1	11.7	0
	1	βi	5	138.9	15.6	0

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