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The Contribution of the Environment and Genes to the Development of Personality in Three-Spined Stickleback (*Gasterosteus aculeatus*)

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Aggression and Attraction: Aggressive Three-spined Stickleback (*Gasterosteus aculeatus*) Males Show Increased Sexual Signalling Effort

MRes Animal Behaviour

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General introduction

Personality research

Behavioural flexibility is presumed to be advantageous, allowing individuals to respond to others' behaviours and react to ever changing environmental conditions (Bergmüler 2010). However, individuals within the same species have been found to show consistent behavioural differences often described as personality. Personality is characterised by between-individual differences in behaviour that are consistent across time and context (Niemelä *et al*., 2012). Species often appear to show high inter-individual and low intra-individual variation in behaviour (Schuett, Tregenza and Dall, 2010). For example, within a population, some individuals are consistently bolder (more willing to take risks) than others (Wilson *et al.*, 1994). This suggests behaviour may not be as plastic as previously assumed. These behavioural differences have been found in many different species including birds (Groothuis and Carere, 2005), amphibians (Wilson and Krause 2012), mammals (Dammhahn, 2012), reptiles (Rodríguez-Prieto, Martín, Fernández-Juricic, 2010), fish (Bell, 2005) and invertebrates (Chapman *et al.*,2011).

Many personality traits are correlated with each other; this is referred to as a behavioural syndrome (Sih, Bell and Johnson 2004). A behavioural syndrome can be defined as a suite of correlated behaviours consistent across contexts (Bell, 2005; Sih, Bell and Johnson, 2004). A common behavioural syndrome widely observed is the boldness-aggression syndrome where bolder individuals also tend to show higher levels of aggression (Bell, 2005; Brick and Jakobsson, 2002; Moretz, Martins and Robinson, 2007).

Animal personality is an emerging area of research within the field of behavioural ecology. Both the origin and maintenance of animal personalities are not fully understood. To gain a proper understanding, both functional and mechanistic approaches must be considered (Dingemanse and Réale, 2005). The first experiment focuses on the proximate mechanisms of personality with an aim to further understand the contribution of the environment and genes on personality and the stability of personality across ontogeny. The second experiment is concerned with the ultimate

outcomes of personality i.e. fitness and life history consequences and also explores whether sexually selected traits are correlated with personality traits boldness and aggression.

Stickleback as model species

The Three-spined stickleback (*Gasterosteus aculeatus*) is a well-studied model organism used widely in evolutionary, (Jones *et al.*,2012), physiological (Pottinger, Carrick and Yeomans, 2002) and behavioural (Dingemanse *et al.*, 2007) research. Stickleback are commonly used in research due to their wide distribution, ecological flexibility, phenotypic diversity and ease of housing (Bell and Foster, 1994; Huntingford and Ruiz-Gomez, 2009). They are distributed across the whole of the Northern Hemisphere, inhabiting fresh, brackish and saltwater habitats (Bell and Foster, 1994).

Variation in behavioural (Dingemanse *et al*., 2007), life history (Baker, 1994), phenotypic (Lavin and McPhail, 1986) and morphological (Lavin and McPhail, 1986) traits has been widely observed in three-spined stickleback (Huntingford and Ruiz-Gomez, 2009). They are famous for their phenotypic diversity shaped by a broad geographical range and ecological flexibility resulting in thousands of isolated populations (McKinnon and Rundle, 2002). These isolated populations are ideal for comparative studies of natural selection (De Roij and MacColl, 2012).

The Three-spined stickleback is a model species for behavioural studies as it shows a diverse range of behaviours (Dingemanse *et al.*,2007). Nikolaas Tinbergen was the first to establish stickleback as a model species in behavioural research (Tinbergen and Van Iersel, 1947). Tinbergen started studying sticklebacks in the 1930's and described stickleback behaviour as simple enough to be easily measured but complex enough to be of interest (Huntingord and Ruiz-Gomez, 2009).

When male stickleback reach breeding condition (March to August in the UK) they develop red throat colouration and blue eyes (Kraak, Bakker and Mundweiler, 1999). The males establish a territory which it defends from other males and builds a nest using sand, gravel and plant materials (Páll, Mayer and Borg, 2002). Breeding males

produce a glue within the kidneys called spiggin which they use to build the nest (Páll, Mayer and Borg, 2002). They perform an elaborate courtship dance to attract females to their nest (Candolin, 1999). The courtship dance includes a zigzag dance where the male moves swiftly from side to side in a zigzag pattern (Cabdikub, 1999). Once the male has the female's attention he lures her towards the nest by displaying fanning, pecking, gluing and creeping behaviours (Cabdikub, 1999). If the female chooses to spawn, she crawls through a hole in the nest and deposits her eggs. The male follows behind to fertilise the eggs. After spawning, the female leaves and the male cares for the eggs. The male guards the fertilised eggs and fans oxygenated water at them. Males are highly polygynous and may care for eggs from several females within a single nest. Once the eggs hatch the male will defend the fry for several weeks (Kraak, Bakker and Mundweiler, 1999).

The Contribution of the Environment and Genes to the Development of Personality in Three-Spined Stickleback (*Gasterosteus aculeatus*)

Abstract

Personality, once a term restricted exclusively to *Homo sapiens,* is now a widespread subject in the field of behavioural ecology. A major area of debate for personality researchers is one which questions the relative contributions of environment and genes on personality, mirroring the "nature versus nurture" debate in human personality studies. Another major question considers the development of personality and its stability over ontogeny. This study aimed to investigate the role of genes and the environment on the development of personality and behavioural syndromes. Wild stickleback from five geographically separated lochs on North Uist, Scotland, were analysed for boldness, activity and exploration. Offspring of these wild fish were laboratory reared in standardised conditions and assayed in their juvenile and adult stage to investigate personality change across development. The only differences between the populations of lab-reared fish was the loch their parents originated from. Results indicated clear behavioural differences between adult wild and adult lab-reared populations despite similar genotypes. Adult lab-reared fish exhibited lower levels of activity, exploration and boldness than wild fish. There were no significant differences in behaviour between the populations of adult lab-reared stickleback. However, wild fish and juvenile lab-reared fish showed significant behavioural variation between populations. These results tentatively point towards a leading role of the environment in shaping behaviour. However, behaviour was not stable across development. Juvenile behaviour showed some similarities to wild adult behaviour, but these similarities were lost over maturation. These results suggest that initial gene effects may be compounded by experience/environmental influences. Personality may be subject to developmental plasticity allowing for individuals with similar genotypes to show differing behavioural phenotypes.

1. Introduction

Behavioural plasticity is presumed to be advantageous, allowing individuals to display the optimal behaviour for each situation (Bergmüler, 2010). However, individuals within the same species have been found to show consistent differences in behaviour, often described as personality (Schuett, Tregenza and Dall, 2010). Within-species variation in behaviour, or personality, is widely researched in the field of behavioural ecology (Dall, Houston and McNamara, 2004).

Personality and behavioural syndromes (population-level correlations between personality traits) have recently received much research focus because they imply behaviours may not be as plastic as previously assumed (Bell, 2005; Dingemanse and Réale, 2005). Behavioural syndromes can lead to behavioural carryovers across contexts and can explain why some individuals show behaviours seemingly inappropriate or non-adaptive for the situation, e.g. high levels of aggression in the presence of potential mates (Sih, Bell and Johnson, 2004).

Personality can have both ecological and evolutionary consequences for example, on species' distributions and rates of speciation (Ingley and Johnson, 2014). The fitness consequences of personality are likely context dependent (Coleman and Wilson, 1998) and vary with environmental variables such as food availability (Dingemanse *et al*., 2004) and predation (Réale and Festa-Bianchet, 2003). For example, aggression may be advantageous in intra-specific competition for food and/or mates, but may be costly in the presence of a predator. Smith and Blumstein (2008) conducted a meta-analysis and found a general trend for bolder individuals to have increased reproductive success but higher survival costs thus demonstrating fitness consequences of boldness.

Boldness (exhibiting more risky behaviours) and shyness (exhibiting more reserved behaviours) are common personality traits researched in personality studies and these traits have been found to vary greatly between individuals (Brown, Jones and Braithwaite, 2005; Frost *et* al., 2007; Ward *et al*., 2004). Both shy and bold personalities experience trade-offs (Wolf *et al.,* 2007). 'Bold' individuals are more likely to accept risks in return for increased foraging or reproductive gains (Wilson *et al*., 1994). Whereas 'shy' individuals are risk-averse, and in doing so may consequently have lower foraging or reproductive gains (Wilson *et al*., 1994). The

position of an individual along the shy-bold axis dictates whether the trade-off is predation risk or resource gain (Ward *et al*., 2004). This has been demonstrated in bighorn sheep (*Ovis canadensis*) (Réale *et al*., 2000), swift fox (*Vulpes velox*) (Bremner-Harrison *et al*., 2004), guppy (*Poecilia reticulata*) (Dugatkin, 1992) and great tits (*Parus major*) (Dingemanse *et* al., 2004).

1.1. Why show personality?

There have been several major shifts in thinking within the field of behavioural ecology (Bergmüller and Taborsky, 2007). Behavioural ecologists previously considered behaviour as plastic, enabling the optimal behaviour to be exhibited in each situation. However, the existence of personality and behavioural syndromes implies that individuals may be limited in their behavioural plasticity (Dingemanse *et al*., 2010). Animals within a population were previously predicted to evolve towards an adaptive population average phenotype to produce the highest fitness under specific environmental challenges (Dingemanse *et al.*, 2010). Behavioural variation between individuals was viewed as a set of non-adaptive anomalies surrounding a mean optimal phenotype (Bergmüller, 2010; Dall, Houston and McNamara, 2004), i.e. personality was viewed as an evolutionary constraint limiting an individual's ability to respond freely to stimuli (constraint hypothesis) (Bergmüller and Taborsky, 2007). The constraint hypothesis predicts that personality and behavioural syndromes are difficult to uncouple due to proximally linked behaviours or pleiotropy (Bergmüller and Taborsky, 2007). Therefore, personality and behavioural syndromes should be typical characteristics of a species, or prevalent across different populations regardless of environmental differences (Brydges *et al*., 2008).

More recently, researchers have begun to describe personality as adaptive and maintained by natural selection rather than as a consequence of pleiotropy or proximal links (adaptive hypothesis) (Bergmüller and Taborsky, 2007). The adaptive hypothesis posits that if behavioural syndromes exist they are likely a combination of traits which function well together and therefore may be a result of selection (Bell, 2005). Furthermore, we are unlikely to see the same behavioural syndromes across same-species populations in different selective environments (Brydges *et al*., 2008). This is because different selection pressures may favour a different combination of traits or

may uncouple behaviours (Brydges *et al*., 2008). Numerous species have been found to show varying behavioural traits across different geographical locations (Bell, 2005; Bell and Sih, 2007; Brown, Burgess and Braithwaite, 2007; Dingemanse *et al*., 2007) For example, Dingemanse *et al*., (2007) found stickleback personality and behavioural syndromes to vary in 12 different populations based on differences in the selective environment. However, more research is needed to identify key selective factors (such as predator abundance and competition for resources) that favour behavioural syndromes and personality under certain conditions.

1.2. Genes vs the environment

It is often debated whether between and within population differences in behaviours are caused by environmental factors or genes (Van Oers *et al*., 2005). Nature versus nurture studies on humans have revealed evidence for both genes and the environment influencing personality traits (McCrae *et al.*, 2000). However, there is relatively little research on the contribution of heritable and experiential effects on personality type in other animals.

The adaptive hypothesis predicts that there will be an association between personality traits and the type of environment from which a population originates (Bergmüller and Taborsky, 2007). Environmental conditions, both biotic and abiotic, such as predator abundance and food availability, can act as strong selection pressures which influence behaviour (Brown, Burgess and Braithwaite, 2007). Repeated exposure to specific selection pressures has been found to significantly influence personality traits such as boldness, and increase the presence of behavioural syndromes (Brown, Burgess and Braithwaite, 2007). For example, Dingemanse *et al*., (2009) experimentally showed that population differences in stickleback can be explained by variation in selection induced by predators. Populations exposed to predators were more inclined to show behavioural syndromes, therefore having limited plasticity across contexts compared to predator-naïve populations. Predators appear to act as selective agents affecting the evolution of their prey, which supports the adaptive hypothesis (Dingemanse *et al*., 2009). Variation in environmental conditions can cause fluctuating selection pressures which may be one of the reasons behavioural variation within species is maintained (Adrianenssens and Johnsson, 2010).

How an individual behaves will influence life history traits e.g. reproductive success, and therefore behaviour is assumed to be at least partially heritable due to natural selection (van Oers *et al*., 2005). Research has found evidence of heritability in several personality traits in a number of species (Dingemanse *et al*., 2002; Drent *et al.*, 2003 and Saetre *et al*., 2006). For example, Dingemanse *et al.,* (2002) found variation in exploratory behaviour to be heritable in wild great tits (*Parus major*).

According to the "rules" of frequency-dependent selection, phenotypic fitness is dependent on the frequencies of other phenotypes within a population (Dall, Houston and McNamara, 2004) and this may offer support for the constraint hypothesis and explain differing behavioural strategies within populations (Dall, Houston and McNamara, 2004). However, the social environment is also a selection pressure (Cote and Clobert, 2007) and therefore frequency-dependent selection may also be a mechanism of the adaptive hypothesis. For example, bold individuals may be better able to exploit food in risky areas than shy individuals. The benefits of being bold however, will decrease with the frequency of bold individuals due to density-dependent resource competition (Wolf *et al*., 2007). Boldness is therefore negatively frequency-dependent, promoting the coexistence of shy and bold individuals (Dall, Houston and McNamara, 2004; Wolf *et al*., 2007).

Studies have found behavioural syndromes to be heritable (Dingemanse *et al*., 2002) and also to vary between populations of the same species across different environmental gradients (Dingemanse and Wolf, 2013). Brown, Burgess and Braithwaite, (2007) found lab-reared fish to show similar personality traits to their wild-caught parents indicating a heritable component to personality. However, they also found boldness to be related to environmental experiences, offspring regularly chased with a net (imitating predation) tended to show bolder behaviours (Brown, Burgess and Braithwaite, 2007). It is likely that both genes and the environment contribute to the development of personality type. Epigenetic effects involve both genes and environmental influences and are likely to contribute to personality development (De Winter *et* al., 2016).

1.3. The ontogeny of personality

It is often assumed that personality traits and behavioural syndromes are at least

partially heritable (Van Oers *et al*., 2005). This assumption accompanies the assumption that personality is temporally stable during development (Groothuis and Trillmich, 2011; Stamps and Groothuis, 2010). Research into personality is biased towards adults as many behaviours are age dependent (Niemelä, *et al*., 2012). However, genes are unlikely to be the only influence on personality. For example, aggression is not commonly seen until sexual maturity (Peiman and Robinson, 2007). Therefore, it is important to study personality across extended periods of time or development. Few studies have looked into personality changes during ontogeny. Bell and Stamps, (2004) examined developmental stability of activity, aggression and boldness in two populations of lab-reared stickleback measured as juveniles, sub-adults and adults. They found individual behaviours were not stable through time, but behavioural correlations/syndromes were (Bell and Stamps, 2004).

Behavioural syndromes are likely to remain stable throughout ontogeny if the proximate mechanisms linking the behaviours are not uncoupled during development (Bell and Stamps, 2004). Behavioural syndromes may, however, break down over ontogeny, particularly during any major physical changes such as metamorphosis or sexual maturity (Sinn and Moltschaniwskyj, 2005). It is also possible that individuals may change their behaviours with changes in environmental conditions, e.g. food availability and this may be adaptive (Wolf and Weissing, 2010).

There is an ongoing debate as to the origins and maintenance of personality. Several studies have attempted to unravel the influence of genes and the environment on personality (Ahlbeck and Holliland, 2012; Bell, 2005; Brown, Burgess and Braithwaite, 2007; Dingemanse *et al*., 2007; McCrae *et al*., 2000; Saetre *et al*., 2006; Stamps and Groothuis, 2010). This study explored this by looking at behavioural differences between wild and lab-reared populations of stickleback originating from five different geographically isolated lochs on North Uist. Environmental influence is predicted to be a more powerful influence on personality than genes. Lab-reared adults from different populations are predicted to show no significant difference in behaviour between populations. These predictions are based on the findings of other studies where environmental variables such as exposure to predators appear to drive the development personality and/or behavioural syndromes (Brown, Burgess and Braithwaite, 2007).

Additionally, the study will investigate changes in personality expression between juvenile and adult stages. Behaviours are predicted to be unstable across ontogeny within a controlled lab setting, this prediction is due to two reasons. Firstly, juveniles are under the influence of differing selection pressures to adults, for example, juveniles of several species are more vulnerable to predation than adults (Laurel, Gregory and Brown, 2003; Longland and Jenkins, 1987; Sogard, 1997). Secondly, sexual maturity is an important stage during development where behavioural as well as morphological changes are likely to occur, for example, levels of aggression in males may increase during sexual maturity when males begin to defend a territory (Archer, 2006; Sakakura and Tsukamoto, 1998).

2. Methods

2.1. Study animals and experimental conditions

Between April and May 2015, wild adult three-spined stickleback from five different lochs on the island of North Uist (57.5704° N, 7.2812° W), Scotland, were captured and subsequently assayed for boldness, activity, and exploration (details described below). These wild individuals were stripped of eggs and sperm and the resultant offspring of these wild populations were reared under common garden conditions in the laboratory. The offspring were subsequently tested for boldness, activity and exploration while they were still juveniles (between October and November 2015) and later when they had reached maturity (between July and August 2016) to see if behavioural differences between populations were still expressed.

During the experimental period of five weeks, 30 fish per week (six from each group) were assayed. During this period, the 30 fish were all held in individual tanks (12cm X 20cm X 12cm). They were moved to their individual tanks on Monday afternoon and left to acclimatize for one day before trials. Trials were conducted between Tuesday and Friday. On Friday afternoon, once trials had ceased, the length of each fish was measured (mm) and the fish were then returned to larger, group tanks (100cm X 45cm X 30cm). The experimental room was held at a controlled temperature of 15°C constantly.

2.2. Data collection

Every individual was assayed twice for each of the three behaviours (boldness, activity and exploration) within a four-day period. Assays were conducted twice to test for temporal consistency. Table 1 below shows the number of fish assayed in each condition and population. Observer effect was minimised using white, opaque plastic liners stuck around the sides of the experimental tanks. This also prevented any visual signals being passed between the fish being assayed which could otherwise potentially alter their behaviours.

Table 1. The number of fish used in analysis from each population and condition

Population	Juvenile lab	Adult lab	Adult wild
CHRU	24	11	39
HOST	29	26	30
REIV	30	24	30
STRU	28	30	40
TORM	16	9	40

Data previously collected by De Winter *et al*., (2016) on environmental characteristics of each loch the wild fish were collected from included information on presence/absence of brown trout (*Salmo trutta*) (the main predator of three-spined stickleback), parasite density and resource availability (see table 2).

Table 2. The environmental characteristics of sampled lochs on North Uist, Scotland.

Loch	Parasites	Predators	Resource
CHRU (Chada Ruaidh)	Medium	No	Low
REIV (na Reival)	High	No	High
STRU (nan Struban)	High	Yes	Medium
TORM (Tormasad)	Low	Yes	Medium
HOST (Hosta)	High	Yes	?

All fish were assayed for boldness twice over a period of two days (Tuesdays and Wednesdays). The fish were placed into (20 X 40 X 30cm) tanks and left to acclimatise for three minutes. Pilot studies indicated that fish freeze in the presence of a fright stimulus and vary in the length of time it takes them to resume movement.

Latency to move was used as an indicator of an individual's “boldness” as used in De Winter *et* al., 2015. Once the acclimatisation period was over, a piece of cardboard was placed over the top of the tanks as a fright stimulus (simulating a potential predator overhead) and held for three seconds before being swiftly removed. Stop watches were used to time how long it took for the fish to begin moving again after the fright stimulus (the timer was stopped when movement was a continuous motion for a distance greater than the focal individual's body length). A maximum recording period of 300 seconds was used as very few fish in the pilot study took longer than 300 seconds to move after the fright stimulus. Boldness scores were calculated by taking the score (i.e. number of seconds before movement) away from 300 (the ceiling value) so bolder fish had larger values and any fish taking more than 300 seconds to move scored 0. Two fish were trialled at one time in separate tanks. Between each trial a quarter of the water from the trial tanks was removed and replaced with fresh filtered water to avoid any olfactory cues from other fish (De Winter *et* al., 2015; De Winter *et al*., 2016).

Activity and exploration were measured twice over a period of two days (Thursday and Friday) at the same time. Ethovision software (Ethovision XT recording, Noldus Information Technology, Wageningen, Netherlands) was used to track the movement of the subjects. Four fish were trialled at the same time with four separate cameras (Panasonic WV-CP314E CCTV) and four arenas (55cm X 45cm X 20cm). The arenas all contained two white plastic cups as novel items and refuge. Fish were introduced to the arenas and given 3 minutes to acclimatise before the live tracking began. The tracking period lasted for five minutes during which both activity (total time moving out of 300 seconds) and exploration (the area of the arena covered in meters) were measured. Data for activity was received instantly once the trial had stopped. Data for exploration required heat maps being processed through ImageJ (https://imagej.nih.gov/ij/) at a later date. Ethovision tracking was performed under low light conditions to prevent light reflections disrupting tracking.

2.3. Statistical analysis

Data were found to be non-normally distributed via a Shapiro-Wilk test so non-parametric tests were adopted. IBM SPSS Statistics 23 and R x64 3.3.1 were used for

statistical analysis throughout.

To check for behavioural consistency between trials one and two, Spearman's rank correlation tests were conducted for each behaviour in each population. A total of 15 tests were conducted. Due to a large number of tests being conducted, it was necessary to perform a sequential Bonferroni correction (Peres-Neto, 1999). Spearman's rank correlation was also carried out on the three behaviours within each population to investigate the presence of any behavioural syndromes.

Generalised linear mixed models were carried out on adult wild, juvenile lab and adult lab data looking at Population, Length and Population:Length interactions. Post-hoc Tukey test and Spearman's rank tests were used to determine the direction of any significant results. Kruskal-Wallis tests were conducted to explore whether behaviours differed between conditions (adult wild, adult lab and juvenile lab) originating from the same population. Generalised linear models were used to determine whether parasite burden, predator presence and resource availability from the lochs the wild parents originated from influenced behaviours in each condition.

3. Results

3.1. Consistency

Results of Spearman's rank correlation tests for behavioural consistency between trials found all significant correlations in juveniles, to be either weak or moderate. To reduce the chances of type 1 errors by multiple testing, a sequential Bonferroni correlation was conducted. None of the corrected P values were significant, indicating no consistency between trials in the juveniles.

Wild adult data were also tested for consistency. After Bonferroni correction, boldness was found to be consistent in CHRU (R_s=0.492, P=0.001) and TORM (R_s=0.733, P<0.0001), Activity was consistent in REIV (R_s=0.617, P<0.0001) and STRU (R_s=0.558, P<0.0001), no consistency was found in exploration after correction. Adult data appears to be more consistent between trials than juvenile data.

Adult lab-reared fish were also analysed for consistency and again, little consistency was shown with only REIV (R_s=0.575, P=0.003) and STRU (R_s=0.526, P=0.003)

showing consistency in exploration. Due to lack of consistency between trials only data from trial one was used for any further analysis.

3.2. Behavioural syndromes

Spearman's rank correlation tests were conducted to test for the presence of behavioural syndromes (correlations between behaviours). For the lab-reared juveniles, correlations between behaviours were found between activity and exploration in STRU (R_s=0.558, P=0.002) and HOST (R_s=0.831, P<0.0001). Boldness and exploration were significant in CHRU (R_s=0.678, P<0.0001). In the adult wild populations activity and exploration were correlated in CHRU (R_s=0.691, P<0.0001) and TORM (R_s=0.476, P=0.002). For the adult lab-reared fish, activity and exploration were correlated in REIV (R_s=0.608, P=0.002), STRU (R_s=0.707, P<0.0001), HOST (R_s=0.725, P<0.0001) and TORM (R_s=0.845, P=0.004).

3.3. General linear models

Generalised linear models (with Gamma error structure) were conducted to investigate which factors explain variation in boldness/activity/exploration. Tests were conducted for each condition (juvenile lab, adult lab and adult wild) and for each behaviour (boldness, activity and exploration). The model incorporated Population (HOST, CHRU, STRU, TORM and REIV), Length and the Population:Length interaction.

Table 3 contains results of Juvenile lab-reared fish. Population was significant for boldness and exploration (as seen in adult wild fish). Post-hoc Tukey's HSD tests found boldness to be significantly different between populations (P=0.023). For exploration, there was also a significant difference between populations (P=0.005).

Table 3. Results of generalised linear models conducted for juvenile lab fish demonstrating a significant effect of population on boldness and exploration

Behaviour	**Population:Length**	**Length**	**Population**
Boldness	P=0.6938, Chi^2=0.6707, DF=4	P=0.398, Chi^2=0.2028, DF=1	*P=0.0437, Chi^2=2.754, DF = 4
Activity	P=0.2655, Chi^2=2.7169, DF=4	P=0.114, Chi^2=1.2357, DF=1	P=0.253, Chi^2=2.608, DF=4

Exploration	P=0.3607, Chi^2=0.017569, DF=4	P=0.4494, Chi^2=0.0023, DF=1	*P=0.0035, Chi^2=0.062, DF=4

Spearman's rank tests confirmed a highly significant positive correlation between length and activity in wild adults (P<0.0001, R_s=0.490). Length and exploration also showed a positive correlation (P=0.029, R_s=0.179).

Post-hoc Tukey's HSD tests were conducted to investigate where population differences in boldness and exploration lie. Results found between population variation in exploration to be highly significant (P<0.0001). Boldness also showed high between-population variation (P<0.0001).

Table 4. General linear models conducted for adult wild fish demonstrating the effect of population and length on behaviour (note HOST was removed from analysis in activity and exploration)

Behaviour	**Population:Length**	**Length**	**Population**
Boldness	P=0.2456, Chi^2=0.9171, DF=4	P=0.2748, Chi^2=0.18673, DF=1	*P=0.0003, Chi^2=3.2112, DF = 4
Activity	P=0.0902, Chi^2=7475.6, DF=3	*P<0.0001, Chi^2=52425, DF=1	P<0.08674, Chi^2=10087, DF=3
Exploration	P=0.3191, Chi^2=0.00333, DF=3	*P=0.0011, Chi^2=0.0101, DF=1	*P<0.0001, Chi^2=0.02986, DF=3

Table 5 shows results for adult lab-reared fish. The Population-Length interaction was significant for activity and exploration, meaning the effect of body length on activity and exploration varied between populations. Length had a significant impact on boldness. Spearman's rank correlation tests were conducted on length and boldness and results indicated a significant negative correlation (P=0.007, R_s=-0.276). Population did not have a significant effect on behaviour indicating that there was little difference in behaviours between populations from different founding lochs.

Table 5. General linear models for each behaviour conducted for adult lab fish demonstrating the effect of length and population:length interaction on behaviour

Behaviour	**Population:Length**	**Length**	**Population**
Boldness	P=0.2333,	*P=0.0221,	P=0.3662,

	Chi^2=5.7941, DF=4	Chi^2=5.1971, DF=1	Chi^2=4.274, DF = 4
Activity	*P=0.0199, Chi^2=32.512, DF=4	P=0.3681, Chi^2=1.748, DF=1	P=0.4974, Chi^2=7.093, DF=4
Exploration	*P=0.0001, Chi^2=24.515, DF=4	P=0.8433, Chi^2=0.0450, DF=1	P=0.8678, Chi^2=1.458, DF=4

Generalised linear models were conducted to investigate whether parasite burden, predator abundance and resource abundance influenced activity, exploration or boldness behaviours in each condition. Results indicated no significant effect of either variable on adult lab-reared fish. Results from adult wild fish indicated parasite (P <0.001, DF = 148) and resource (P=0.002, DF = 148) abundance to have a significant effect on exploration behaviour but not boldness or activity. No significant effect was found in juvenile lab-reared fish.

3.4. Behavioural variation between conditions

Tests were conducted to discover whether behaviours differed between conditions (adult wild, adult lab and juvenile lab) originating from the same population (CHRU, STRU, TORM, HOST, REIV). Significant values (denoted by *) indicate behaviours differed between the three conditions. Overall there appear to be high levels of variation between the three conditions, particularly in activity. Adult lab-reared fish generally scored lower boldness, activity and exploration scores, whereas adult wild fish scored highest in these behaviours.

Table 6. Results from a Kruskal Wallis test demonstrating the effect of condition (adult lab, adult wild and juvenile lab) on behaviour.

Population	P value	Chi^2 value	DF
	BOLDNESS		
CHRU	0.599	1.025	2
STRU	0.006*	10.229	2
TORM	0.578	1.095	2
HOST	0.011*	9.054	2
REIV	0.000*	19.712	2
	ACTIVITY		
CHRU	0.000*	24.865	2
STRU	0.000*	49.687	2
TORM	0.000*	24.617	2

HOST (EXCLUDING WILD)	0.020*	5.444	2
REIV	0.000*	42.054	2
	EXPLORATION		
CHRU	0.000*	19.876	2
STRU	0.000*	23.445	2
TORM	0.001*	13.137	2
HOST (EXCLUDING WILD)	0.476	0.508	2
REIV	0.001*	13.078	2

Figure 1. Median exploration with error bars of +/-SE across the five populations and three conditions.

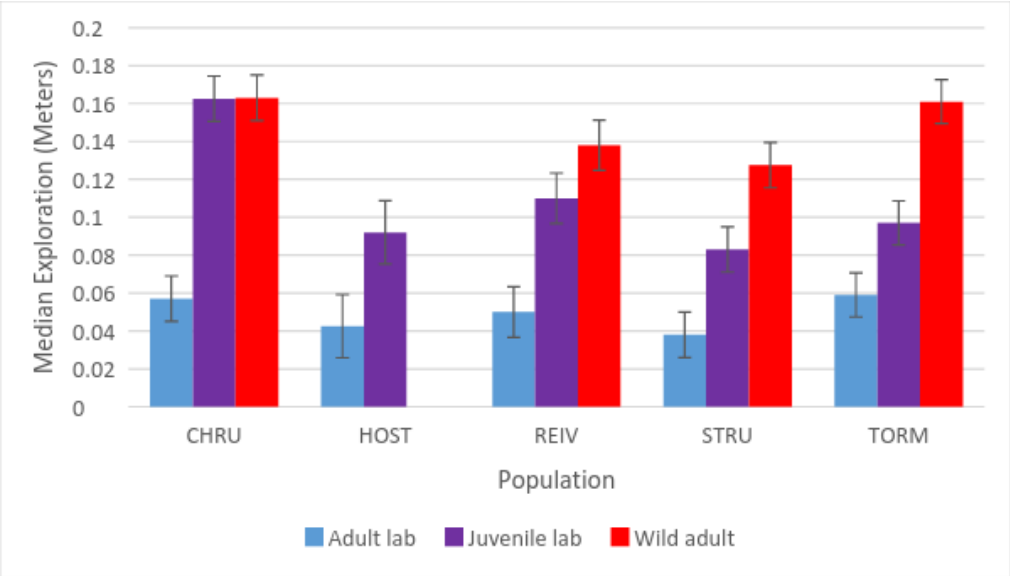


Figure 2. Median Activity with error bars of +/-SE across the five populations and three conditions.

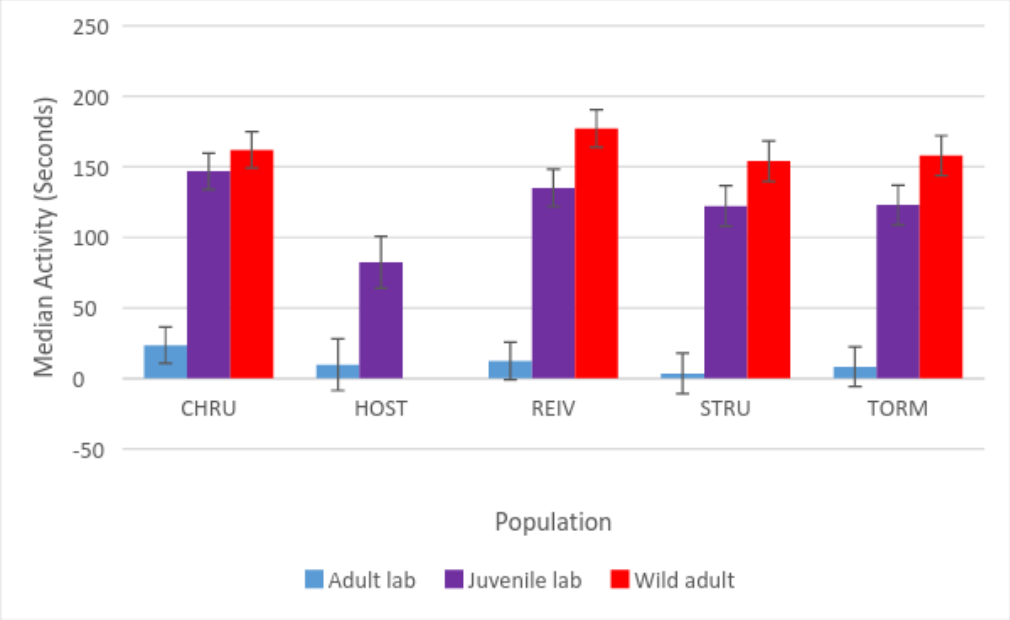
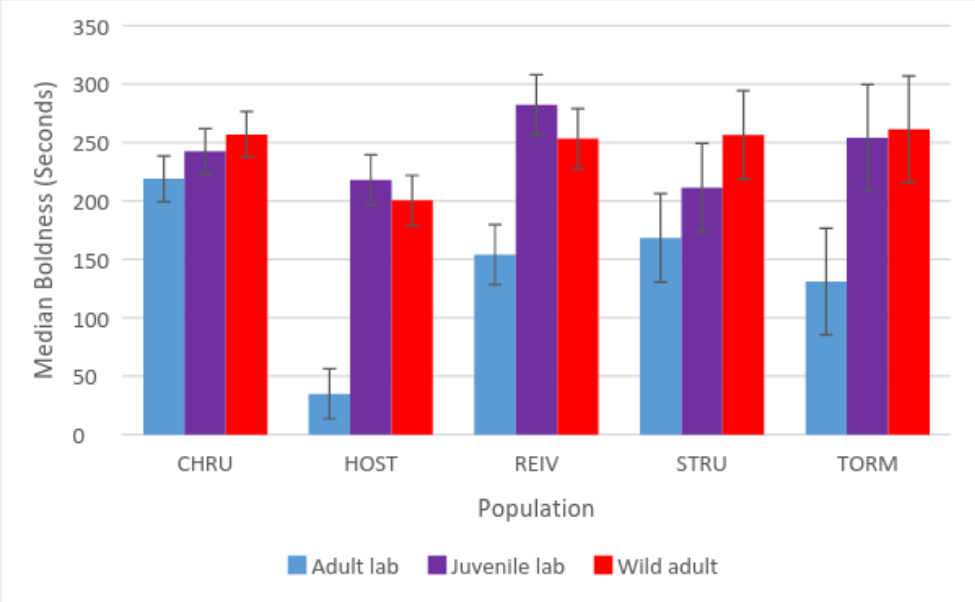


Figure 3. Median Boldness with error bars of +/-SE across the five populations and three conditions.



4. Discussion

This study shows clear behavioural differences between wild and lab-reared adult fish with similar genotypes. Lab-reared adult fish appeared to show more passive behaviour than wild fish by showing much lower levels of activity and exploration.

Furthermore, as predicted there were no significant differences between the populations of adult lab-reared stickleback, but adult wild fish showed significant variation between populations. These results provide tentative support that rearing environment may be more influential on personality than genes. Behaviour was not stable across development indicating personality may be more plastic than previously assumed.

4.1. Environment vs Genes

Behavioural differences between populations were found in the adult wild and juvenile lab fish but not the adult lab-reared fish. Different populations of the wild fish were expected to show behavioural differences due to differing selection pressures between lochs. Indeed, parasite and resource abundance had a significant impact on exploratory behaviour in wild adults. Adult lab-reared fish were expected to show no or little behavioural variation between populations due to standardised environments. The results appear to be in line with these predictions. These results tentatively point towards a leading role of the environment on shaping personality traits.

The results of this study suggest that behaviour of captive stickleback is not analogous to the behaviour of their wild counterparts. Environmental complexity may play a major role in determining behaviour. Ahlbeck and Holliland (2012) reared Pikeperch (*Sander lucioperca*) in semi-natural ponds and indoor tanks to investigate whether the different environments influenced behaviour. Fish reared in the pond condition were faster foragers and more active than the indoor fish. Their results, similarly to the ones from this experiment, suggest that rearing environment plays a vital role in shaping behaviours such as activity.

Key to determining the presence of personality is consistency in behaviours across time (and/or context) therefore personality was not expressed by all populations and conditions. An explanation for why there were low levels of behavioural consistency may be down to habituation to the behavioural assay. For example, in activity/exploration the environment was only truly novel in the first trial as the environment was not changed between the two trials.

Stickleback are regularly kept in laboratory environments for behavioural research.

Standardised artificial environments as seen in lab-reared fish may lack environmental complexity and certain selective factors that may be important for the development of personality traits. It is important to understand the effect a standardised environment can have on behaviours. Although this study focussed on fish that were in a laboratory setting their whole lives, other research has found evidence of much shorter periods in laboratory settings altering behaviours. For example, Butler *et al*., (2006) looked at habituation to captivity in wild chaffinch (*Fringilla coelebs*). The longer the birds were in captivity the less inclined they were to show foraging behaviour. This supports a level of phenotypic plasticity, that individuals (or whole populations) alter their behaviours to suit their current environment.

The results of this study point towards the environment playing a major role in the development of personality. Further research looking into behavioural change over specific environmental gradients may help with predictions of the potential impact of climate change (Cockrem, 2013). Additionally, identifying important selective agents in the environment that impact personality traits (e.g. predator presence) would aid understanding of the mechanisms of personality.

4.2. Ontogeny

Adult wild fish showed the highest levels of boldness, activity and exploration, and adult lab fish the lowest. Behavioural differences between juvenile and adult lab-reared fish may be due to a number of different reasons. For example, tank area per-fish; juvenile fish were smaller than adults and thus had more free space in the tanks. Another possible explanation is that adult lab fish may show lower levels of each behaviour as they are habituated to the lab environment where there is little novelty. A more complex explanation may be that genes play a more prominent role during early development and experience and the environment play a role in shaping behaviour later in life. Personality may be subject to developmental plasticity allowing for individuals with similar genotypes to show differing phenotypes in terms of personality (Carere *et al*., 2005). Some behaviours shown by juveniles may be specific to early life and may be lost or modified during development via mutations (Groothuis and Trillmich, 2011). For example, Bell and Stamps (2004) demonstrated how behavioural syndromes can be uncoupled over development due to environmental

factors such as predation risk. This indicates a potential role of epigenetic factors in the development of personality (Carere *et al*., 2005).

An interesting area for further research may be to look into sex differences in behaviour over ontogeny. In some species, males show higher levels of aggression when they reach sexual maturity (Archer, 2006; Dixon and Cade, 1986). It is therefore likely that behavioural changes over ontogeny may differ between the sexes. Additionally, looking at changes over ontogeny at an individual level may yield more relevant data.

4.3. Conclusions

It is hard to decipher the relative contributions of genes and environmental influences on the development and maintenance of personality traits. The results of this study tentatively point towards a leading role of the environment and plasticity in the development of personality traits. However, there is likely a contribution of genes to behaviour, particularly early on in development. Behaviour was not stable over ontogeny, juvenile lab fish showed population differences that were similar to wild adult behavioural differences between populations, but adult lab fish behaviour did not reflect juvenile or wild behaviour. It is highly probable that epigenetic effects may have an influence on personality, i.e. environmental factors may be modulating the expression of genetic traits. Further research should aim to identify important selective agents within the environment that impact personality development.

Aggression and Attraction: Aggressive Three-spined Stickleback (*Gasterosteus aculeatus*) Males Show Increased Sexual Signalling Effort

Abstract

Personality imposes a limitation on behavioural plasticity resulting in trade-offs dependent on personality type. For example, aggressive males may put more effort into reproduction at a cost to survival (Smith and Blumstein, 2008). Recently, researchers have begun to look into how personality interacts with aspects of life history. One approach is to look at how personality traits correlate with traits influencing aspects of life history such as sexual signalling. This study aims to investigate whether personality traits boldness, and aggression, correlate with behavioural and ornamental sexual signals in the three-spined stickleback (*Gasterosteus aculeatus*). In this study, male stickleback in breeding condition from a single population exhibited consistent individual variation in boldness, aggression and sexual signals. All sexual signals measured (colouration, nest building and courtship behaviours) correlated with each other to form a "reproductive syndrome". Aggression correlated positively with all sexual signals and signalling quality appeared to increase with aggression levels. It is hypothesised that this is likely due to aggressive males putting more effort into signalling/reproductive behaviour with a trade-off of reduced survival. Furthermore, aggression and signalling effort were significantly greater in males that built nests during the experimental period. Finally, males appeared to modulate their signalling effort based on the presence of competitive rivals. Males held in single sex tanks prior to the experimental trials expressed higher levels of aggression and sexual signalling behaviours than males in mixed sex tanks. Whilst some research has investigated the role of personality in mate choice, little research has explored the relationship between signal quality and personality. The results of this study demonstrate that personality and sexual signalling are intricately linked. Further research incorporating a wider grouping of personality traits is needed to better understand the trade-offs between personality type and sexual signalling.

5. Introduction

5.1. Personality

Individuals within the same species have been commonly found to show consistent between-individual variation in behaviour across time and context (Coleman and Wilson, 1998; Dingemanse *et al*., 2007). This is commonly referred to as "animal personality" and is widespread in numerous wild and domestic species from domestic dogs (*Canis lupus familiaris*) (Svartberg and Forkman, 2002) to ants (*Myrmica*) (Chapman *et al.,* 2011). The concept of personality implies a restriction in the behavioural plasticity of an individual (Sih, Bell and Johnson, 2004). This limited plasticity is likely to have both ecological and evolutionary consequences, for example on distribution and rates of speciation (Ingley and Johnson, 2014). Until fairly recently, individual differences in behaviours were considered random variation around optimal mean behaviours (Bergmüller and Taborsky, 2007). However, researchers now favour a more adaptive explanation (Bergmüller and Taborsky, 2007).

It is widely questioned why personality types are maintained over time. Personality types have been found to have associated costs to fitness, therefore to persist these costs must be balanced with significant benefits e.g. increased reproductive output. Boldness and shyness are traits which vary greatly between individuals and are commonly tested for in personality studies (Ward *et al*., 2004). Both shy and bold personalities have been found to have trade-offs. 'Bold' individuals are more likely to trade risks to survival for increased foraging or reproductive gains. Whereas 'shy' individuals are risk-averse, and in doing so may consequently have lower foraging or reproductive gains. For example, Smith and Blumstein (2008) conducted a meta-analysis and found that bolder individuals across numerous species had increased reproductive success but lower survival than shyer individuals.

5.2. Natural and Sexual selection

The influence of natural selection on personality has been acknowledged in numerous species (Dingemanse *et al*., 2002; Dingemanse and Réale, 2005; Groothuis *et al*.,

2008). For example, the adaptive hypothesis is now widely accepted as an explanation of natural variation in personality between populations of the same species (Sih, Bell and Johnson, 2004). However, few studies have considered the role of sexual selection in the evolution and maintenance of personalities (Dingemanse and Réale, 2005; Schuett, Tregenza and Dall, 2010). One study which has investigated this link is Godin and Dugatkin (1996) who conducted a study on personality traits and sexual selection in the Trinidadian guppy (*Poecilia reticulata*). Brighter males were found to be bolder than drab males and females showed preference for bolder males irrespective of their brightness (Godin and Dugatkin, 1996).

Darwin (1859), noting that natural selection alone could not explain traits that were detrimental to survival, theorised sexual selection as an explanation. Sexual selection leads to the development of ornamental signals which demonstrate fitness. These signals are often costly to the individual e.g. a red chest may work to attract females, but may also attract predators. Females (the limiting sex) show preferences for these signals due to direct fitness benefits (e.g. to survival or fecundity) and indirect fitness benefits (e.g. genes) (Candolin, 2003; Fisher, 1915; Zahavi, 1975). Sexual signals exist either to enhance their competitive ability for access to females (intra-sexual selection) or to make the male more attractive to females (inter-sexual selection) (Bakker and Milinski, 1993). In both forms of sexual selection, more ornamented males are usually at an advantage (Hill and Yasukawa, 2014; Wells, Safran and Dale, 2016).

Mate choice by females acts as a selective force driving the evolution of male sexual signals. Sexual selection causes different selection pressures on males and females leading to sex differences in physiology (hormones, carotenoids) which is then expressed as behavioural (e.g. aggression) and morphological (e.g. ornamental traits) differences.

Evidence is beginning to emerge that social environment and the presence of rival males may also influence sexual signalling (Dijkstra *et al*., 2007). The presence of rival males reduces an individual male's chances of successfully mating due to increased competition (West-Eberhard, 1979). Therefore, males may invest more energy into sexual signalling when other males pose a threat to territory or access to

females (Candolin, 2000; Dijkstra *et al*., 2007).

Fundamental to sexual selection is communication via signals. Signals come in various forms including tactile, visual, chemical and acoustic (Dirienzo and Hendrick, 2014). They can act alone or in concert with other signals. Despite a single sexual signal being sufficient in some species, many species showing sexual dimorphism display multiple sexual signals to attract mates (Candolin, 2003). Males often display multiple morphological and behavioural cues, and even resources such as territory and nests, to attract females (Candolin, 2003). Species showing multiple sexual signals tend to show greater variation in these traits (Møller and Pomiankowski, 1993). Species with only one signal are subject to stronger selection restricting both the number of signals (to a single signal) and how much the signal varies between individuals (Møller and Pomiankowski, 1993).

There are numerous plausible hypotheses to explain the benefits of multiple signals, and these hypotheses are not always mutually exclusive. The hypotheses fall into three main categories; content based signals, efficiency based signals and inter-signal interactions (Dirienzo and Hendrick, 2014). Content based signalling involves signals conveying information on the quality or condition of the signaller. For example, Hughes (1996) studied claw signals in snapping shrimp (*Alpheidae*) and found males to alter their response to an open claw signal depending on whether male or female chemical signals were present. Efficiency based signalling involves different components aiding signal transmission or detectability. For example, Leonard *et al.*, (2011) found bumble bees presented with rewarding and unrewarding plants differing in both colour and scent to better at distinguishing between colours than bees only exposed to colour without scent. Finally, with inter-signal interactions, different signals combine to elicit a response that is not achievable from a single signal (Dirienzo and Hendrick, 2014; Lozano, 2009; Heuschele *et al*., 2009).

5.3. Sexual selection and personality

Personality and sexual selection as separate fields are heavily researched. However, relatively little research has been conducted on the relationship between personality and sexual selection (Schuett, Tregenza and Dall, 2010). Both inter and intra-sexual

selection have the potential to influence the maintenance of personality. Both male-male competition and non-random mate choice by females impose selection either directly or indirectly for personality traits. For example, in male-male competition (intra-sexual selection) more aggressive males may be favoured. And in inter-sexual selection, a female may be indirectly (or directly) acquiring a bolder male when choosing the male on the basis of the male's colouration - if a correlation between boldness and colouration exists. This may then lead to the development of behavioural syndromes.

Several studies have found mate choice can not only be based on sexual signals but also on personality traits (Kortet *et al*., 2012; Schuett *et al*., 2011) For example, Godin and Dugatkin (1996) experimentally demonstrated a positive correlation between a colouration (sexual signal), and boldness (personality trait), in the Trinidadian guppy (*Poecilia reticulata*). They found females to prefer colourful males, but chose bolder males irrespective of colour if able to watch their behaviours. Additionally, assortative mating by personality type has been found in several species (Ariyomo and Watt, 2013; Gabriel and Black, 2012; Kralj-Fišer *et al*., 2013). For example, aggressive bridge spider (*Larinioides sclopetarius*) males have a preference for mating with aggressive females (Kralj-Fišer *et al*., 2013). These results are surprising given the assessment costs and difficulties of observing personality type in the field.

Personality has associated costs which vary between personality type, for example bolder individuals have reduced survival rates, but tend to benefit from increased reproductive success (Smith and Blumstein, 2008). Sexual signals are costly to produce, both energetically and in terms of predation risk, but higher signal quality has been found to increase reproductive success (Bakker and Mundwiler, 1994). With both personality and sexual signals demonstrating costs and benefits there may be trade-offs between the personality of a signalling individual and the quality of their signal. For example, exploratory stickleback may be at a greater risk of predation so may reduce the intensity and area of their red throat colouration in favour of behavioural signals such as courtship dances (Dirienzo and Hendrick, 2014).

It seems likely that a correlation between personality traits and sexual signals may exist. Personality is already linked to reproductive success, for example, bold males having increased reproductive success (Smith and Blumstein, 2008) and assortative mating based on personality type in some species (Kralj-Fišer *et al.*, 2013). Given that some personality types are riskier than others (for example, high boldness and aggression), it is of interest to look into their relationship with sexual selection. What benefits are these males gaining to balance their costs? Are males showing riskier behaviours also showing stronger sexual signals? Costs of signalling and personality may result in personality-signal trade-offs, for example, shy individuals may spend a large proportion of their time hidden away so may lower their investment in visual signals. Variation in signaller personality may therefore influence signal quality.

Stickleback males display multiple sexual signals including red colouration on the throat, blue eyes, nest building, physical length, aggressive defence of territory, and an elaborate courtship dance (Kraak, Bakker and Mundweiler, 1999). They have also been found to show a wide range of personality traits (Dingemanse *et al.,* 2007). Personality traits and sexual signals have both been found to vary greatly between individual sticklebacks (Candolin, 1999).

This study aims to examine the relationship between two personality traits (boldness and aggression) and multiple sexual signals (colouration and courtship behaviours) in the three-spined stickleback. The study aims to address three key questions: 1. Does boldness and/or aggression correlate with sexual signals? 2. Do males that build nests within the study period show different personality and sexual signals to males which do not build their nests within the study period? And 3. Does the social environment prior to trials influence sexual signalling? It is predicted that there will be a positive correlation between one or more personality trait (aggression and/or boldness) and one or more sexual signal (colouration and courtship behaviours). Males that build nests within the study period or earlier on in the study period are predicted to be bolder and more aggressive than non-building males as they will be defensive of their territories. Finally, males housed within mixed sex tanks are predicted to show higher rates of sexual signalling due to the presence of potential mates and higher levels of

aggression due to the presence of rival males.

6. Methods

Male sticklebacks were collected in late March 2016 from Tottle Brook, Highfields Park, Nottingham (52°56′06”; -1°11′41″) using minnow traps. The fish were transferred to the laboratory and stored in groups of 15-20 individuals in (100 x 45 x 30 cm) glass tanks at approximately 15 degrees Celsius with a 16:8 light dark cycle. They were fed bloodworm in the morning, daily.

Only males showing breeding colouration were used in the trials. Their length was measured to the nearest millimetre with callipers. They were housed in isolated tanks (45 x 25 x 25 cm), at 15 degrees Celsius and a 16:8 light dark cycle. Each tank was equipped with gravel covering the whole bottom area of the tank, 150 ml of sand in a pile in the front right corner, multiple (200) 7 cm long black polyester threads for nesting material, two small plastic plants for refuge and a sponge airlift biofilter to maintain water quality throughout the experiment (as used in Barber, Nairn and Huntingford, 2001). To encourage the construction of nests, each male was briefly presented with a female held within a glass jar once daily (excluding weekends).

Each morning before feeding the males, around 10% of the tank water was replaced with fresh filtered water to prevent waste build-up. The outside of three sides of the tank were covered with blue plastic posters to reduce observer effect and the influence of any social cues from conspecifics. The posters also minimise reflections which may influence behaviour. The remaining side of the tank was temporarily covered when trials were not being conducted.

6.1. Boldness

All fish were assayed for boldness on two consecutive mornings, the same protocol was followed for both. The fish were placed into 20 x 40 x 30 cm tanks half-filled with water with a plastic plant as refuge and left to acclimatise for three minutes. Pilot studies indicated that fish freeze in the presence of a fright stimulus and consistently vary in the length of time it takes them to begin moving again. Once the

acclimatisation period was over, a piece of cardboard was placed over the top of the tanks as a fright stimulus (simulating a potential predator presence overhead) and held for three seconds before being swiftly removed. Stop watches were used to time how long it took for the fish to begin moving again after the fright stimulus (the timer was stopped when movement was a continuous motion for a distance greater than the focal individual's body length). A maximum recording period of 300 seconds was used. Boldness scores were calculated by subtracting the score (i.e. number of seconds before movement) away from 300 (the ceiling value) so that bolder fish had larger values and any fish taking more than 300 seconds to move scored 0. Two fish were trialled at one time in separate tanks. Between each trial, a quarter of the water from the tanks was removed and replaced with fresh filtered water to avoid any olfactory cues from other fish influencing behaviour (De Winter *et al*., 2015; De Winter *et al*., 2016).

6.2. Aggression

A mirror was placed into each male's experimental tank in an attempt to trigger aggressive responses as the male defends its territory. The response to the mirror was filmed from within the tank using a DBPOWER 1080P Waterproof Action Camera for five minutes. The use of a mirror standardises the experiment, each male perceives its reflection as an equally matched rival. The video footage was viewed after the trial and the frequency of aggression was recorded and which is the number of times the fish showed an aggressive response aimed towards the reflection within five minutes. An aggressive response was either charging at the mirror to within 1 body length, biting at the mirror, or chasing the reflection on the mirror.

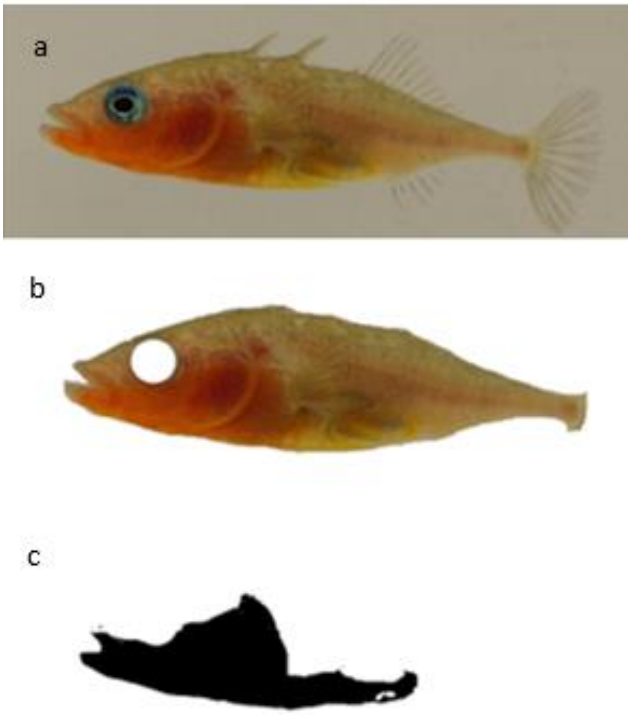
6.3. Colouration

Photographs of the fish were taken at the end of the trials under standardised conditions using a Pentax K-50 16.3 MP DSLR Camera with an 18-55mm lens. Fish were placed in a small tank with a white background under full lighting conditions and photos were taken within a two-minute period to avoid stress altering colouration. Fish were returned to their individual tanks after being photographed. A colour card (MacBeth Kodak) was positioned on the white background for calibration of scale and

colour during later analysis. The left lateral side was photographed each time.

The area of redness was analysed using ImageJ software 1.50i (https://imagej.nih.gov/ij/) using 'threshold colour', 'threshold' and 'analyse particles' functions. The area of redness on the left side of the fish was divided by the total area on the left side of the fish and multiplied by 100 to obtain a percentage for red throat coverage.

Figure 4. An example of how area of redness is calculated in imageJ. a. is the starting photograph of the whole fish, b. background, fins and the eye are removed and the area of the whole fish is calculated. c. the area of the red colouration is selected and the area calculated.



The intensity of redness was also measured using ImageJ software. Intensity scores of the males' throats were calculated using the calculation devised by Frischknecht (1993) for red index. The 'colour histogram' function was used to obtain the red, green and blue (RGB) values for the throat area. The darkest red area on the fish was selected and used in the analysis of intensity (for example, if a male had orange, red and red-purple colouration the values red-purple would be analysed). To control for the effect of lighting on colouration RGB values were also calculated for black on the colour card. RGB values for the males' throats were divided by RGB for black on the

colour card to obtain standardised RGB values. The standardised brightness value (0-255) of the red component of the males' throats was then divided by the sum of the standardised red, green and blue values to obtain an intensity value (Frischknecht, 1993).

6.4. Nesting

Males were left to construct nests under controlled conditions using standardised materials provided. Gravel, sand and 200 pieces of black polyester string approximately 7 cm long were provided to each fish. A female was exposed to each experimental male daily (excluding weekends) to induce nest building. Ten days were given for nest building as pilot studies indicated the majority of males built nests within ten days. All nests were photographed next to a scale after behavioural trials of aggression, boldness and courtship behaviours were complete.

6.5. Courtship

On days 10 and 11 of the experiment, a female contained within a glass jar was presented to each male for a ten-minute period and the response was filmed using a Pentax K-50 16.3 MP DSLR Camera with an 18-55mm lens for later analysis. The variables recorded are listed in Table 7.

Table 7. Detailed descriptions of each component of the courtship dance measured

Variable	**Description**
Latency to approach female	The number of seconds for the male to approach within one body length of the female in the jar.
Fanning	Fast ventilating movement of pectoral and caudal fins whilst remaining positioned over the nest.
Pecking	The male is in a head down position prodding the nest, or around the nest with his snout.
Gluing	The male is in a head up position where his pelvis is almost touching the nest. He slowly moves forwards while secreting glue from his kidneys.

Creeping	The male crawls through a hole in the nest.
Zigzag frequency	Zig-zag is where the male swims back and forth and side to side in often rapid movements. This measures the number of times a male performs a zig-zag dance.

6.6. Statistical analysis

Shapiro-Wilk tests revealed data were not normally distributed (except for length and area of redness) so non-parametric tests were carried out. All data analysis was carried out using IBM SPSS statistics version 23.

Spearman's rank correlation tests were used to test whether behaviours were consistent between first and second trials. Mann-Whitney-U tests were then conducted to determine whether males that built nests showed different traits to non-nest building males. Mann-Whitney-U tests were also conducted to investigate the effect of social environment on nest building behaviours.

A Principal Components Analysis (PCA) was conducted to investigate the relationships between sexual signals (fanning, pecking, gluing, creeping, zigzag frequency, area of redness (%) and intensity). Finally, PC1 scores from the PCA were used to test for correlations between personality measures (boldness and aggression) and sexual signals using Spearman's rank correlations.

7. Results

All sexual behaviours (excepting latency to approach a female and creeping behaviour) were found to be consistent between trials ($P<0.01$). Means of the two trials were therefore used for any further analysis. All values remained significant after Sequential Bonferroni correction. Table 8 contains the range of each personality and sexual signal trait measured. The broad ranges demonstrate how varied personality and sexual signals were at a population level despite being consistent within individuals.

Table 8. The range of each personality and sexual signal trait measured including consistency between trials

Trait	Range	Correlation coefficient	P value for consistency
Intensity of redness	0.399 – 0.738	N/A	N/A
Area of redness (%)	1.5 – 44.6	N/A	N/A
Fanning	0 - 28	0.845	0.0001
Pecking	0 - 55	0.903	0.0001
Gluing	0 - 13	0.854	0.0001
Creeping	0 - 1	0.278	0.087
Zigzag Frequency	0 - 58	0.905	0.0001
Boldness	0 - 298	0.435	0.006
Frequency of aggression	0 - 23	0.828	0.0001
Latency to approach female	1 - 460	0.111	0.366

7.1. Personality

Consistency between the trials for both boldness and frequency of aggression indicates the presence of personality. Spearman's rank correlation tests between mean boldness and mean frequency of aggression were statistically significant ($R_s = 0.447$, P=0.004).

7.2. Do multiple cues correlate?

A principal components analysis was conducted on the sexual signals (fanning, pecking, gluing, creeping, zigzag frequency, area of redness (%) and intensity of redness). All of the seven signals assayed correlated with one another with the exception of creeping and area of redness (%) (see correlation matrix table in appendix 1).

PC1 explained 64.982% of the variance in the data. Furthermore, all values were positive and above 0.6 (Table 9), this indicates that sexual signals were strongly clustered and inter-correlated. PC1 showed negative loadings, the inverse of PC1 (-PC1) was therefore used so higher scoring fish were showing more pronounced sexual behaviours. -PC1 is referred to as "sexual selection performance score", as it

summarises the multivariate components of the correlated signals. Sexual selection performance score is used as a proxy for the intensity of sexual signalling in further analysis. High sexual selection performance scores indicate individuals that have shown high levels of courtship vigour (including zigzag frequency, fanning, pecking, gluing and creeping behaviours) and high colour scores (higher percentage area of redness and higher intensity).

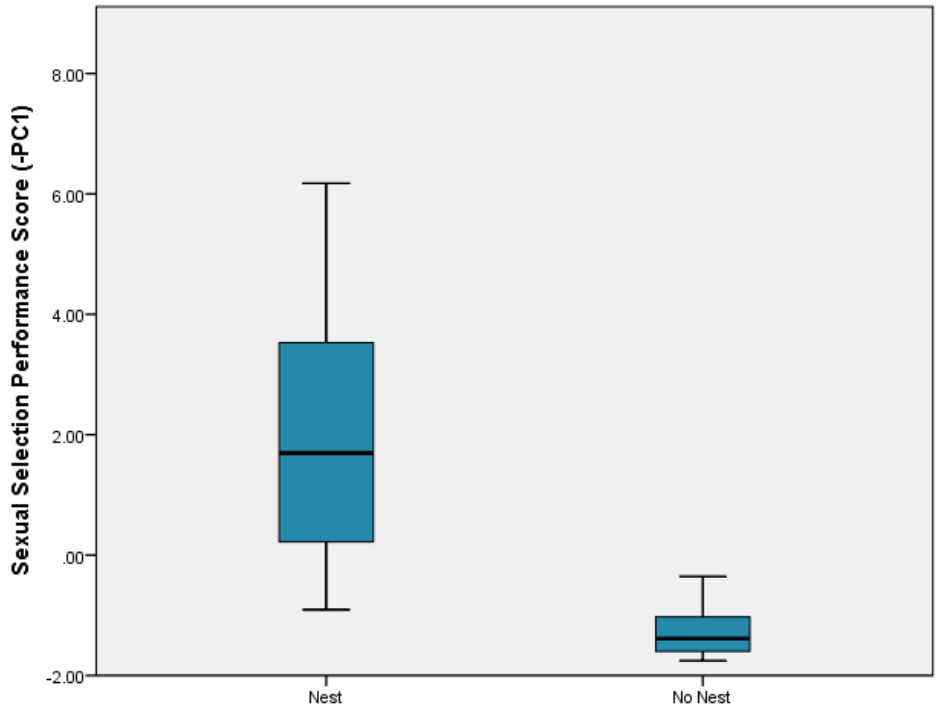
Table 9. PCA loadings for components 1 and 2

Behaviour	Component 1	Component 2
Fanning	0.929	
Pecking	0.927	
Gluing	0.914	-0.314
Zigzag frequency	0.913	
Creeping	0.604	
Area of redness	0.618	0.665
Intensity of redness	0.641	0.654

7.3. Characteristics of nest building males

Nest building males showed significantly higher frequencies of aggression (Mann-Whitney U = 62.5, P<0.0001, median 9.5 for nesting and 0 for non-nesting) than non-nesting males. Boldness however, had no significant effect on nest building (Mann-Whitney U = 148, P=0.368). Mann-Whitney U tests found nesting behaviour and sexual selection performance score (-PC1) to be highly significant (Mann-Whitney U = 5, P<0.0001, DF 38). Males that built nests during the study period (15 out of 39 males) had a higher sexual selection performance score than non-nest-building males (Figure 5).

Figure 5. The median and inter-quartile range of nesting (15) and non-nesting (24) males' sexual selection performance score.



7.4. Social environment

Tank composition prior to trials (whether males were held in single sex, N=20 or mixed sex, N = 19 tanks) had a highly significant effect on nest building (Mann-Whitney U = 67, P<0.0001, DF = 38). Fourteen of the fifteen males who built nests came from single sex tanks before trials. Males that were previously in single sex tanks showed higher sexual selection performance scores, (Mann-Whitney-U score = 95, P=0.007, DF = 38). Furthermore, aggression was also higher in males from single sex tanks (Mann-Whitney U = 94.5, P=0.006, DF = 38).

7.5. Personality and sexual selection

Using the sexual selection performance scores, Spearman's rank correlation tests were conducted. Boldness scores did not correlate with sexual selection performance scores (R_s = 0.263, P=0.105). Aggression, however showed a strong positive correlation (R_s = 0.705, P<0.0001) with the score (Figure 6). Figure 7 shows correlations between aggression and each individual signal within the sexual selection performance scores.

Figure 6. Sexual selection performance score correlated against mean frequency of aggression with a trend line demonstrating the positive relationship.

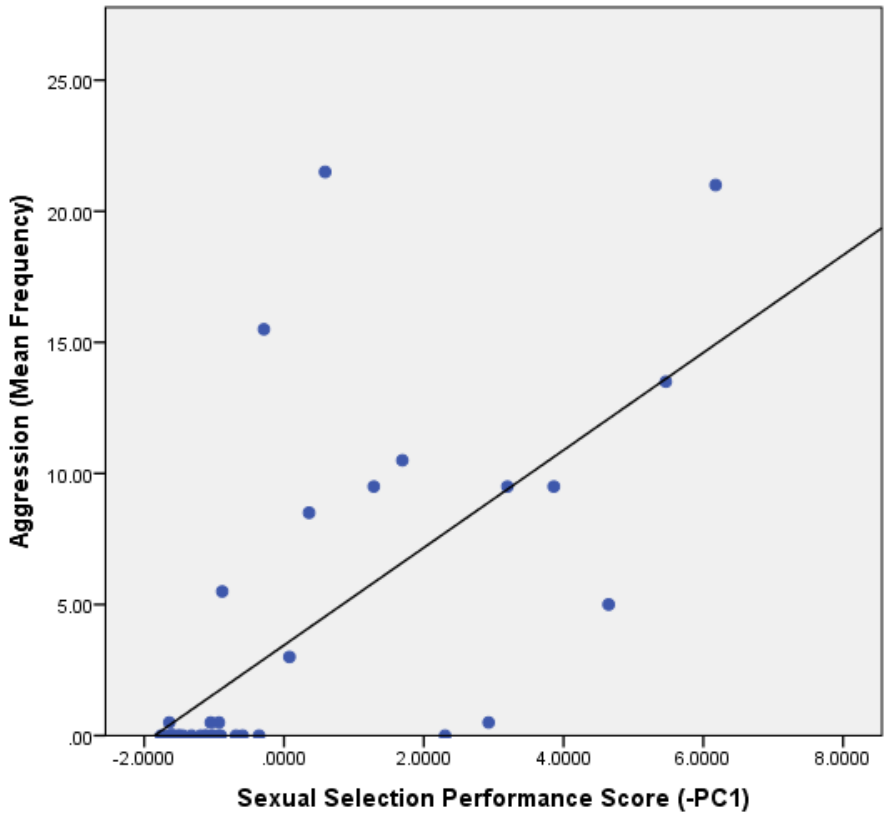
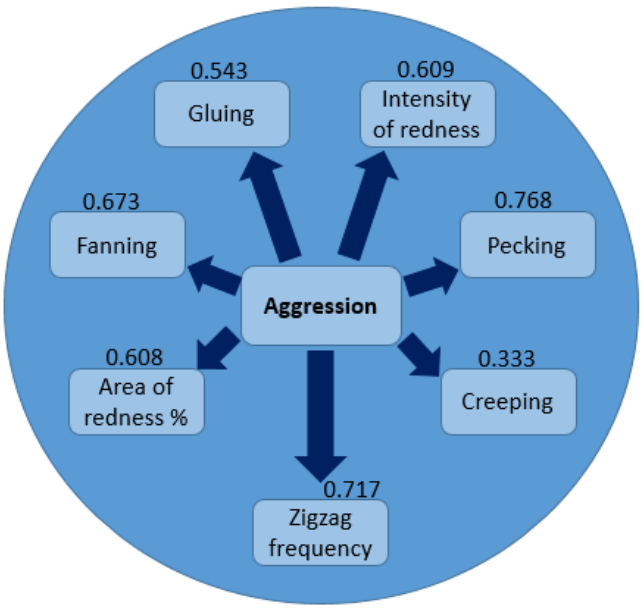


Figure 7. Correlation matrix of aggression and sexual signals demonstrating that aggression was most highly correlated with zigzag frequency and nest pecking behaviour.



8. Discussion

Male three-spined stickleback in breeding condition from a single population exhibited variation in numerous sexual signals and personality measures. Aggression was found to have a highly positive correlation with sexual selection performance score. Several factors are positively related to nest building including aggression and sexual selection performance score. Furthermore, males held in single sex tanks prior to trials showed much higher sexual selection performance scores and levels of aggression.

8.1. Personality and sexual selection

As predicted, a relationship between a personality trait and sexual signalling was found. Aggression was found to correlate with all sexual signals measured (Intensity of redness; Area of redness; Zigzag frequency; Fanning; Pecking; Gluing; and Creeping). Aggression is a personality trait which is subject to both inter and intra-sexual selection. Several studies have found evidence of females choosing aggressive males to mate with (Barlow, 1986; Breden and Stoner, 1987; Smith and Blumstein, 2008). For example, Barlow (1986) found female midas cichids (*Cichlasoma citrinellum*) to select more aggressive males due to their ability to guard young and territories. Additionally, there is a wide selection of studies on male-male aggression between rivals (Wong and Candolin, 2005; Elias *et al*., 2009). However, research investigating the relationship between aggression and sexual signals is somewhat lacking.

One probable explanation for why aggressive males were found to express more pronounced sexually selected behaviours may be due to trade-offs between reproductive success and survival similar to those found in bold individuals in Smith and Blumstein's (2008) study. Aggressive males may take more risks in order to gain better reproductive success. Therefore, aggressive males may focus most of their energy into sexual ornaments and behaviours to attract females with risks to their survival. Additionally, male visual signals such as red chest colouration correlating with aggression levels, may also function to aid a male's decision whether to avoid or threaten a rival.

Another explanation for the correlation may be a hormonal link. The endocrine system has been found to be a significant mechanism for integrating behavioural, morphological and physiological traits (Hoffman *et al*., 2014). Rowland (1984), in line with the results of this study found a positive correlation between colouration, courtship intensity and aggression and suggested a hormonal (testosterone) link between the three.

As aggression is advantageous when rival males are present, it is likely males may increase aggressive responses when in breeding condition. It would be interesting to investigate whether male stickleback are consistent in their personality traits in and out of breeding condition. An area for further research would be to explore whether males increase levels of aggression evenly across a population when in breeding condition.

8.2. Social stimulus and mating effort

Males in this study were housed either in mixed sex groups or in single sex groups prior to trials. An individual male's social environment prior to being isolated for trials was found to have a significant effect on nest building, aggression and sexual selection performance score. Males kept in single sex groups produced a significantly higher sexual selection performance score, higher frequency of aggression and built significantly more nests than males from mixed sex tanks.

Sexual signals such as size and throat colouration in male stickleback not only function as a signal in inter-sexual selection, but can also function in intra-sexual selection. In male-male competition, males assess rivals' competitive abilities based on these traits (Bakker and Milinski, 1993). Males from this study appear to make flexible mating decisions based upon social conditions, more specifically on the presence of competitive rivals. It is widely documented that higher male densities result in greater competition between rivals in numerous species (Jirotkul, 1999). It is likely that males in male-only tanks are competing with each other for territory. Testosterone rises in response to the presence of other rival males and this can lead to higher levels of aggression, this is known as the challenge hypothesis (Archer, 2006). Therefore, sexual and agonistic signals show higher rates of expression in the male-only conditions.

Sexual signals are sometimes assumed to be fixed throughout the breeding season. This is unlikely to be the case as evidence from this study points towards males modulating signalling effort according to conditions. Both male-male competition and female choice are likely to be modulated according to male density (Tinghitella, Stehle and Boughman, 2015). When male density is high, there is a larger percentage of unsuccessful males. Therefore, males are predicted to be more aggressive towards rivals and increase their mating effort. Furthermore, females are likely to be highly discriminating when given the choice of a large number of males. Higher competition leads to more pronounced signal expression suggesting signal expression is more plastic than previously thought and that males may attempt to reduce costs by modulating effort according to male density. Kim and Velando (2014) found stickleback males strategically regulate the effort they put into courtship according to the presence of rival males. They found male stickleback to increase the area of red throat colouration and courtship performance when exposed to brightly coloured dummy males. Kim and Velando (2014), in line with the results of this study, suggest that male stickleback increase their investment in mating effort (thus increasing costs) when competitive rivals pose a threat to territory and/or mating success. An alternative explanation as to why males from male-only tanks expressed higher rates of aggression may be due to a stress response from the housing conditions.

8.3. Conclusions

Little research has investigated the relationship between sexual signals and personality traits. Studies which have looked into sexual selection and personality have focused on mate choice. This study found high levels of within individual consistency and inter-individual variation in both personality traits and reproductive traits. Furthermore, behavioural and reproductive syndromes were present. Social environment influenced nest building behaviour, levels of aggression and sexual signal quality. Males appear to modify their reproductive strategy depending on the presence of competitive rivals. Aggression and sexual performance were positively correlated - more aggressive males were found to demonstrate stronger sexual signalling than less aggressive males. Furthermore, aggression also predicted which males built nests. This relationship between aggression and sexual signals may be due

to trade-offs between reproductive success and survival. Aggressive males are more risk prone and appear to put more energy into signal effort and reproduction than survival. Trade-offs between personality and life history traits such as reproduction may maintain personality variation within populations. There is opportunity for further study investigating the links between personality variation and life-history trade-offs for a number of life history characteristics such as growth and survival. Further research on the interactions will aid understanding of both fields.

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APPENDIX 1

	Fanning	**Pecking**	**Gluing**	**Creeping**	**Zigzag frequency**	**Area of redness**	**Intensity of redness**
Fanning	1.000	.929	.936	.404	.900	.419	.397
Pecking	.929	1.000	.864	.436	.808	.502	.508
Gluing	.936	.864	1.000	.468	.890	.369	.392
Creeping	.404	.436	.468	1.000	.527	.333	.391
Zigzag frequency	.900	.808	.890	.527	1.000	.382	.446
Area of redness	.419	.502	.369	.333	.382	1.000	.703
Intensity of redness	.397	.508	.392	.391	.446	.703	1.000