Does sexual selection favour consistent behavioural differences in bi-parental cichlids?

DISSERTATION

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CONTENTS

Summary

In a constantly changing world behavioural flexibility should be favoured by selection allowing individuals to adjust their behaviour to various situations. Yet, consistent between-individual differences in behaviour (also called personality differences) are prevailing in the animal kingdom. But although the existence of personality differences is empirically well supported we are just starting to understand their adaptive significance. Recently, it has been suggested that sexual selection may have profound effects on personality variation observed within populations. Depending on the direction of mate choice and associated variance in reproductive success of different behavioural types (or the combination of them), sexual selection may have the power to erode or stabilise personality variation. However, the hypotheses proposed largely remain to be tested. In my thesis, I aimed to contribute to a better understanding of how sexual selection can shape personality variation by testing how individual differences in aggression and boldness, two of the most prominent personality traits, affect mate choice and reproductive success in the rainbow krib, *Pelvicachromis pulcher*, a bi-parental cichlid from West Africa. Individual aggression and boldness are thought to be sexually selected in this species because both traits are important during parental care (i.e. they affect whether and how parental fish protect their offspring from con- and heterospecific brood-predators).

I tested female mate choice for male boldness and aggression and male mate choice for female boldness (level and consistency of behaviour, respectively). Further, I tested how personality differences in pre-determined boldness and aggression, and in parental care behaviour affect reproductive success (number and size of offspring). For all experiments, I followed up two alternative hypotheses regarding the level and consistency of behaviour: I either expected selection for individual quality (indicated by directional selection) or for pair compatibility (indicated by (dis-) assortment).

I found differences in mate choice between the sexes (female choice but no male mate choice) and, for female mate choice, between personality traits. That is, females showed a directional preference for consistent high-aggression males, a dis-assortative preference for the level of male boldness, and an assortative preference for the consistency of male boldness. The dis-assortative female preference for male level of boldness was associated with increased reproductive success in terms of a higher number of offspring. However, also positive assortment in the level of aggression- and boldness-like parental care behaviour was associated with increased reproductive success in terms of larger offspring.

My results suggest that sexual selection may erode personality variation in the level of aggression and boldness via directional and dis-assortative selection, respectively (given that there are no other selective pressures). But my data also support the hypothesis that personality variation is maintained via reproductive benefits resulting from positive

behavioural assortment during parental care. My data suggest that the strength of sexual selection may vary between the sexes (as I found female but no male mate choice). Further, my data indicate that not only the level of behaviour but also behavioural consistency is sexually selected (as indicated by female mate choice) though I could not detect reproductive consequences being associated with behavioural consistency. I provide evidence that sexual selection affects personality variation in rainbow kribs. However, the direction of selection is rather complex calling for further investigations the shed light on the overall direction of selection.

Zusammenfassung

In einer sich ständig verändernden Welt sollte Verhaltensflexibilität einen selektiven Vorteil bieten, da sie Individuen erlaubt sich unterschiedlichsten Situationen anzupassen. Trotzdem können wir bei einer großen Anzahl von Tierarten konsistente Unterschiede im Verhalten zwischen Individuen beobachten (auch Persönlichkeitsunterschiede genannt). Obwohl die Existenz dieser Persönlichkeitsunterschiede empirisch gut belegt ist, fangen wir gerade erst an zu verstehen, welche adaptive Bedeutung Persönlichkeitsvariation haben könnte. Sexuelle Selektion könnte hierbei eine entscheidende Rolle spielen. Das heißt, in Abhängigkeit der Richtung der Partnerwahl und der damit verbundenen Variation im Reproduktionserfolg der verschiedenen Verhaltenstypen (oder deren Kombination), könnte sexuelle Selektion eine erodierende oder stabilisierende Wirkung auf die Persönlichkeitsvariation innerhalb von Populationen haben - wobei diese Hypothesen erst noch empirisch untersucht werden müssen. Mit dieser Dissertation möchte ich zu einem besseren Verständnis des Einflusses sexueller Selektion auf die Evolution von Persönlichkeitsunterschieden beitragen. Zu diesem Zweck habe ich getestet, welche Rolle individuelle Verhaltensunterschiede in Aggressivität und Mutigkeit, zwei der prominentesten Persönlichkeitsmerkmale, bei Partnerwahl und Reproduktionserfolg des Pupurprachtbuntbarsches, *Pelvicachromis pulcher*, einem bi-parentaler Cichliden aus Westafrika, spielen. Ich hatte vermutet, dass individuelle Verhaltensunterschiede in Aggressivität und Mutigkeit in dieser Art sexuell selektiert werden, weil beide Verhaltensmerkmale während des Elternfürsogeverhaltens wichtig sind (d.h. sie beeinflussen ob und wie die Nachkommen vor artgleich und artfremden Brutpredatoren beschützt werden).

Ich habe getestet, welche Paarungspräferenzen Weibchen für Aggressivität und Mutigkeit bei Männchen zeigen und welche Paarungspräferenzen Männchen für Mutigkeit bei Weibchen haben (dabei habe ich jeweils sowohl das Verhaltenslevel als auch die Verhaltenskonsistenz berücksichtigt). Außerdem habe ich getestet, welchen Einfluss Persönlichkeitsunterschiede in Aggressivität und Mutigkeit (vor und während der Jungenfürsorge) auf den Reproduktionserfolg (Anzahl und Größe der Nachkommen) haben. In allen Experimenten habe ich zwei alternative Hypothesen hinsichtlich des Verhaltenslevels und der Verhaltenskonsistenz verfolgt: ich hatte gerichtete Selektion für individuelle Qualität erwartet oder Selektion auf (Un-) Ähnlichkeit (positiv oder negativ assortative Selektion) für Kompatibilität.

Das Partnerwahlverhalten hat sich zwischen den Geschlechtern unterschieden (Weibchen- aber keine Männchenwahl) und bei der Weibchenwahl unterschied es sich auch zwischen den Verhaltensmerkmalen. Das bedeutet, Weibchen zeigten eine gerichtete Präferenz für konsistent hoch-aggressive Männchen. Bezüglich des Mutigkeitsverhaltens zeigten Weibchen eine Paarungspräferenz für Männchen mit einem unähnlichen

Verhaltenslevel aber mit ähnlicher Verhaltenskonsistenz (im Verhältnis zum Weibchen). Die Paarungspräferenz für Unähnlichkeit im Level des Mutigkeitsverhalten stand im Zusammenhang mit einem erhöhten Reproduktionserfolg, d.h. eine erhöhte Anzahl von Nachkommen. Allerdings hatten auch Brutpaare, die sich während der Jungenfürsorge ähnlich verhalten hatten einen erhöhten Reproduktionserfolg, d.h. größere Nachkommen.

Meine Ergebnisse legen nahe, dass sexuelle Selektion Persönlichkeitsvariation in Aggressivität und Mutigkeit durch gerichtete und negativ-assortative Selektion erodieren könnte (vorausgesetzt es wirkt kein anderer Selektionsdruck auf diese Verhaltensmerkmale). Meine Daten stützen allerdings auch die Hypothese, dass Persönlichkeitsunterschiede durch positiv-assortative Selektion erhalten bleiben, da Ähnlichkeit im Verhalten während der Jungenfürsorge reproduktive Vorteile hatte. Die Stärke der Selektion scheint sich zwischen den Geschlechter zu unterscheiden, da ich Weibchen- aber keine Männchenwahl nachweisen konnte. Außerdem zeigen die Resultate meiner Weibchenwahl-Experimente, dass nicht nur das Verhaltenslevel sondern auch die Verhaltenskonsistenz sexuell selektiert wird; wobei ich keinen Effekt von Verhaltenskonsistenz auf den Reproduktionserfolg nachweisen konnte. Ich zeige, dass sexuelle Selektion Persönlichkeitsvariation im Pupurprachtbuntbarsch beeinflusst. Die Richtung der Selektion ist allerdings komplex, daher bedarf es weiterer Untersuchungen, um den Zusammenhang zwischen Persönlichkeitsunterschieden und sexueller Selektion in dieser Art besser zu verstehen.

CHAPTER 1

GENERAL INTRODUCTION

A couple days ago, I met an old friend from school. Little Brigitte. I have not seen her in years. But Brigitte hasn't changed a bit: a bit overweight (food was always her soft spot) but very smart and witty. And I have not changed either: not caring much about food and still being absorbed by her fascinating way of thinking. Meeting her felt like no time had passed. No one will be surprised by this little anecdote. In fact, it represents a rather common incident that you most probably have experienced and heard about yourself many times. In essence, we broadly agree on the fact that we, humans, are different to one another, and that these behavioural differences persist over a very long time, if not even a life time (Buss and Greiling 1999; Gosling 2001). In non-human animals, however, we have neglected the existence of such consistent between-individual differences in behaviour - also called personality differences, temperaments or coping styles (Réale et al. 2010a; Réale et al. 2007) - for a long time. Some pioneering work has been done in the 1970-80s (Clark and Ehlinger 1987; Huntingford 1976) but we continued to believe that the behavioural variation observed within populations represents non-adaptive, random noise that surrounds an adaptive mean, rather than being adaptive itself (Wilson 1998). According to Réale et al. (2010a), it was an article published by Wilson et al. (1994), 'Shyness and boldness in humans and other animals', that caused a tenfold increase in the number of publications concerning animal personalities during the following two decades. Since the 1990s, much effort has been made to characterise the distribution of personality differences and to define its proximate and ultimate causes, yet, we are still just starting to understand (a) the evolution of stable personality variation and (b) the consequences resulting from the observed pattern.

(a) Today, the existence of stable personality variation is not surprising anymore: we know that personality differences are not exceptional in the animal kingdom, but universal (see the below 'Descriptive background'). However, the existence of stable personality variation brings a whole new set of questions with it: Why do individuals behave so differently from one another (even when they are in the same situation)? And why do these differences persist over time? What is the adaptive benefit of the distinct behavioural polymorphism that we can observe? We need to establish (and empirically verify) quantitative frameworks to identify the evolutionary forces that generate and maintain stable personality variation and to find out how these evolutionary forces shape personality differences (direction and strength of selection). Answers to these questions are central to our general understanding of animal behaviour at the individual as well as at the population level. Yet, conceptual frameworks developed so far are still in their 'infancy' (Wolf and McNamara 2012) and the validity of the theories proposed has often yet to be empirically tested (Schuett et al. 2010; Wolf and McNamara 2012).

(b) Personality differences affect a wide array of ecologically relevant processes (Réale et al. 2007), e.g. decision making (Chang et al. 2018; Chang et al. 2017; Mamuneas et al. 2015), habitat use (Schirmer et al. 2019), disease and parasite transmission (Keiser et al. 2016; Sih et al. 2018), dispersal behaviour (Cooper et al. 2017; Luna et al. 2019), and collective movements (del Mar Delgado et al. 2018). Thus, we may have to rethink the general validity of classic and widely excepted concepts that assume individuals (should) behave more or less equally (Schirmer et al. 2019; Wilson 1998). For example, classic optimal foraging theory assumes that there is one optimal strategy that should be applied by all individuals (Wilson 1998). But individuals differ consistently in their foraging behaviour depending on their behavioural type (Ioannou and Dall 2016; Patrick et al. 2017; Schuett and Dall 2009), suggesting they are following different strategies. Not least, personality differences should also be considered in conservation issues as they can affect a population's adaptive potential (McDougall et al. 2005; Watters and Meehan 2007). For example, in captive-bred swift foxes, *Vulpes velox*, boldness was negatively associated with survival in the wild after release (Bremner-Harrison et al. 2004). Finally, integrating insight from animal personality research could increase both productivity and animal welfare in economic sectors like agriculture, fisheries, zoos, and pet shops (Kelleher et al. 2018; McDougall et al. 2005; Watters and Powell 2012; Wielebnowski 1999). Productivity could be increased considering the behavioural type of individuals within a breeding pair as some behavioural types have been shown to be more successful in producing offspring than others (reviewed in Tetley and O'Hara 2012). Also, when grouping animals in a social context outside of breeding, the composition of behavioural types within the group can significantly contribute to the well-being of group members, thus the consideration of individual behavioural types in husbandry can contribute to animal welfare (reviewed in Tetley and O'Hara 2012). In sum, conclusions drawn from animal personality research are relevant to ecological research, conservation, and all economic sectors handling live animals. Yet, animal personality research is far from providing conclusive evidence that can be readily applied (see above).

Our very limited understanding of (a) *why* and *how* personality differences are generated and maintained in combination with (b) the relevance of personality variation for other research fields like ecology and conservation, economics and animal welfare promotes the strong need for further animal personality research. I hereby want to contribute to this matter. More precisely, in my dissertation, I investigated the potential role of sexual selection on the evolution of personality differences. But before I introduce the conceptual framework underlying my work and my main study aims, I shortly give some descriptive background on personality traits and present major concepts on the evolution of personality differences that have been proposed so far. I further outline the gaps and limitations of these concepts and explain why the consideration of sexual selection is important for our understanding of how personality differences are generated and maintained.

Animal personalities: Descriptive background

Repeatable differences in individual behaviour have been shown in a wide range of taxa including mammals, birds, fish, reptiles, amphibians, insects, spiders, and cephalopods (reviewed in: Bell et al. 2009; Gosling 2001; Stamps 2007). Although the personality measures obtained vary a lot between species and studies, they can broadly be summarized into five categories: (1) shyness-boldness, (2) exploration-avoidance, (3) activity, (4) aggressiveness, and (5) sociability (proposed by Réale et al. 2007). These categories differ from the standard five-factor model (also called the *OCEAN*-model) used in psychological personality research in humans; which comprises *O*penness, *C*onscientiousness, *E*xtraversion, *A*greeableness, and *N*euroticism (Allport and Odbert 1936; Gosling and John 1999; McCrae and Costa 1999). However, the human model is not ideally suited for application in animal personality research as the psychological mechanisms underlying a behavioural expression may differ between humans and non-human animals (Réale et al. 2007). Often, a correlation between personality measures of different categories has been reported (Conrad et al. 2011; Kelleher et al. 2018; Sih et al. 2004a; Sih et al. 2004b; but see Broecke et al. 2018, and Thys et al. 2017). For example, boldness and exploration behaviour are positively correlated in the convict cichlid, *Amatitlania siquia* (Mazué et al. 2015) and in nesting rodents, *Octodon degus* (Chock et al. 2017). Due to these correlations between behaviours, individual behaviour is sometimes even further simplified, and categorized on a single axis, the proactive-reactive continuum (Ibarra-Zatarain et al. 2019; Laubu et al. 2016). However, we do not entirely understand why behaviours show such correlations across contexts, given that this may result in often mal-adaptive behaviour (Sih et al. 2004a; Sih et al. 2004b).

Personality differences have a genetic component with modest to moderate heritability of up to approx. 30-60% (Araya-Ajoy and Dingemanse 2017; Ariyomo et al. 2013; Dochtermann et al. 2015; Drent et al. 2003; Petelle and Blumstein 2015; Reif and Lesch 2003; van Oers et al. 2005) and they are associated with diverse fitness consequences (reviewed in: Biro and Stamps 2008; Dingemanse et al. 2004; Smith and Blumstein 2008). For example, high levels of both boldness and aggressiveness are associated with increased reproductive success in a number of species (Ariyomo and Watt 2012; Ballew et al. 2017; Kontiainen et al. 2009; Sinn et al. 2008). Furthermore, aggression is a good predictor for dominance and competitive ability (Houpt et al. 1978; Muller and Wrangham 2004; Wilson et al. 2013). But, on the other hand, aggressive behaviour is metabolically costly (Briffa and Sneddon 2007; Castro et al. 2006), can decrease survival (Dufty 1989), and the risk of being predated (Hess et al. 2016) or injured (Dufty 1989). Also for boldness, there is mixed evidence regarding its adaptive benefit. That is, bold individuals in the common yabby, *Cherax destructor* (Biro and Sampson 2015), and in the sole, *Solea solea* (Mas-Muñoz et al. 2011), show faster growth rates compared to their shyer conspecifics, whereas bold brown trouts, *Salmo trutta*, grow slower (Adriaenssens and Johnsson 2010). Similarly, boldness is associated with increased - (Foster et al. 2017; Godin and Dugatkin 1995; Piquet et al. 2018; Smith and Blumstein 2010) as well as with decreased survival (Ballew et al. 2017; Smith and Blumstein 2008) in a number of species, respectively. In great tits, *Parus major*, explorative behaviour had a rather complex effect on survival showing differences between the two sexes and between years (Dingemanse et al. 2004). In short, personality traits show a manifold of fitness effects that vary between and even within species depending on environmental conditions (Ballew et al. 2017; Dingemanse et al. 2004; Dingemanse and Réale 2005; Teyssier et al. 2014).

Evolution of personality differences: An overview of conceptual frameworks

Personality traits show, per definition, polymorphic variation, they are heritable and they affect an individual's fitness (see the above 'Descriptive background') - building the basis for evolution to act on (Barrett and Schluter 2008; Bijma 2011; Dingemanse and Réale 2005). Thus, we have good reason to believe that stable personality variation observed within populations is not just evolutionary raw material but may be a product of selection. Frameworks addressing the evolution of personality variation tackle three characteristics of personality differences, i.e. the existence of (1) within-individual consistency, (2) between-individual variation, and (3) within-individual correlations between different behaviours (behavioural syndromes) (Roberts and DelVecchio 2000). Below, I outline main concepts regarding the first and second issue. However, I do not go into detail regarding the third issue, as the evolution of behavioural syndromes does not specifically fall within the scope of the present project.

Evolution of within-individual consistency

Behavioural flexibility is generally assumed to be advantageous over consistency (Bell and Aubin-Horth 2010; Dingemanse et al. 2012; Sih et al. 2004a; Sih et al. 2004b), this is because flexibility allows an individual to adjust its behavioural expression fitting the needs of a situation, whereas behavioural consistency constrains an individual's capacity to optimize its behaviour. For example, a male that consistently expresses a high level of aggression shows high competitive ability (potentially adaptive) but, as this behaviour is expressed consistently, he may also show increased aggression against females or predators (mal-adaptive) (Sih et al. 2004b). Similarly, a shy individual that spends a lot of time hiding, instead of feeding, shows adaptive behaviour if the predation risk is high but not if it is low (because it misses out on feeding opportunities) (Sih et al. 2004b). However, there are at least four mutually non-exclusive mechanisms that may promote the evolution of within-individual consistency by either constraining flexibility or by favouring consistency.

First, behavioural flexibility can be constrained by physiological and morphological factors (Bell and Aubin-Horth 2010; Wolf and McNamara 2012). For example, body size is a strong predictor for locomotor activity in fishes (Bainbridge 1958; Fry and Cox 1970; Webb et al. 1984). In female Dumpling squids, *Euprymna tasmanica*, boldness was positively affected by body size (Sinn et al. 2006). Also, metabolic rates (reviewed in Biro and Stamps 2010), and the neuroendocrine system including the serotonergic system (reviewed in: Boissy 1995; Reif and Lesch 2003) can restrain behavioural flexibility. However, constrains of flexibility alone cannot explain why there is such persisting polymorphism in behaviour (Wolf and McNamara 2012).

Second, state-dependent positive feedback loops can be stabilising on individual behaviour (Petelle et al. 2019; Wolf and McNamara 2012). The state comprises all characteristics that determine an individual's ability to survive and reproduce, e.g. body condition, age, and energetic reserves; but also factors like territory size, parasite load, or experience determine an individual's state (McNamara and Houston 1996). Please note, an individual's state also includes physiological characteristics - causing some overlap with the above mentioned constrains of flexibility. But different to the above constrains of flexibility, state-dependent feedbacks represent an adaptive process.

Third, behavioural consistency can be advantageous in social interactions - on condition of social responsiveness or awareness (Dall et al. 2004; McNamara et al. 2009; Wolf and McNamara 2012). That is, behavioural consistency allows individuals to make predictions about their conspecifics' future behavioural responses. For example, social responsiveness in combination with behavioural consistency can prevent costly fight escalation in conspecific agonistic encounters via eavesdropping, i.e. the focal individual observes its counterpart's previous fights and makes an informed decision by estimating a potential fight's outcome based on what it saw: do not fight if it is likely to lose (Dall et al. 2004). Thus, behavioural consistency may allow for a more effective coordination of behaviours, reducing stress and conflict, leading to behavioural specialisation into social niches (Bergmüller and Taborsky 2010; Montiglio et al. 2013). Indeed, there is empirical support for this social niche specialisation hypothesis. Von Merten et al. (2017) compared the agonistic behaviour of a social shrew mouse with three closely related solitary shrew species and found more pronounced personality differences in the social species. Similarly, Laskowski and Pruitt (2014) showed that social spiders, *Stegodyphus mimosarum*, which familiarized with the conspecifics in their social group showed higher among-individual variation and higher within-individual consistency of boldness.

Fourth, life-history trade-offs may promote both within-individual consistency as well as between-individual variation in behaviour (Dammhahn 2012; Schuett et al. 2015; Wolf et al. 2007). This is because individuals vary in their assets (i.e. fitness expectations). Thus, 'high-asset' individuals that show risky behaviour have more to loose compared to their 'low-asset' conspecifics showing the same level of behaviour. Therefore, individuals with high assets should behave risk-averse whereas individuals with low assets should be more prone to risks (Wolf et al. 2007). And as these between-individual differences in future fitness expectations persist over a long time we expect individuals to consistently vary in their risk-prone vs. risk-averse behaviour (Wolf et al. 2007), forming distinct life-history strategies (Petelle et al. 2019; Réale et al. 2010b; Schuett et al. 2015). Please note, an individual's state mediates its life-history trade-offs (McNamara and Houston 1996; Schuett et al. 2015), therefore, this fourth point raised relates to the above third one.

Evolution of between-individual variation

Beside the afore-mentioned effect of life-history trade-offs on the evolution of stable personality differences, negative frequency-dependent selection is one of the major concepts explaining between-individual variation (Roff 1998; Wolf and McNamara 2012). Here, the fitness benefits of a behavioural strategy depend on how many other individuals play the same strategy (Ayala and Campbell 1974; Dugatkin and Reeve 2000; Maynard Smith 1982). For example, Lichtenstein and Pruitt (2015) found reproductive success of aggressive and docile individuals in three species of social spiders to be the highest for groups that were composed of a mixture of the two behavioural types (compared to groups composed of a single behavioural type).

Further, environmental fluctuations (temporal and spatial) may contribute largely to the maintenance of between-individual variation in behaviour (Dingemanse et al. 2004; Dingemanse and Réale 2005). For example, Schuett et al. (2018) showed that the explorative tendency of three species of ground beetles from natural populations differed between habitat types with beetles in urbanized areas being more explorative compared to beetles in less urbanized areas. Also, Haage et al. (2013) found boldness and exploration in the European mink, *Mustela lutreola*, to differ between the breeding - and non-breeding season.

Gaps and limitations

Although the above outlined frameworks are highly relevant to our understanding of the evolution of stable personality variation they lack two crucial considerations that need to be taken into account when thinking about personality differences and their evolution. First, the frameworks listed so far cover possible evolutionary forces that generate and maintain within- and between-individual variation in the level of behaviour (i.e. the magnitude in the expression) but between-individual differences in behavioural consistency (i.e. the variation in the expression) and their potential fitness consequences have not been considered (but see Dall et al. 2004). However, recent empirical evidence does not only suggest that individuals vary with respect to their behavioural consistency, but also that there are fitness consequences associated with these differences (Dingemanse et al. 2010; Stamps et al. 2012). Second, there are two components affecting an individual's fitness: survival and reproductive success. Yet, the latter has not been addressed by the above frameworks. Given the evolutionary importance of an individual's reproductive success and the prevalence of sexually reproducing species there is a strong need for both conceptual and empirical work regarding the effect of sexual selection on the evolution of personality differences (Schuett et al. 2010).

Personality differences: A role of sexual selection

Sexual selection, that is selection driven by differences in individual reproductive output (Andersson 1994; Jones and Ratterman 2009), may have the power to both generate and maintain personality differences. A first conceptual framework considering the effect of sexual selection on the evolution of stable personality variation was proposed by Schuett et al. (2010). Most importantly, Schuett et al. (2010) considered sexual selection to affect two aspects of personality: the level and consistency of individual behaviour. Schuett et al. (2010) outlined how the two key mechanisms of sexual selection, i.e. intra-sexual selection (often referred to as male-male competition to access to potential mates, though also female-female competition exists), and inter-sexual selection (mate choice) may generate (only differences in consistency) and maintain (level and consistency differences) stable personality variation. Intra-sexual selection may affect the level and consistency of behavioural traits that mediate competitive ability, e.g. a high level of aggression as well as the consistent expression of this high level can be favoured by intra-sexual selection if it secures a high hierarchy position and therefore access to mates. However, this benefit comes at a cost driven by more fight escalations and increased vigilance, therefore, the pay offs of this behavioural strategy may depend on the frequency in which the strategies are played (Schuett et al. 2010). See below for possible effects of mate choice on the evolution of personality differences.

Mate choice for personality differences

Mate choice for the level of behaviour

According to the proposed framework (Schuett et al. 2010), mate choice can lead to a directional selection for (or against) a high level of behaviour if there is an inter-individual agreement in mating preference. This is the case if the behavioural expression is an honest indicator for quality, being it either genetic or phenotypic quality. Benefits associated with genetic quality (indirect benefits) require that the expression of a behavioural level is associated with a measure of fitness by a shared genetic basis that affects both (the behaviour and the fitness measure) in a similar manner, thus providing benefits in turns of increased offspring fitness (Jones and Ratterman 2009; Schuett et al. 2010). For example, indirect evidence comes from Ariyomo and Watt (2012), who found that high levels of boldness and aggressiveness were associated with increased egg fertilisation rates in the zebrafish, *Danio rerio*. Benefits of phenotypic quality (direct or behavioural benefits) in behaviour are mediated by long-term pair bonds and/or (bi-) parental care behaviour (Schuett et al. 2010). For example, aggressiveness and exploration behaviour have been shown to go hand in hand with effort in nest defence (reviewed in Chira 2014).

Alternatively, individuals may differ in their preference depending on their own behavioural background leading to (dis-) assortment in their mating preference. Again, this may have genetic and phenotypic benefits resulting from increased compatibility between mates (Schuett et al. 2010). Behavioural compatibility may improve within-pair coordination and cooperation in that it could ease sexual conflict over the amount of parental provisioning (Schuett et al. 2011; Schuett et al. 2010). In that regard, positive assortment in birds is associated with synchronisation of feeding rates and higher reproductive success (Mariette and Griffith 2012; van Rooij and Griffith 2013). Laubu et al. (2016), found that dis-assortative breeding pairs in convict cichlid, *Amatitlania siquia*, could increase their reproductive success by achieving post-pairing similarity. However, also dis-assortment may be advantageous depending on the species' biology, e.g. if parental care comprises more than one activity and a specialisation into different parental roles increases efficiency, and thus reproductive success (Schuett et al. 2010).

Although the amount of empirical work considering mate choice for personality differences (in the level of behaviour) is rising, our understanding of how sexual selection shapes personality variation remains very incomplete. Existing studies vary in the direction found. That is, depending on the species of interest and the behaviour, studies have found either positive (Barlow 1986), negative (Ophir and Galef 2003), assortative (Kralj-Fišer et al. 2013), or dis-assortative (indirect evidence from van Oers et al. 2008) female mating preferences. But also within species considerable variation in mating preferences for behavioural traits can be observed, depending on environmental conditions (e.g. Teyssier et al. 2014). The empirical evidence collected so far implies a high complexity in the interplay between personality differences and sexual selection calling for further empirical work.

Also, we know very little about the reproductive consequences being associated with the mate choice patterns we can observe (i.e. behavioural vs. genetic benefits). This is especially interesting for (bi-) parental species as parental care effort strongly affects reproductive success (Clutton-Brock 1991; Schneider and Lamprecht 1990) though the style and amount of care depend on individual behavioural types (reviewed in Chira 2014), i.e. personality differences can affect parental care and reproductive success and should therefore be under sexual selection. In bi-parental species, both sexes engage into costly parental care, therefore, we would expect not only females but also males to be choosy. But existing studies considering mate choice for personality in care giving species mainly focus on female mate choice though studies considering male mate choice for female personality are scarce (but see Laubu et al. 2017).

Mate choice for behavioural consistency

Similarly to the above outlined mate choice trajectories for the level of behaviour, also behavioural consistency can be selected via directional vs. (dis-) assortative mate choice (Schuett et al. 2010). Directional mate choice for high consistency is expected when consistency in the expression of a behaviour indicates genetic quality (because it is costly to produce) or when the resulting predictability of behaviour provides behavioural advantages. That is, a male that shows consistent high-aggression may signal genetic quality because it is costly to be constantly aggressive (e.g. in terms of an increased metabolic rate or injuries). Further, it may signal parental ability (behavioural advantage) to a female if it is likely that the male continues to be consistent in this behaviour until the time comes to defend potential future progeny (Royle et al. 2010; Schuett et al. 2010).

Positive assortment for behavioural consistency may arise if individuals profit from either flexible negotiation over the amount of care provided vs. sealing the bid (Royle et al. 2010). However, also (dis-) assortment in behavioural consistency can be adaptive, for example in the following scenario: imagine a bi-parental system where male-male competition selects for consistent high-aggression males. And further assume female choice points towards the same direction because consistent high-aggression males protect the offspring in a high and predictable manner. Then, females could increase reproductive success via flexible adjustment of their behaviour depending on environmental conditions (Schuett et al. 2010). This would result in a reproductive benefit of 'skewed' dis-assortment for behavioural consistency (i.e. higher reproductive success for breeding pairs with an inconsistent female and a consistent male).

Given the above reasoning it seems worthwhile to investigate a potential role of individual differences in behavioural consistency during mate choice. However, empirical work addressing mate choice for behavioural consistency (and its reproductive consequences) is scarce (but see Schuett et al. 2011). Further, existing studies addressing sexual selection for the level of behaviour have often neglected between-individual differences in behavioural consistency. This is non-trivial as a potential preference for (and benefits from) the behavioural level may depend upon the consistency in which it is expressed (see above).

Project outline

Study aims

In my dissertation, I investigated whether personality differences in the rainbow krib, *Pelvicachromis pulcher* are sexually selected. This study species is particularly well suited to test for a potential role of sexual selection on the evolution of personality differences because it is a bi-parental species (see below). That is, both sexes heavily invest into reproduction (indicating mutual mate choice) and they repeatedly interact with each other (allowing to test for behavioural benefits being associated with personality differences). My main study aims were to find out (I) whether male personality differences are sexually selected via female choice and (II) whether female personality differences are sexually selected via male choice. For (I) female - and (II) male mate choice, I considered individual differences in both personality aspects, level and consistency of behaviour, to be important; and I considered two possible selection trajectories: directional selection for individual quality vs. (dis-) assortment for pair compatibility (Schuett et al. 2010). Further, I wanted to know whether (III) the mating preferences I found translate into reproductive success. Here, I was specifically interested in testing whether reproductive advantages resulting from pairing by personality are mediated via behavioural benefits (increased compatibility or quality in parental care activities) or genetic benefits. In my studies, I focussed on two personality traits, aggression and boldness. Both traits were thought to be sexually selected in the rainbow krib because they are important during parental care (see below).

With regard to my study aims, (a) I conducted two female mate choice experiments, one to test for female preference for male aggression (Chapter 2), the other one to test for female preference for male boldness (Chapter 3) (level and consistency of behaviour, respectively). These two experiments were conducted on a correlative basis, i.e. females could eavesdrop on the level and consistency of the behaviour males naturally express. However, such correlative evidence does not imply causality (Schuett et al. 2010). I therefore performed two follow-up female mate choice experiments where I manipulated either male aggression or male boldness (level and consistency of behaviour, respectively) (Chapter 4). (b) Further, I tested for the male perspective, that is, I tested male mating preference for the level and consistency of female boldness (correlative set-up) (Chapter 5). (c) To test for an effect of personality differences on parental care behaviour and on reproductive success I conducted a breeding experiment where I set up breeding pairs that varied in their behavioural composition (regarding the level and consistency of boldness) (Chapter 6). General implications resulting from this experimental work are discussed (Chapter 7). Finally, I present some methodological work testing the suitability of applied methods, supplied in Appendix 1 (VIE colour marking as a tool for individual identification), Appendix 2 (mirror tests as a tool to test for individual aggressiveness), and Appendix 3 (simple computer animations to test for individual aggressiveness and boldness).

Figure 1. Male (to the left) and female (to the right) rainbow krib with fry.

Study species

The rainbow krib is a relatively small (more or less palm-sized) and, like its name tells, colourful cichlid (Figure 1). The species naturally occurs in streams and rivers around West Africa. Rainbow kribs are socially monogamous and perform extensive bi-parental care for several weeks. Breeding pairs form territories including a breeding cavity (consisting of stones, little rocks and/or shells) where eggs are laid and where wrigglers (free-embryos, developmental stage between eggs and free-swimming fry) stay until they become free-swimming (U. Scherer, personal observation). Eggs develop into wrigglers within three day, wrigglers need another five days to develop into free-swimming fry (approximate durations). The egg- and wriggler stage are characterised by one parent mostly staying in the breeding cave to provide direct care to eggs/wrigglers (oxygenation, cleaning) while the other parent stays outside (but within the territory) protecting the offspring (vigilance, chasing away intruders). Free-swimming fry are guarded and protected by both parents (U. Scherer, personal observation). Commonly, a classical role division has been reported in cichlids with the male engaging more into protective behaviours (offspring and territory defence) and the female being the primary direct-care giver (McKaye and Murry 2008; Richter et al. 2010). But both parents can do and do the same parental behaviours indicating that role allocation is flexible (see Itzkowitz (1984), Lavery and Reebs (1994), Sasvari (1986), and Storey et al. (1994) for studies on sex roles in cichlids and see Royle et al. (2014) for a review on flexibility of parental care behaviour). Individual aggressiveness is important during parental care because brood and territory are defending via aggressively attacking con- and heterospecific intruders. Also boldness is thought to be important during parental care as it affects an individuals willingness to approach and inspect a potential thread (Godin and Dugatkin 1996).

Empirical work on rainbow kribs is scarce (but see: Martin and Taborsky 1997; Nelson and Elwood 1997; Seaver and Hurd 2017) and, to the best of my knowledge, no work has been done on mate choice in this species. However, existing studies on a closely related sister species, *P. taeniatus*, found female and male mate choice for relatedness (Thünken et al. 2007), body size (Baldauf et al. 2009a) and colouration (Baldauf et al. 2011; Baldauf et al. 2009b). Mutual mate choice is further suggested by the intense, but sexually dimorphic body colouration of rainbow krib males and females. Also, both sexes in the rainbow krib show courtship behaviour (including a change in colouration, fin displays, for females: flaunting of the belly) (U. Scherer, personal observation).

Note

For chapters including supplemental material, the supplement is provided directly following the reference list. For published articles, supplemental material may contain raw data or R codes that are not provided in the current transcript but can be found online.

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CHAPTER 2

Predictability is attractive: Female preference for behaviourally consistent males but no preference for the level of male aggression in a bi-parental cichlid

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ABSTRACT

Although personality traits can largely affect individual fitness we know little about the evolutionary forces generating and maintaining personality variation. Here, we investigated the hypothesis that personality variation in aggression is sexually selected in the monogamous, bi-parental cichlid *Pelvicachromis pulcher*. In this species, breeding pairs form territories and they aggressively defend their territory and offspring against con- and heterospecific intruders. In our mate choice study, we followed up two alternative hypotheses. We either expected females to show a directional preference for a high level and high consistency of aggression (potentially indicating mate choice for male parental quality) or, alternatively, we expected females to choose males for (dis-) similarity in the level/consistency of aggression (potentially indicating mate choice for compatibility). Individual level and consistency of aggression were assessed for males and females using mirror tests. After eavesdropping on aggressive behaviour of two males (differing in level and consistency of aggression) females were then allowed to choose between the two males. Males, but not females, showed personality variation in aggression. Further, females generally preferred consistent over inconsistent males independent of their level of aggression. We did not detect a general preference for the level of male aggression. However, we found an above average preference for consistent high-aggression males; whereas female preference for inconsistent high-aggression did not deviate from random choice. Our results suggest behavioural consistency of aggression in male rainbow kribs is selected for via female mate choice. Further, our study underlines the importance of considering both the level and the consistency of a behavioural trait in studies of animal behaviour.

INTRODCUTION

Consistent between-individual differences in behaviour (aka personalities, coping styles or temperaments; Schuett et al. 2010) have far-reaching fitness consequences (reviewed in Chira 2014; Réale et al. 2007; Smith and Blumstein 2008). For example, boldness and aggressiveness have been shown to affect egg fertilization rates (Ariyomo and Watt 2012), survival (Ballew et al. 2017; Dufty 1989), growth (Adriaenssens and Johnsson 2010; Grant 1990), and foraging success (Dyer et al. 2008; Grant 1990). Especially in (bi-) parental species, consistent behavioural differences are thought to heavily affect fitness (reviewed in Schuett et al. 2010) because the reproductive success largely depends on parental care behaviour (Clutton-Brock 1991; Mutzel et al. 2013). Parental care behaviour, in turn, is often closely associated with individual personalities (Budaev et al. 1999; Cain and Ketterson 2013; Chira 2014).

Because personality traits can largely affect individual reproductive success and overall fitness they should likely be considered during mate choice (Chira 2014; Qvarnström and Forsgren 1998; Schuett et al. 2010; Teyssier et al. 2014). However, existing studies investigating the link between personalities, or non-sexual behaviour in general, and mate choice are rare and deliver divergent results. Some studies found a general preference for (Bierbach et al. 2013; Doutrelant and McGregor 2000; Herb et al. 2003) or against (Ophir and Galef 2003; Spritzer et al. 2005) certain behavioural traits among females of a species. Other studies found females to differ in their mating preference, depending on their own behavioural type, leading to positive assortment (Kralj-Fišer et al. 2013; Montiglio et al. 2016; Schuett et al. 2011b) or dis-assortment (Scherer et al. 2017). In addition, existing studies on the role of behaviour during mate choice have often neglected the role of between-individual differences in behavioural consistency (but see: Schuett et al. 2011a), although this is an important personality component that can have diverse fitness implications itself (Dingemanse et al. 2010; Laubu et al. 2016; Stamps et al. 2012). Behavioural consistency in exploration behaviour, for example, is positively correlated with reproductive success in zebra finches, *Taeniopygia guttata,* (Schuett et al. 2011a) and consistency in boldness positively correlates with food consumption and collective foraging behaviour in three-spined sticklebacks, *Gasterosteus aculeatus* (Ioannou and Dall 2016). Accurate assessment of a potential mate's behaviour (level and consistency) is demanding and costly (Bleu et al. 2012; Castellano and Cermelli 2011; Fawcett and Johnstone 2003) because it requires careful observation. High assessment costs could sometimes outweigh social and reproductive benefits of behavioural consistency promoting also flexible behaviour (Laubu et al. 2016). Clearly, we need more studies to identify the evolutionary forces shaping the diverse preference pattern, consequently helping us to understand the existence of animal personality variation. Further, a more comprehensive approach is needed including all aspects of behavioural traits (behavioural level and consistency) to fully describe the relation between personality traits and mate choice.

In the present study, we used a correlative approach to investigate the effect of individual aggression (level and consistency) on female mate choice in a bi-parental West African cichlid, the rainbow krib, *Pelvicachromis pulcher*. Breeding pairs of this species raise their offspring in territories and, among other parental duties (e.g. searching for foraging grounds, keeping the brood together), both parents aggressively defend their offspring and territory against any kind of intruders. Therefore, individual differences in aggression are likely to affect reproductive success and should thus be considered during mate choice. We assessed level and consistency of aggression (total number of aggressive behaviours) for all males and females over two mirror tests. Aggressive behaviour directed towards a mirror image reliably reflects aggression towards conspecifics in *P. pulcher* (Scherer et al. 2016). Females were allowed to choose between a high- and a low-aggression male (differing in their consistency) after prior eavesdropping on male aggressive behaviour. If females choose males for their ability to defend offspring and territory (mate choice for male parental quality) we would expect females to generally prefer high- over low-aggression males. Also, several studies found high aggression to be associated with high genetic quality (e.g. Ariyomo and Watt 2012; Grant 1990). For the behavioural consistency, we expected females to show a general preference for consistent males because this would indicate the reliability of the behaviour allowing a female to predict future parental performance (Royle et al. 2010; Schuett et al. 2010). Also, high behavioural consistency could ease sexual conflict over parental investment through facilitated negotiation over the amount of parental provisioning (Royle et al. 2010). Further, high consistency in highly aggressive behaviour can serve as a signal for eavesdropping individuals lowering the number of escalating fights (Dall et al. 2004). Alternatively, we expected females to prefer the male being more (dis-) similar to themselves, which could ease synchronisation and/or specialisation of parental abilities and facilitate care coordination (mate choice for compatibility; discussed in Schuett et al. 2010).

MATERIAL & METHODS

Ethics statement

This work was approved by the German 'Behörde für Gesundheit und Verbraucherschutz Hamburg' (permission number 52/16). Stimulus males were used twice in order to reduce the number of animals needed and we used animated conspecifics instead of live conspecifics to reduce stress. Avoiding the risk of injuries during actual fights we determined individual aggressiveness using mirror tests. The number of aggressive behaviours is a good proxy for the probability of fight winning (Ophir and Galef 2003; Schlinger et al. 1987) and therefore represents a biologically relevant measure of aggressiveness.

Fish maintenance

We used laboratory bred rainbow kribs from a breeding stock at the Universität Hamburg, a local supplier (Atlantis Aquarium; Hamburg, Germany; 53°60′58.39″N 10°07′72.39″O) and a wholesaler (Dietzenbach Aquarium GmbH; Dietzenbach, Germany; 50°02′27.32″N 8°80′19.71″O). All fish were maintained in same-sex sibling groups of approx. 20-30 individuals per tank and were fed on 5 days a week with *Artemia* spp. Holding conditions were standardised using a 12:12 hours light:dark cycle and 100 L fish holding tanks (100 x 50 x 25 cm) containing a layer of sand and plastic aquarium plants. The water (26 ± 1°C water temperature) was internally aerated and filtered and changed once a week. One day prior to the start of the experiment, all fish were measured for their standard length (mean ± SE; males = 4.6 ± 0.1 cm, N = 40; females = 3.8 ± 0.1

cm, N = 39) using ImageJ (Schneider et al. 2012) and transferred to individual housing tanks (25 x 50 x 25 cm, holding conditions as above) for the duration of experimental trials. Each tank was endowed with half a clay pot (8 x 8 x 4 cm) as shelter.

Experimental outline

We assessed the level and consistency of aggression for all males (N = 40) and females (N = 39) using mirror tests (see '*Mirror tests*'). Mirror tests were performed twice (5 days in between tests) in order to assess the mean level as well as the degree of individual consistency in aggression and to test for consistent between-individual differences in aggression at population level (repeatability). For mate choice trials, males were paired up to dyads (N = 20) always consisting of two males differing in their level and consistency of aggression, based on their aggression shown during mirror tests (for more details please see '*Mirror tests*'). Male dyads were used twice during mate choice trials, except one dyad that was only used once. Females were tested for their mate preference once.

Female mate choice trials were conducted in two steps: an observation and a subsequent choice (see '*Mate choice trials*'). During the observation, females were allowed to eavesdrop on the aggressive behaviour of the two males of a dyad; directed towards their mirror images. Females could then choose between these two males in a dichotomous choice test, a standard procedure suitable to predict mating preferences in cichlids (Dechaume-Moncharmont et al. 2011; Thünken et al. 2007). Several studies have shown that individuals in many fish species gain social information through observing conspecific interactions and later use this information during their own social interactions (Aquiloni et al. 2008; Doutrelant and McGregor 2000; Ophir and Galef 2003; Schlupp et al. 1994; Witte and Godin 2010).

Mirror tests

Mirror tests were performed according to Scherer et al. (2016). We started a mirror test by removing filter and heater from an individual's housing tank, and setting up a video camera in front of the tank, one day after introducing fish into their individual housing tanks. After an acclimation of 15 min, a mirror (25 x 50 cm) was introduced on one long side of the tank facing the opening of the clay pot. The focal fish's behaviour was video-recorded for 12 min. To avoid disturbances, no human was present during recordings and tanks were covered with black plastic foil on three sides. Individuals were tested at the same time of day ± 15 min to avoid potential effects of hunger level or time of day on individual aggression (Ariyomo and Watt 2015; MacPhail et al. 2009) in repeated trials.

Following Scherer et al. (2016), the number of all restrained (frontal displays, left lateral displays, right lateral displays, s-shaped bendings, fast approachings) and overt aggressions (bites) were manually counted from the videos for a duration of 10 min, starting 2 min after the beginning of a video. We calculated the mean aggression level for each individual as the sum of all restrained and overt aggressions (average over both mirror tests). Individual consistency was calculated as behavioural inconsistency: the absolute difference in the number of all aggressive behaviours between the first and second mirror test (Ioannou and Dall 2016).

Based on the mirror tests, we formed male dyads: the two males within a dyad were matched for size (size difference < 10% of standard length; mean difference ± SE = 2.1 ± 0.3 mm) and family, but were otherwise chosen to have a maximum possible contrast in their aggressive behaviour (mean difference ± SE; level of aggression: 207 ± 28 aggressive behaviours; behavioural inconsistency: 110 ± 18 difference in the number of aggressive behaviours; N = 20 male dyads). Accordingly, males within a dyad were classified into high (mean ± SE = 277 ± 25 aggressive behaviours) and low-aggression males (mean ± SE = 70 ± 15 aggressive behaviours). High- and low-aggression males differed significantly in their mean level of aggression (unpaired Wilcoxon signed-rank test; $W = 309$, $P < 0.0001$; N = 20 male dyads). Likewise, the two males within a dyad were classified into consistent (lower within-individual variation) and inconsistent (higher within-individual variation). Consistent (mean ± SE = 32 ± 8 difference in the number of aggressive behaviours) and inconsistent (mean ± SE = 141 ± 22 difference in the number of aggressive behaviours) males significantly differed in their behavioural inconsistency (unpaired Wilcoxon signed-rank test; $W = 38$, $P < 0.0001$; N = 20 male dyads). For all individuals, the first mirror test was performed before mate choice trials and the second mirror test was performed after mate choice trials. In order to form male dyads used for mate choice trials, we pre-classified males according to their behaviour shown during the first mirror test but final classification was performed a posteriori based on the results of both mirror tests. Differences in the behavioural contrast between the two males of a dyad did not affect female preference. That is, female preference for the preferred male was neither affected by how much the two males of a dyad differed in their mean level of aggression during the mirror tests (linear mixed-effects model; $\chi^2_1 = 1.631$, $P = 0.202$; N = 35 mate choice trials, with male pair ID as random effect) nor was it affected by how much the two males of a dyad differed in their behavioural inconsistency (linear mixed-effects model; $\chi^2_1 = 0.281$, $P = 0.596$; N = 35 mate choice trials, with male pair ID as random effect).

When considering both male level and consistency classification, our set up resulted in a crossed design with four different male types: consistent high-aggression males, inconsistent high-aggression males, consistent low-aggression males and inconsistent low-aggression males (please see Table 1). Male dyads consisted either of one consistent-high and one inconsistent-low aggression male or, alternatively, they consisted of one inconsistent-high and one consistent-low aggression male.

Table 1. Descriptive statistics on male classification of aggression. Given are sample sizes and mean ± SE for the level (number of aggressive behaviours) and inconsistency (absolute difference in the number of aggressive behaviours) of aggression within each of the four classifications of male ($N_{males} = 40$, resulting in N = 20 male dyads) behaviour.

	High-aggression males		Low-aggression males	
	Consistent	Inconsistent	Consistent	Inconsistent
N	9	11	11	9
Mean ± SE level	305 ± 43	254 ± 28	42 ± 15	105 ± 22
Mean ± SE inconsistency	44 ± 8	148 ± 33	22 ± 13	133 ± 29

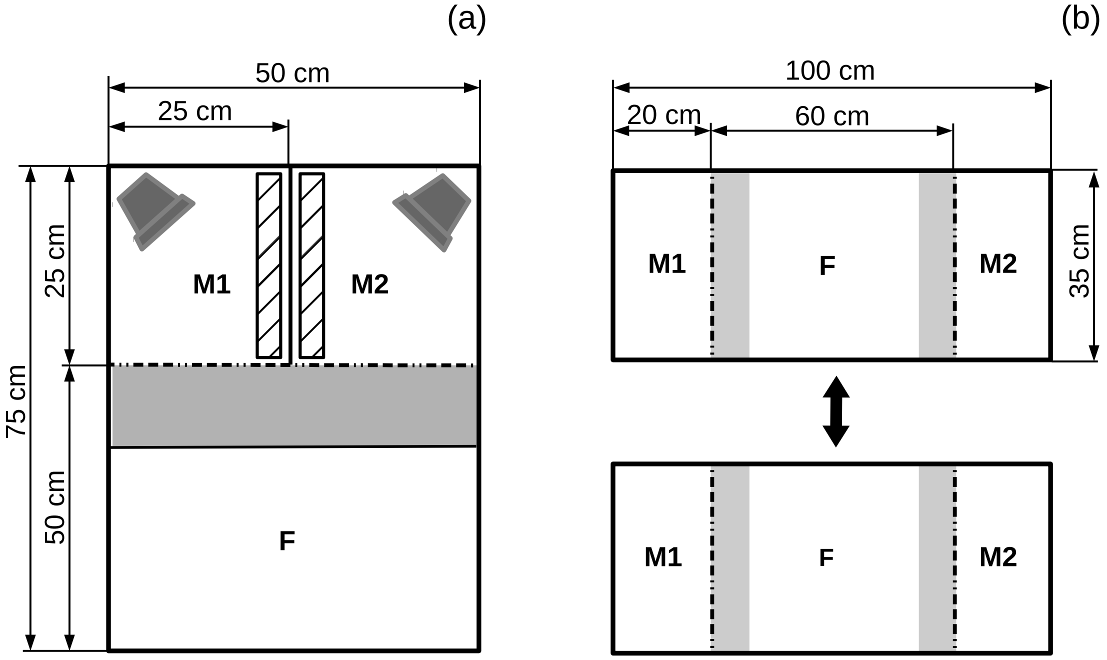

Figure 1. Experimental setup for testing female mating preference. Apparatus for (a) female eavesdropping on male aggression and (b) subsequent female choice. (a) The observation tank (height = 50 cm, water level 10 cm) was divided into three compartments: (F) a female compartment and (M1 and M2) two male compartments. Each male compartment was provided with a half a clay pot (8 x 8 x 4 cm) in a standardised position (objects in dark grey) and a removable mirror (objects with hatching). The female was hidden behind a one-way mirror (slope of 45° to avoid males seeing their mirror image; grey area). (b) The choice chamber (height = 35 cm, water level = 10 cm) was divided into three compartments with the female compartment being in the middle and two male compartments covering the edges of the tank. The female compartment was subdivided into three zones, with the neutral zone being in the middle and the two preference zones for the males on the adjacent sides, each zone alongside the concomitant male compartment (width = 12 cm, refers to approx. two fish lengths; light grey areas). Compartments of observation tank and choice chamber were separated using clear Plexiglas (dashed lines) and/or white Plexiglas (solid lines). Tanks were surrounded with white Plexiglas.

Mate choice trials

Before each mate choice test, females were allowed to observe male aggressive behaviour. To start the observation, we introduced two males of a dyad into an observation tank (Figure 1a), one male into each of two male compartments. Also, we transferred a randomly chosen female (non-sibling and non-familiar to the males) into the female observer compartment, visually separated from the male compartments using a white partition. After an acclimation period of 15 min, a mirror (25 x 50 cm) was introduced into each male compartment covering the partition between the male compartments (Figure 1a) and the partition visually separating the female compartment from male compartments was removed. Hidden behind a one-way mirror (Figure 1a), the female could observe the two males interacting with their mirrors for 12 min without being seen by males.

Immediately after the observation, the two males and the female observer were transferred to a mate choice chamber (Figure 1b). The two males were randomly assigned to two male compartments and the female was introduced to a female compartment. After an acclimation of 10 min without visual contact, white partitions separating the compartments were gently removed and the first mate choice test period of 12 min started. To take account for a potential side-bias, the trial was repeated immediately after with the males being switched between the male compartments. All fish were again allowed to acclimate for 10 min (under visual separation) before the second test period of 12 min started. During experiments, no observer was present to avoid disturbances. Both test periods were video-recorded from above.

We assessed the association time (female time spent in a male's preference zone (Figure 1b), sum of both test periods, sec) for each male from the videos using Ethovision XT 11 (Noldus, Wageningen, The Netherlands). Videos were analysed for 10 min, starting 2 min after the beginning of a video. Female mating preference was then quantified from both test periods as the strength of preference for each male: the association time for one male was divided by the association for both males (e.g. Dugatkin 1996; Makowicz et al. 2010; Scherer et al. 2017). Further, we calculated female side bias as the time a female spent in one preference zone relative to the amount of time spent in both preference zones (sum of both test periods). A female was considered side-biased when she spent more than 80% of the test time in just one preference zone (Poschadel et al. 2009; Scherer et al. 2017; Schlüter et al. 1998). We decided a priori to exclude side-biased preference data from the analysis (N = 1 mate choice trial) (e.g. Scherer et al. 2017; Schlupp et al. 1999). Another three mate choice trials were excluded because of damaged video files, resulting in final N = 35 trials used for preference analyses (including N = 18 male dyads).

Statistical analysis

All statistical analyses were performed in R version 3.4.0 (R Core Team 2017). Repeatability of aggressive behaviour was calculated for males and females separately using linear mixed effects models (LMMs) implemented in the *rptR*-package (Stoffel et al. 2017). Repeatability calculations were performed with 1000 bootstrapping runs and 1000 permutations. Significant repeatability was given when the 95% confidence interval (CI) did not include 0. Further, we tested for a sex difference in the mean level and inconsistency of male and female aggression (N_{total} = 79, consisting of N_{males} = 40 and $N_{females}$ = 39) fitting two linear models (LMs): one model was fit on the level of aggression and the other model was fit on the behavioural inconsistency, both models contained the sex as predictor variable.

We tested for a general preference for high- over low-aggression males using an LMM (lme4-package, Bates et al. 2014) with female preference for high-aggression males (including both consistent and inconsistent high-aggression males; N_{trials} = 35) as response variable. We included male dyad ID as random effect but otherwise did not include any fixed effects (aka null model). Deviation from random choice would be revealed if the 95 % CI of the mean does not include 0.5. Further, we tested for a preference for consistent over inconsistent males (including both low- and high-aggression; N_{trials} = 35) using the same approach: we ran a null model with female preference for consistent males as response and included male dyad ID as random effect.

In order to test the possibility that female preference for the consistency and the level of male aggression are interdependent we further assessed deviation from random choice of female preference for consistent high-aggression (N_{trials} = 17) and consistent low-aggression (N_{trials} = 18) using the above method. To avoid redundancy, we did not analyse the remaining behavioural combination (inconsistent high-aggression and inconsistent low-aggression) in the same way. Due to our experimental design, female preference for consistent high-aggression males were directly inverse to female preference for inconsistent low-aggression males. Likewise, female preference for inconsistent high-aggression males and for consistent low-aggression males were directly inverse.

Also, we tested for a difference in female preference between consistent high- and consistent low-aggression males. We fit an LMM on female preference for consistent males (N_{trials} = 35) including male behavioural type combination (consistent high-aggression and consistent low-aggression) as fixed effect and male dyad ID as random effect. Similarly, we tested for a difference in female preference between consistent and inconsistent high-aggression males (N_{trials} = 35) fitting an LMM on female preference for high-aggression males, again, including male behavioural type combination (consistent high-aggression and inconsistent high-aggression) as fixed effect and male dyad ID as random effect. For all models, we calculated effect sizes (partial R^2) for fixed effects following Nakagawa and Schielzeth (2013) using the *r2glmm*-package (Jaeger 2016). For non-significant fixed effects we report the partial R^2 deriving from the model before the term was dropped. Model assumptions were visually assured using model diagnosis plots. For all analyses, female strength of preference was *arcsine-square root*-transformed for normality of the residuals.

The prediction that females might show a preference for behavioural (dis-) similarity in aggression is based on the assumption that females show personality variation for aggressiveness. However, overall female aggression was not repeatable (please see '*Results*'). Therefore, we cannot present a reliable measure of female aggressive behaviour. Thus, we only present an analysis of female preference for (dis-) similarity in the supplement (Supplemental Material 1).

We provide our raw data including behavioural data of mirror test (Supplemental Material 2) and behavioural data obtained during mate choice trials (Supplemental Material 3). Also, we supply our R code used for preference analyses (Supplemental Material 4).

RESULTS

Male aggressive behaviour (mean ± SE = 166 ± 15 aggressive behaviours) was significantly repeatable (R ± SE = 0.660 ± 0.092, CI = [0.452, 0.807]; N_{trials} = 80, N_{males} = 40). In contrast, female aggressive behaviour (mean ± SE = 196 ± 17 aggressive behaviours) was not repeatable (R ± SE = 0.176 ± 0.135, CI = [0.000, 0.460]; N_{trials} = 78, $N_{females}$ = 39). Males (mean ± SE = 170 ± 22 aggressive behaviours) tended to be less aggressive than females (mean ± SE = 219 ± 17 aggressive behaviours) (LM; estimate ± SE = -62 ± 35, $F_{1,77}$ = 3.110, P = 0.082; R^2 = 0.039, CI = [0.160, 0.100]; $N_{males+females}$ = 79 males and females; Figure 2a). Further, females (mean ± SE = 0.252 ± 0.032) were less consistent than males (mean ± SE = 0.143 ± 0.024) (LM; estimate ± SE = 80 ± 26, $F_{1,77}$ = 9.581, P = 0.003; R^2 = 0.111, CI = [0.016, 0.263]; $N_{males+females}$ = 79; Figure 2b).

Females did not show a general preference for high- over low-aggression males (including consistent and inconsistent males; mean preference = 0.492; CI = [0.416, 0.567]; N = 35). In contrast, females

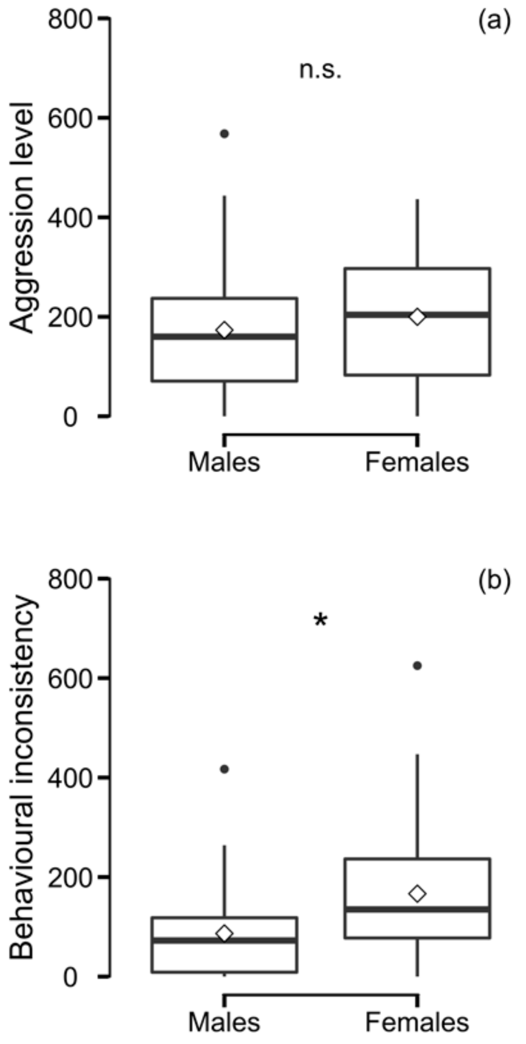

Figure 2. Comparison of male and female (a) level and (b) inconsistency of aggression. Level (number of aggressive behaviours) and inconsistency (absolute difference in the number of aggressive behaviours) of male and female aggressive behaviour. Boxplots present original data with mean (◊), mean (-) and 1.5 interquartile ranges, significance indicated (significant: *; non-significant: n.s.).

generally preferred consistent over inconsistent males (including high- and low-aggression males; mean preference = 0.577; CI = [0.511, 0.641]; N = 35). Female preference for consistent males was mainly driven by a significant above average preference for consistent high-aggression males (mean preference = 0.571; CI = [0.508, 0.633]; Figure 3) whereas female preference for consistent low-aggression males did not deviate from random choice (mean preference = 0.584; CI = [0.459, 0.704]; Figure 3). However, preference scores for consistent-high and consistent low-aggression males did not statistically differ from each other (LMM; estimate ± SE = 0.014 ± 0.064, χ^2 = 0.054, P = 0.816; R^2 = 0.002, CI = [0.000, 0.148]; N = 35; Figure 3). Female preference for consistent high-aggression males was significantly higher than female preference for inconsistent high-aggression males (LMM; estimate ± SE = -0.154 ± 0.064, χ^2 = 5.057, P = 0.025; R^2 = 0.191, CI = [0.022, 0.441]; N = 35; Figure 3). Inconsistent low-aggression males were the least preferred (Figure 3).

DISCUSSION

We found that only male but not female *P. pulcher* showed personality variation in aggressiveness. Males and females did not differ in their level of aggression but males showed significantly higher behavioural consistency. Further, females did not show a general preference for high- over low-aggression males. Instead, female preference for the level of male aggression was dependent on the consistency in which male aggression was expressed; that means consistent high-aggression males received significant above average preference scores but inconsistent high-aggression males did not. Finally, females generally preferred consistent over inconsistent males no matter whether these males were classified as high- or low-aggression.

The sex difference in the consistency of aggression might indicate there are different selective regimes acting on male and female behavioural consistency. Although both rainbow krib parents engage in offspring and territory defence a typical division of labour can be observed with males usually doing a greater proportion of territory defence and females providing more direct offspring care. Such a division of labour with specific sex roles during parental care can be commonly observed in cichlid fish species (e.g. Itzkowitz 1984; Lavery and Reebs 1994; McKaye and Murry 2008). Due to this parental role allocation, the selective pressure on consistent aggression might be higher for male than for female *P. pulcher*. In other words, while females may benefit from choosing consistent high-aggression males in terms of better offspring and territory defence, there may not be such benefits for consistent female aggression. Instead, selection may actively favour flexibility of female aggression due to possibly high costs of consistent aggression in close proximity to the offspring (Nandy et al. 2016; Smith and Harper 1988); i.e. during direct offspring care female aggression could easily be misdirected towards offspring when expressed consistently. In a closely related sister species, the convict cichlid, *Amatitlania siquia*, behavioural flexibility increased reproductive success of behaviourally mismatched breeding pairs through behavioural convergence (Laubu et al. 2016). In the present study, female inconsistency might potentially allow them to flexibly adjust their behaviour to the needs of that very moment.

Because both sexes in the rainbow krib provide parental care, we expected mate choice for behavioural compatibility to be likely. But females did not prefer behaviourally (dis-) similar males (see Supplemental Material 1), which may be attributed to the fact that we did not find personality variation for female aggression. Instead, females showed a preference for consistent high-aggression males suggesting mate choice for (parental) quality. A high level of aggression could ensure a male's ability to defend offspring and territory, while behavioural consistency could signal a female how reliable the information is. High consistency in aggressiveness could allow a female to predict male future parental care performance (in defence behaviour) from male aggression shown prior to reproduction (Dall et al. 2004; Royle et al. 2010; Schuett et al. 2010). Also, females could benefit from choosing consistent high-aggression males if these males provide genetic benefits for the offspring. For instance, aggressiveness has been shown to correlate with food intake and growth (reviewed in Biro and Stamps 2008), reproductive success (reviewed in Schuett and Dall 2010) and fat storage (in zebra finches, Schuett and Dall 2010). Notably, we did not set up a choice condition testing female preference for consistent high-

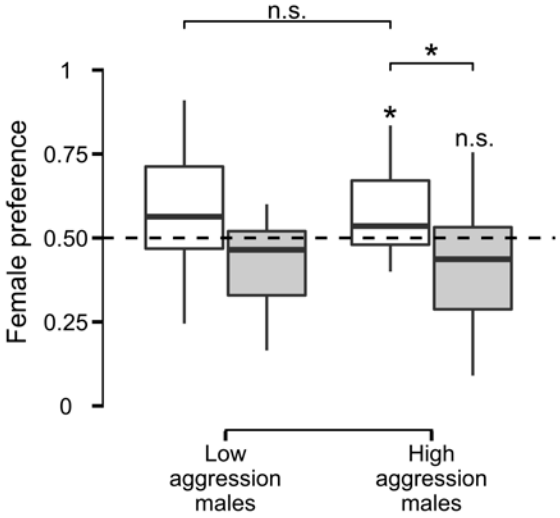

Figure 3. Female preference. Deviation from random choice (female preference = 0.50; dashed line) for high- and low-aggression males, split into consistent (white filling) and inconsistent (grey filling). Boxplots present original data with median (-) and 1.5 interquartile ranges, significance indicated (significant: *; non-significant: n.s.). Please note, female preference for consistent high-aggression and inconsistent low-aggression males are directly inverse, as well as inconsistent high-aggression and consistent-low preference scores.

aggression vs. consistent low-aggression. Hence, we cannot conclusively prove a directional female preference for the level of male aggression.

We found male behavioural consistency to affect female mate choice although females were allowed to observe male aggressive behaviour only once. Possibly, the behavioural consistency within one trial correlates with the behavioural consistency between repeated behavioural measurements. That is, an individual that behaves homogenously at one time (e.g. number of aggressive behaviours evenly distributed throughout the observation) might possibly also behave homogenously throughout time (thus showing a similar number of aggressive behaviours anytime later). On the contrary, an individual that behaves very heterogeneously within one observation (e.g. high fluctuation in the frequency of performing aggressive behaviours) might show higher heterogeneity between observations. Such a transition from within- to between-observational consistency would allow a female to predict future (parental) behaviour from just one observation. Due to our correlative experimental design, female preference could also be related to consistency in a different behaviour (e.g. general swimming behaviour, activity), or even to a non-behavioural trait (e.g. colouration) that might be correlated to behavioural consistency in aggressiveness. Further examinations using experimental manipulations of the natural behaviour are inevitable to disentangle the behaviour from other, possibly correlated traits.

The strength of our study lies within the consideration of both personality compounds: level and consistency of aggression. In animal personality research, large attempts have been made to understand the evolution of individual differences in the level of behaviour. However, the effects of individual differences in the behavioural consistency have mostly been unattended (but see: Ioannou and Dall 2016; Scherer et al. 2017; Schuett et al. 2011a). Our study shows the effects of the behavioural level can be tightly linked to the consistency in which the behaviour is expressed. We highlight the importance of considering both aspects of a personality trait (the level and the consistency) and encourage future research to use a more holistic experimental design in studies on animal personality. Clearly, the power of our experimental design is limited by a lack of male behavioural data from the observation phase of mate choice trials. Although males were behaviourally consistent on population level, individuals differed in their degree of consistency. While the behaviour of ‘consistent’ males should confidently match their classification we cannot be conclusively sure that the behavioural level shown by ‘inconsistent’ males during the observation matched their classification. The uncertainty in ‘inconsistent’ male aggression might have interfered with our testing of female preference for male aggression level and could have weakened the signal. However, the classification ‘inconsistent’ does not necessarily mean a male shows high behavioural instability. Instead, it solely means that individual's behavioural consistency is lower compared to the consistency of the other male within one male dyad. Generally, the behavioural consistency of all males, including ‘consistent’ and ‘inconsistent’ was relatively high.

CONCLUSION

In summary, we found males and females to be equally aggressive but females were less consistent in their aggressiveness, which might be attributed to the parental roles during offspring care leading to sexual selection favouring consistent male aggression (advantage in offspring and territory defence) and disfavouring consistent female aggression (dangerous for offspring). Female preference for consistent high-aggression males might indicate female choice for parental care quality or male genetic quality. However, in order to determine, which of these two non-exclusive evolutionary mechanisms (mate choice for parental care or mate choice for intrinsic quality) is relevant follow-up breeding experiments disentangling direct behavioural from genetic benefits are necessary. Females generally preferred high consistency though a high level of aggression was only preferred in combination with high consistency. This might indicate that the behavioural consistency (indicating the quality of the signal) is more important than the behavioural level. However, the adaptive benefit of behavioural consistency (independent of the behavioural level) remains to be tested in our target species. This would be especially worthwhile with regard to parental performance as behavioural consistency is expected to provide reproductive benefits associated with the predictability of behaviour: facilitation of parental role specialisation and/or eased negotiation over amount of offspring care (Royle et al. 2010; Schuett et al. 2010). Our results highlight that behavioural consistency is an essential component of personality traits that should not be overlooked in the behavioural sciences.

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CHAPTER 2 - SUPPLEMENTAL MATERIAL 1

Analysis of female preference for behavioural (dis-) similarity

Statistical analysis

Data analysis was performed in R version 3.4.0 (R Core Team 2017). To test for an effect of male-female behavioural (dis-) similarity on female preference (please see main text for the assessment of female preference) we fit an LMM (linear mixed-effects model) on female preference for high-aggression males (consistent and inconsistent) (N = 35). The model included relative similarity in the behavioural level and relative similarity in the behavioural consistency as fixed effects; male ID was included as random effect. Relative similarity (for level and consistency, respectively) was calculated as the female's similarity with the low-aggression/inconsistent male (absolute value of the difference) minus the female's similarity with the high aggression/consistent male (absolute value of the difference) (Scherer et al. 2017). Thus, positive values indicate the female is behaviourally more similar to the high aggression/consistent male than to the low-aggression/inconsistent male and vice versa. Before analysis, predictor variables were z-transformed for standardization using the *GenABEL*-package (GenABEL project developers 2013). For modeling, we used the *lme4*-package (Bates et al. 2015). The minimum adequate model was fit using a backward model selection approach. Effect sizes (partial R^2) with CIs were calculated for fixed effects following Nakagawa and Schielzeth (2013) using the *r2glmm*-package (Jaeger 2016). For insignificant fixed effects, R^2 and CIs of the model before the term was dropped were reported. Raw data used for this analysis are provided in Supplemental Material 3. Our R script for running the preference analysis is presented in Supplemental Material 4.

Results

Female preference for high aggression males was neither affected by relative similarity in the behavioural level (LMM; standardized estimate ± SE = -0.011 ± 0.027, $\chi^2_1 = 0.142$, $P = 0.707$; $R^2 = 0.004$, CI = [0.000, 0.153]; N = 35) nor was it affected by relative similarity in consistency (LMM; standardized estimate ± SE = -0.031 ± 0.030, $\chi^2_1 = 0.954$, $P = 0.329$; $R^2 = 0.028$, CI = [0.000, 0.222]; N = 35).

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CHAPTER 3

Different or alike? Female rainbow kribs choose males of similar consistency and dissimilar level of boldness

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ABSTRACT

Although the existence of consistent between-individual differences in behaviour ('personality differences') has been well documented during the last decade, the adaptive value of such behavioural limitations remains an open field for researchers of animal behaviour. Personalities clearly restrict individuals in their ability to adjust their behaviour to different conditions. However, sheer costs of flexibility cannot explain the polymorphism created by personality variation. In a correlative approach, we here tested whether mate choice might act as a major driving force maintaining personality variation in the monogamous, bi-parental rainbow krib, *Pelvicachromis pulcher*. We personality-typed all males and females for their boldness (activity under simulated predation risk) and allowed females to choose between two males that differed in their boldness (behavioural level and consistency). Prior to the choice, females were allowed to observe both males, expressing their natural boldness towards a video-animated natural predator. Both sexes showed personality differences in boldness over the short and long term. Furthermore, when removing side-biased females, we found a dis-assortative mating preference for the behavioural level and an assortative preference for behavioural consistency in boldness. These preference patterns might facilitate effective parental role allocation during offspring care and/or provide genetic benefits. Our results suggest that sexual selection plays an important role in the evolution of personality differences.

INTRODUCTION

Individuals must cope with a wide array of environmental challenges. Therefore, flexibility in the expression of behavioural responses towards different and changing conditions should be favoured by selection (Sih et al. 2004). Yet, individuals often show considerable consistent between-individual differences in behaviour over time and/or contexts (Boissy 1995). Such personality differences are common throughout the animal kingdom (reviewed in Gosling 2001; Kralj-Fišer and Schuett 2014) and have been shown for various behavioural traits, such as activity pattern, aggressiveness, exploratory tendencies, boldness and fearfulness (reviewed in Dall et al. 2004; Gosling 2001; Sih et al. 2004). Personality traits are moderately heritable (Ariyomo et al. 2013; Patrick et al. 2013; Reif and Lesch 2003; van Oers et al. 2005) and have fitness consequences (e.g. Ariyomo and Watt 2012; Dingemanse and Réale 2005; Smith and Blumstein 2008), suggesting they are not merely non-adaptive noise that surrounds an adaptive optimum (Wilson 1998). Nevertheless, underlying mechanisms that generate and maintain behavioural polymorphisms are largely unclear and many aspects of the growing body of theoretical frameworks have yet to be empirically tested (reviewed in e.g. Schuett et al. 2010; Wolf and Weissing 2010).

Recently, Schuett et al. (2010) pointed out that sexual selection may be important in generating and maintaining personality variation although this possibility has rarely been tested (but see e.g. Montiglio et al. 2016; Schuett et al. 2011a; Schuett et al. 2011b). According to the proposed framework (Schuett et al. 2010), personalities are expected to play an important role in mate choice when a potential mate's behavioural phenotype is either associated with good/compatible genes that increase offspring fitness (Dingemanse et al. 2004; Ihle et al. 2015; Mays and Hill 2004) or provides non-genetic benefits increasing the reproductive success through parental ability and/or behavioural compatibility between mates. While mate choice for genetic quality and parental ability should favour inter-individual agreement in the preference for a behavioural trait, mate choice for genetic or behavioural compatibility should depend on an interaction between male and female genotypes or phenotypes (Schuett et al. 2010). Thus, mate choice for compatibility would lead to inter-individual differences in mating preferences, creating either an assortative or dis-assortative mating pattern (Schuett et al. 2010).

Few studies have investigated the effect of personality traits on mate choice (reviewed in Schuett et al. 2010) and some have only assessed the behaviour of the chosen but not the choosing sex (Godin and Dugatkin 1996; Ophir and Galef 2003). The few studies considering a potential interplay between male and female personality during mate choice have often found assortative mate choice for various behavioural traits, in correlative (Gonzaga et al. 2010; Kralj-Fišer et al. 2013; Mascie-Taylor and Vandenberg 1988; Montiglio et al. 2016) or experimental settings (Schuett et al. 2011b), and an increased reproductive success of assortative pairs (e.g. Ariyomo and Watt 2013; Schuett et al. 2011a). However, in studies that found increased success of assortative pairs, personality data were often obtained after pairing (Both et al. 2005; Harris and Siefferman 2014; Laubu et al. 2016), which did not allow the authors to determine whether mate choice was affected by individual personalities or whether behavioural similarity was achieved after pairing in highly successful pairs (Laubu et al. 2016). Indirect evidence that dis-assortment for personality can sometimes be beneficial is provided by van Oers et al. (2008), who found that assortative pairs of great tits, *Parus major*, had higher rates of extra-pair paternity. Generally, positive assortment for genotypic or phenotypic traits is far more prominent in the animal kingdom than evidence for dis-assortment (reviewed in Jiang et al. 2013).

Personality traits consist of two measures: the behavioural level and the degree of behavioural consistency. Although there is considerable variation in within-individual behavioural consistency (Dingemanse et al. 2010) the effect of such individual differences in consistency on mate choice has rarely been considered (Schuett et al. 2011a). Behavioural consistency might be sexually selected for if it reflects individual quality (i.e. consistency is costly under changing conditions) or if choosing a predictable (i.e. consistent) mate provides reliable information about future parental care behaviour prior to mating (Dall et al. 2004; Royle et al. 2010; Schuett et al. 2010). For example, a female might be able to predict a male's ability to protect prospective offspring from the consistency in boldness expressed prior to mate choice.

In the present study, we investigated the influence of male and female boldness (propensity to engage into risky behaviour; Wilson et al. 1994) on female mate preference in a socially monogamous, bi-parental cichlid from West Africa, the rainbow krib, *Pelvicachromis pulcher*. In this species, pairs are highly territorial: they defend territories and offspring aggressively against conspecifics and heterospecifics. Therefore, we assumed individual boldness to be a trait that these fish are likely to consider during mate choice. Furthermore, boldness has been shown to affect foraging success (Dyer et al. 2008), egg fertilization rates (Ariyomo and Watt 2012), dominance (Dahlbom et al. 2011), survivorship (Smith and Blumstein 2010) and parental care effort (Budaev et al. 1999) in other fish species. We measured male and female boldness (activity under simulated predation risk) repeatedly to test for personality differences. During mate choice experiments, females were first allowed to observe a bolder and a shyer male expressing their natural boldness towards a predator animation. Subsequent female mating preference for the two males was assessed in a standard mate choice scenario. We considered both aspects of male and female personality: the behavioural level and behavioural consistency of each individual.

We expected female preferences to depend on both the behavioural level and behavioural consistency, with our predictions being guided by Schuett et al. (2010). For the behavioural level, we expected that if mate choice is based on male (parental or genetic) quality, females should show a general preference for either bold or shy males (e.g. Godin and Dugatkin 1996; Kortet et al. 2012). Alternatively, if mate compatibility is more important during mate choice, females should not show an overall agreement but also consider their own personality during their choice. Because both rainbow krib parents provide offspring care we considered the second possibility, i.e. mate compatibility, to be more important for mate choice based on boldness. In species with bi-parental care, an assortative mating preference for certain behavioural traits could reduce sexual conflict over parental investment (Royle et al. 2010) and facilitate offspring care coordination by better synchronization of parental activities (Schuett et al. 2011a). Depending on the environmental conditions or the biology of the species, dis-assortative mating might also sometimes have advantages (Schuett et al. 2010). For instance, species that perform several parental activities might also benefit from expressing a dis-assortative mating preference, facilitating role allocation and specialization during offspring care. Often, a sexual dimorphism in role specialization can be observed with the female providing more direct offspring care and the male defending the territory (e.g. Guerra and Drummond 1995; Itzkowitz 1984; Neil 1984; Richter et al. 2010; Solomon 1993). Nevertheless, in many species both partners can or do perform the same behaviours (see Royle et al. 2014 for a review on the flexibility of parental care behaviour), and at least partly compensate for their mate's tasks if needed (Itzkowitz 1984; Lavery and Reebs 1994; Sasvari 1986; Storey et al. 1994) indicating that sex roles might be less fixed. For the behavioural consistency, we followed up two possible mate choice scenarios: a general preference for consistent over inconsistent males, which might indicate predictability of later parental performance, and/or individual quality (Royle et al. 2010; Schuett et al. 2010) or mate choice for compatibility leading to a positive assortative preference (Schuett et al. 2011b; Schuett et al. 2010).

METHODS

Ethical note

In consideration of animal welfare, we followed the '3R' framework (Russell and Burch 1959). To decrease the number of study animals needed we used predator animations instead of live predators and test males for mate choice trials were used twice. During experiments, no animals were harmed or exposed to actual predation risk. Prey fish and predators were kept separately and did not have visual contact during fish maintenance. Permits were provided by the German 'Behörde für Gesundheit und Verbraucherschutz Hamburg'.

Study animals and holding conditions

Study individuals were obtained from a captive breeding stock at the University of Hamburg and local suppliers. Males and females were 1–2 years old and sexually inexperienced. Individuals were maintained in same-sex sibling groups under standardized holding conditions (tanks measuring 100 x 50 cm and 25 cm high and 100 x 50 cm and 50 cm high, 26 ± 1°C water temperature, aerated and filtered water, weekly water changes, 12:12 h light:dark) and were fed once a day on 5 days a week with *Artmia*. On experimentation days, fish were fed after observations. One day before the first personality test, the standard length of individuals was measured (males: 3.8–6.2 cm, females: 3.5–5.1 cm) using ImageJ (Schneider et al. 2012) and all individuals were transferred into individual tanks (25 x 25 cm and 50 cm high) for the duration of experimental trials (5 days per individual). Tanks contained sand, half a clay pot as shelter and an internal filter. For identification, all individuals were marked with visible implant elastomers (Northwest Marine Technology, Shaw Island, WA, U.S.A.). These artificial colour marks have no influence on mate choice in our population (Schuett et al. 2017).

Experimental outline

During personality testing and mate choice trials boldness was measured as activity under simulated predation risk using computer animations of a sympatric predator, the African obscure snakehead,

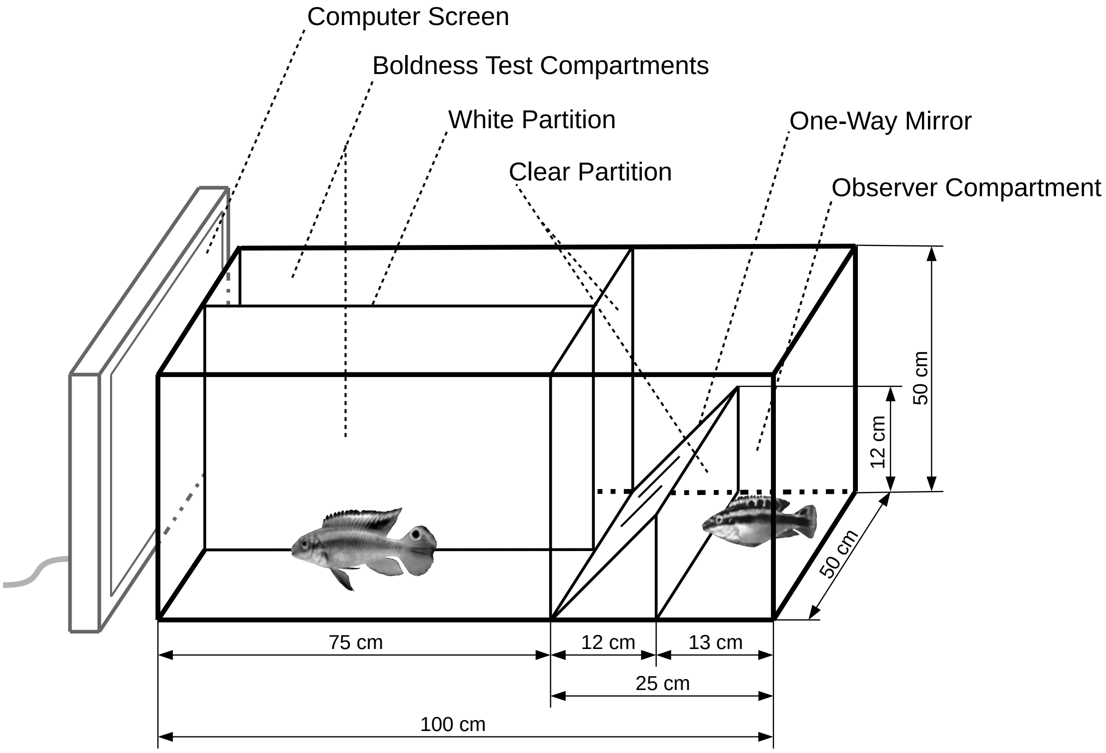

Figure 1. Experimental set-up for the boldness test. Two same-sex focal individuals (visually separated) were exposed to a video animation of a predator. Test individuals were observed by a fish of the other sex but could not see the observer: the observer compartment had a one-way mirror aligned at an angle of 45° towards the test compartments providing a visual cover for the observer. Fish not to scale.

Parachanna obscura. All males (*N* = 48) and females (*N* = 45) used during mate choice experiments were tested for their boldness three times (day 0, day 4, day 33) to assess the behavioural level and consistency for all individuals and short- and long-term repeatability in the population. The first and second test series of male boldness tests were integrated into mate choice trials (*N* = 45), allowing females to observe two males expressing their natural boldness. After the observation, females were allowed to choose between the two males they had just observed in a standard mate choice test (see '*Mate choice trials*'). For the remaining boldness trials (third series of male boldness tests and all female boldness tests) the test procedure was identical to those integrated into mate choice trials to ensure equal test conditions throughout.

Boldness test

Boldness tests were conducted in a test tank (water level 10 cm, water temperature 26 ± 1°C; Figure 1), which was divided into three compartments: two parallel test compartments in which two individuals could be tested for their boldness at the same time and an adjacent observer compartment. A one-way mirror between the observer and the test compartments allowed the observer to see the test individuals but prevented the test individuals from seeing the observer. On the other short side, test compartments faced a computer monitor (Dell, UltraSharp U2412M 61 cm, 24″) for the presentation of predator animations. Removable opaque dividers between the test and the observer compartments as well as between the test compartments and the monitor allowed visual separation during acclimation before trials.

Prior to a boldness test, we put two same-sex individuals (for details see also '*Mate choice trials*') into two clear cylinders (diameter = 11 cm), one per test compartment (test compartments were permanently visually separated from each other). An observer of the opposite sex was put into the observer compartment and allowed to freely swim around. An observer was always introduced (even in male and female personality tests that were not integrated into mate choice trials) because chemical cues could be transmitted from the observer to the test compartments despite physical separation. After 15 min of acclimation, the opaque dividers were removed allowing free view of the animation (test individuals and observer) and test individuals (observer). After another 1 min, the cylinders were removed and the test period of 11 min started. Trials were video-recorded from above with no human present during trials and the test tank was surrounded with white Plexiglas to avoid disturbances. Individuals were always boldness-typed at the same time of day ± 30 min to account for potential effects of time of day and hunger level on individual activity pattern (Ariyomo and Watt 2015; MacPhail et al. 2009). In each boldness test, individuals were exposed to a randomly chosen animation showing a predator specimen they had not seen before.

Predator animations (*N* = 4, each using another specimen) were prepared using PowerPoint following Fischer et al. (2014). Animations displayed a still photograph of the predator swimming back and forth in front of a white background. We have validated this method: *P. Pulcher* decreased their activity in response to predator animations compared to a control while no difference in response towards a live predator and the animation was found (Scherer et al. 2017).

Boldness was measured as individual activity (total distance moved; cm) from the video recordings using the tracking software Ethovision XT 11 (Noldus, Wageningen, The Netherlands). The activity was assessed for a test period of 10 min, beginning 1 min after the start of the video. For all individuals, the behavioural level was defined as the mean activity of the first and second test series. Behavioural consistency was calculated following Ioannou and Dall (2016) as the absolute value of the difference in activity between the first and second boldness test. We further divided the measure of Ioannou and Dall (2016) by the total variation in the population (range of activity within first and second boldness test). As suggested by Dingemanse et al. (2010), this index provides a measure that is standardized in relation to the population. We calculated behavioural consistency for males and females separately. Values for consistency can range from 0 (high consistency) to 1 (low consistency).

Mate choice trials

Mate choice trials consisted of two parts: the observation described above and a subsequent choice. During observation, the female could observe two males showing their natural boldness (see '*Boldness test*'). Subsequent mate choice was conducted immediately after the observation in a standard dichotomous choice test, suitable for predicting mate preference in cichlids from the amount of time spent with a male (Dechaume-Moncharmont et al. 2011; Thünken et al. 2007). The choice chamber (100 x 35 cm and 25 cm high, water level = 10 cm) was separated into three compartments with the female compartment in the middle (60 x 35 cm and

25 cm high) and a male compartment at each side (20 x 35 cm and 25 cm high).

To begin the choice test, we transferred the female and the two males she had just observed from the boldness test tank to the choice chamber. Males were randomly assigned to the two male compartments. All individuals were allowed to acclimate for 10 min while visually separated from each other. Then, the opaque dividers were removed and the first test period of 12 min began. Thereafter, the procedure was repeated with the males switching sides to take account of a potential side bias (again 10 min acclimation followed by a 12 min test period). To avoid disturbance, the choice chamber was surrounded with white Plexiglas and no human was present during trials. Trials were video-recorded from above.

Each female was used once during mate choice trials. The two males used in a mate choice trial were matched for size (standard length difference ≤5%, i.e. ≤3 mm) and family but otherwise randomly chosen. The female observer originated from a different family than the males.

The association time for the two males was determined from both test periods (i.e. 20 min) using Ethovision XT 11. Test periods were analysed for 10 min, starting 2 min after the start of the video. The association time was defined as the time the female spent within 5 cm of each male compartment (which corresponds to ca. one fish length; hereafter 'preference zone'). Female strength of preference was then quantified as the relative amount of time she spent in the preference zone of the bold male (association time for the bold male was divided by the association time for both males; e.g. Dugatkin 1996; Makowicz et al. 2010). For each mate choice test, the bold male was defined as the male that was more active during the boldness test and the shy male was the less active male (mean ± SE for absolute similarity between shy and bold males: behavioural level = 975.95 ± 147.81; behavioural consistency = 0.11 ± 0.02; see '*Statistical analyses*' for calculation of similarity indices). Also, we calculated the side bias for all females and considered a female as side biased when she spent more than 80% of the total time spent in preference zones (both test periods) in just one zone, regardless of which male was there (Poschadel et al. 2009; Schlüter et al. 1998).

Statistical analyses

All data analyses were conducted in R 3.2.3 (R Core Team 2015). To test for personality differences, repeatability of our measure for boldness (activity under simulated predation risk) was assessed with linear mixed-effect models (LMMs) using the rptR-package (Schielzeth and Nakagawa 2013). We assessed short-term repeatability (boldness test: day 0, day 4) as well as long-term repeatability (boldness test: day 4, day 33) for sexes separately with 1000 bootstrapping runs and 1000 permutations. Significance was inferred when the 95% confidence interval, CI, did not include zero. Activity was square root-transformed for normality and models were fitted for Gaussian error structure.

To test for a general preference for bold or shy males, we ran an LMM with female strength of preference for bold males as the response and male ID as a random effect. We did not include any fixed effects. To check for a deviation from random choice (i.e. strength of preference = 50%) we obtained the 95% CI of the estimated mean. A preference for either bold or shy males would be indicated if the CI does not include 0.50. Similarly, we tested for a general preference for behavioural consistency by running a null model with female strength of preference for the male showing the higher consistency during the observation as the response and male ID as a random effect. A preference for either consistency or inconsistency would be revealed if the 95% CI of the mean did not include 0.50.

To test for (dis-) assortative female mate choice we fitted an LMM with female strength of preference for bold males as the response variable and male ID as random term. As fixed effects, we included relative similarity for the behavioural level and relative similarity for the behavioural consistency between the female and the males she saw during the observation phase and mate choice test. To calculate relative similarity (for level and consistency, respectively), we first computed the difference score-based similarity between the female and each of the two males (bold and shy) as the absolute value of the difference in the respective behaviour (Gaunt 2006; Luo and Klohnen 2005; Montiglio et al. 2016) between the female and the bold male, and the female and the shy male. Thus, similarity (in level and consistency, respectively) was highest at zero and dis-similarity increased with increasing values. Relative similarity was then calculated following Gasparini et al. (2015): the similarity between the female and the bold male was subtracted from the similarity between the female and the shy male. Positive values for relative similarity (in level and consistency, respectively) indicate higher similarity between the female and the bold male while negative values indicate the shy male is more similar to the female than the bold male. Prior to the analysis, we *z*-transformed both relative similarity for the behavioural level and for the behavioural consistency for standardization.

We used the lme4 package (Bates et al. 2015) for LMMs. We used stepwise backward model simplification to fit the minimum adequate model. Partial R^2 with confidence level, CL, were calculated for explanatory variables using the approach suggested by Nakagawa and Schielzeth (2013), implemented in the r2glmm package (Jaeger 2016). For non-significant explanatory variables we report regression estimates and partial R^2 of the model before the term was dropped. Model assumptions were visually ensured through model diagnosis plots. For all analyses, female strength of preference was arcsine-square root transformed for normality. We had a priori decided to exclude side-biased females (N = 6) from preference analyses (Dosen and Montgomerie 2004; Hoysak and Godin 2007; Kniel et al. 2015; Schlupp et al. 1999; Schlüter et al. 1998; Williams and Mendelson 2010). By definition, a side-biased female showed contradictory preferences during the two test periods of a choice test. The removal of such inconsistent behaviour that appears random with respect to the presented males is crucial in order to remove females that express a preference not for the presented males but rather for (or against) a specific side of the choice chamber (e.g. because of a lack of motivation). Leaving such biased preference data in the data set would artificially increase the sample size and distort the actual preference pattern. On the other hand, removing side-biased females from the data set can lower the behavioural range represented in this study. As there are different approaches but no common agreement in how to handle side biases in mate choice trials, we performed all preference analyses twice, once with and once without removing side-biased females (N = 45). Although here we consider both approaches, we advocate the removal of clearly biased preference data from analyses and will therefore mainly focus on the presentation of preference analyses

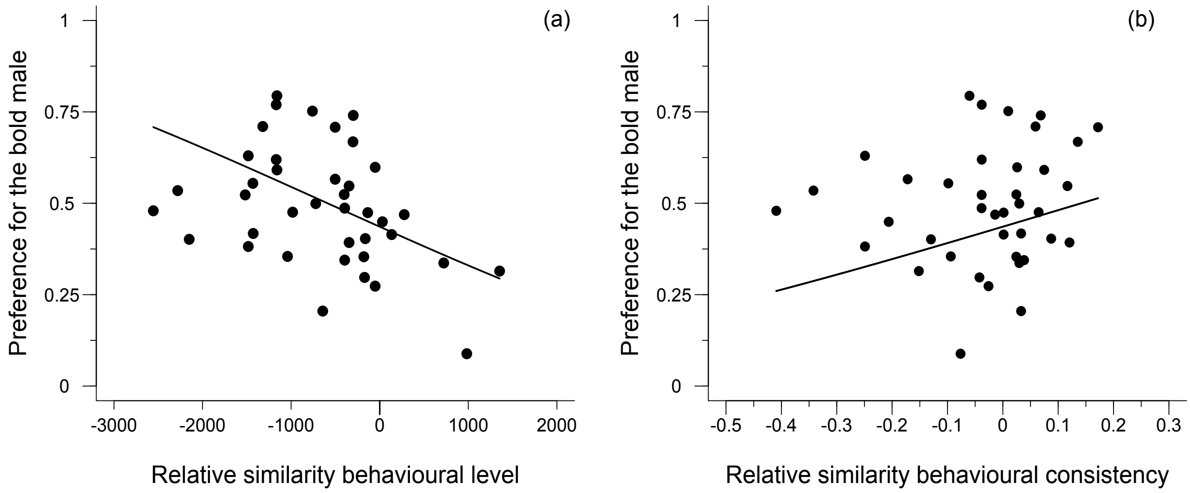

Figure 2. Female strength of preference for the bold male in relation to relative similarity in (a) the level and (b) the consistency of boldness. Positive similarity values indicate the bold male was more similar to the female than the shy male, negative values indicate higher similarity between the female and the shy male. Data visualization was done on the original data; strength of preference was arcsine-square root transformed for analyses.

performed without obvious side biases in the data.

RESULTS

Males and females were significantly repeatable in their boldness over the short term (LMM males: *R* = 0.507, SE = 0.110, CI = [0.246, 0.686], *N* = 48; LMM females: *R* = 0.604, SE = 0.097, CI = [0.380, 0.763], *N* = 45) and long term (LMM males: *R* = 0.463, SE = 0.113, CI = [0.233, 0.657], *N* = 48; LMM females: *R* = 0.557, SE = 0.111, CI = [0.311, 0.732], *N* = 42).

We found no general preference for either bold or shy males (mean preference for bold males: 46.5%, CI = [40.8, 52.1%]). Also, we did not detect a general preference for male consistency (mean preference for consistent males: 53.5%, CI = [47.8, 58.9%]).

Female strength of preference for the bold male decreased significantly with increasing relative similarity in the behavioural level (LMM: χ^2_1 = 10.572, *N* = 39, *P* = 0.001, coefficient ± SE (standardized) = −0.091 ± 0.026; R^2 = 0.242, CL = [0.056, 0.475]; Figure 2a). Further, female strength of preference increased with increasing relative similarity in behavioural consistency (LMM: χ^2_1 = 4.528, *N* = 39, *P* = 0.033, coefficient ± SE (standardized) = 0.058 ± 0.026; R^2 = 0.114, CL = [0.003, 0.341]; Figure 2b).

When performing preference analysis without the removal of side-biased females, we obtained similar results for female strength of preference for bold males (mean preference: 46.5%; 95% CI = [41.5, 51.6%]) and for consistent males (mean preference: 53.9%; 95% CI = 49.1, 59.1%]) with no deviation from random choice. However, in contrast to the analysis with removed side biases, relative similarity in the behavioural level tended to negatively influence female preference for bold males (LMM: χ^2_1 = 2.885, *N* = 45, *P* = 0.089, coefficient ± SE (standardized) = −0.043 ± 0.034; R^2 = 0.066, CL = [0.001, 0.258]) and relative similarity in behavioural consistency did not affect female preference (LMM: χ^2_1 = 2.279, *N* = 45, *P* = 0.131, coefficient ± SE (standardized) = 0.040 ± 0.025; $R^2 = 0.052$, CL = [0.000, 0.235]).

DISCUSSION

Both sexes of *P. Pulcher* showed consistent short- and long-term personality differences for boldness. We did not detect an overall agreement in female mating preference for either male level or consistency of boldness. However, we found dis-assortative female choice for the level of boldness. Also, female preference increased with similarity in behavioural consistency, suggesting assortative choice for consistency in boldness (when side-biased females were removed).

The dis-assortative preference for the behavioural level is contradictory to the results of most other mate choice studies testing for behavioural (dis-) assortment, which have mainly reported assortative mating preferences (e.g. Montiglio et al. 2016; Schuett et al. 2011b). At this point, we can only speculate about possible adaptive benefits of a dis-assortative preference. Behavioural dis-similarity could possibly increase within-pair behavioural and/or genetic compatibility (Schuett et al. 2010). Behavioural compatibility has primarily been discussed for bi-parental species when both parents perform similar parental activity, for instance offspring provisioning in some birds (Royle et al. 2010). In zebra finches, *Taeniopygia guttata*, for instance, similarity in the behavioural level has been shown to increase pair compatibility (e.g. Schuett et al. 2011a). However, when species perform various parental activities they might sometimes benefit from expressing a dis-assortative mating preference, facilitating role allocation during offspring care. In *P. pulcher*, parents typically divide the labour with one individual staying more with the offspring and the other defending the territory. Although sexual dimorphism in role specialization has been described for many cichlids (McKaye and Murry 2008; Neil 1984; Richter et al. 2010), sex roles might not be entirely strict in the species and may rather depend on the interplay between male and female personality. Itzkowitz et al. (2005) have shown that male and female parent convict cichlids, *Archocentrus nigrofasciatum*, changed their defence behaviour in response to the mate's body size, regardless of the sex. This result indicates that, in some species, parental role allocation may depend on the mate's behaviour and physiology rather than on the sex itself. Behavioural dis-similarity in boldness may facilitate labour division with the bolder individual defending the territory and the shyer individual staying with the young. Hence, dis-assortative mating for personality could sometimes lead to inverted parental care roles although this has not yet been investigated. Also, an increased genetic compatibility through dis-similarity could be possible if dis-assortative mating leads to heterozygote offspring that are more viable (Charlesworth and Charlesworth 1987; Dingemanse et al. 2004). For example, Marshall et al. (2003) showed a strong correlation between individual genetic diversity and a behavioural trait, song complexity, in sedge warblers, *Acrocephalus schoenobaenus*. Females chose to mate with males that increased offspring genetic diversity (Marshall et al. 2003). Seddon et al. (2004) found male heterozygosity to be correlated with territory size and song structure in male (but not female) sub-desert mesite, *Monias benschi*.

Further, we found assortative mate choice for the consistency of boldness. The few studies that have assessed the link between behavioural consistency and sexual selection found a positive relationship between consistency and reproductive success (Botero et al. 2009; Byers 2006) and a higher reproductive success of pairs matched for behavioural consistency (Schuett et al. 2011a). Schuett et al. (2011a) have shown that pairs matched for consistency raised foster fledglings in better body condition, indicating the possible mechanism driving assortment for behavioural consistency might be a higher efficiency in the provision of parental care.

Clearly, our study is limited by the correlative design, and does not allow us to specifically address the causality underlying the preference pattern. Further examinations using behavioural manipulations are now needed to decouple boldness from potentially correlated traits that might influence mate choice, to ensure the preference pattern we found is unequivocally related to individual behaviour. Moreover, it should be mentioned that our measure for behavioural consistency was derived from only two measurements. Here, we face a critical trade-off. While multiple measurements can lead to a change in behaviour caused by the number of times tested, e.g. through habituation or sensitization (Bell et al. 2009; Stamps et al. 2012), the measurement error is higher when only two tests are done. In this study, we tested individual responses towards unfamiliar predator animations, presented in a novel situation. Our measurement for boldness would probably have been affected by prior experience and familiarity with test conditions, making it difficult to obtain the same boldness measure in multiple tests. However, the strength of our study is that females could observe male boldness directly before mate choice trials while they were hidden behind one-way glass and partitions. This way, males could express their natural behaviour without being affected by the female's presence. A decoupling of observation and choice ensured female preference was not confounded by the presence of a predator.

Conclusions

In summary, we provide suggestive evidence that sexual selection may play a key role in the evolution of personality differences. Females showed a dis-assortative mating preference for the level of boldness and an assortative preference for the degree of behavioural consistency. Our results indicate mate choice for behavioural and/or genetic compatibility although only assessed in a correlative approach. Such a mating preference might improve parental care efficiency through facilitation of parental role allocation and/or might increase offspring fitness through genetic benefits. Noticeably, the handling of side biases significantly affected our results. While we found an effect of behavioural similarity in level and consistency when removing side biases, we could not detect these effects without removing side-biased females from the data. This discrepancy in results underlines the importance of taking the approach used into consideration when comparing the results of different mate choice studies. The handling of side biases in mate choice studies is not trivial and can largely affect experimental outcomes.

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CHAPTER 4

Do female rainbow kribs choose males on the basis of their apparent aggression and boldness? A non-correlational mate choice study

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(*Submitted manuscript*)

ABSTRACT

Consistent between-individual differences in behaviour, known as personality differences, are heritable and have consequences for individual survival and reproductive success. Therefore, it is likely that personality differences are not just under natural but also under sexual selection. Indeed, the recently developed idea that individuals choose their mate based on its personality finds empirical support. However, most studies on mate choice based on personality traits are correlative pioneering work and there is a paucity of experimental studies that test for causality by disentangling personality measures from other, potentially correlated traits that may be important during mate choice. Here, we tested female preference for the apparent level and consistency of either male aggression (measured as mean distance of approach towards an animated opponent, manipulated by locating males at a fixed distance) or male boldness (measured as activity under a simulated predation threat, manipulated using a gradient in ambient water temperature) in a bi-parental West African cichlid, *Pelvicachromis pulcher*. Females could observe the apparent behaviour of paired stimulus males and were allowed to choose between the two stimulus males in a subsequent dichotomous choice test. Using behavioural manipulations, we expected to confirm the results of our previous, correlative mate choice studies, where we found links between aggression/boldness and female preference. In the current study, we found no direct effect of male apparent behaviour on female choice, but an indirect effect such that female preference for the apparently bold male increased with increasing within-male pair contrast in their apparent level of boldness.

SIGNIFICANCE STATEMENT

Ever since Darwin introduced the conceptual framework of sexual selection, female mate choice has been studied extensively. However, the hypothesis that consistent differences in individual behaviours (known as personality differences) affect mate choice is relatively new. Correlative studies support this idea but provide only suggestive evidence. Here, we used behavioural manipulations to test for a causal link between female choice and personality differences in male aggression and boldness (both in level and consistency of behaviour) in a bi-parental cichlid fish, *Pelvicachromis pulcher*. Females could choose between two paired stimulus males, which were manipulated to differ in their level or consistency of aggression/boldness. Contrary to expectation, we found no overall female preference for male apparent behaviour, but female preference for the bold-appearing male increased the larger the differences between males in their apparent boldness.

INTRODUCTION

Consistent differences in individual behaviour, also known as personality differences, temperaments or coping styles (Gosling 2001; Réale et al. 2007), are moderately heritable (Ariyomo et al. 2013; Patrick et al. 2013) and have diverse fitness consequences (Ariyomo and Watt 2012; Dingemanse and Réale 2005; Smith and Blumstein 2008), including various effects on reproductive success (e.g. Cain and Ketterson 2013; Laubu et al. 2016; Schuett et al. 2011a; Spoon et al. 2006). Therefore, it seems likely that sexual selection may play a key role in shaping stable personality variation within populations (Schuett et al. 2010). Indeed, previous studies showed that personality traits, such as boldness (Godin and Dugatkin 1996; Scherer et al. 2017b), aggression (Kralj-Fišer et al. 2013; Ophir and Galef 2003), and exploratory behaviour (Pogány et al. 2018; Schuett et al. 2011b), affect mate choice in several species.

However, our understanding of how sexual selection shapes personality differences remains very incomplete. The empirical data collected so far are somewhat contradictory, in that they differ regarding the direction of effect found. For example, previous experimental studies have revealed either positive (Bierbach et al. 2013; Herb et al. 2003), negative (Ophir and Galef 2003), assortative (Kralj-Fišer et al. 2013) or no effects (Laubu et al. 2017) of male aggression on female mating preferences. The adaptive benefit of a given personality trait may not only vary between, but also within, species depending on prevailing environmental conditions (Cain and Ketterson 2013; Teyssier et al. 2014). Moreover, existing studies have often focused on potential effects of the level of expression of a given personality trait on female mating preferences while neglecting the importance of individual differences in the consistency of its expression (but see Scherer et al. 2018). Additionally, most previous studies on the potential role of individual personality on mate choice are correlative in nature (e.g. Laubu et al. 2017; Scherer et al. 2017b; Schuett et al. 2011b). To test for causality, we need behavioural manipulations that allow us to decouple the personality trait of interest from other, correlated traits that may influence mate choice.

In the current study, we therefore experimentally manipulated both the level and consistency of two personality traits, aggression (experiment 1) and boldness (experiment 2), in male rainbow kribs (*Pelvicachromis pulcher*) and tested for an effect of these apparent traits on female mating preferences. In the first experiment, we manipulated the level and consistency of apparent male aggression (measured as average distance of approach towards an opponent) by placing a male in a clear Plexiglas cylinder located at a fixed distance from an animated same-sex conspecific opponent presented on a nearby computer screen. The average distance from such an animated opponent is a good proxy for aggressiveness in our model species (Scherer et al. 2017a). In the second experiment, we manipulated the level and consistency of apparent boldness (measured as activity under a simulated predation threat) using a gradient in ambient water temperature to manipulate the activity level of the focal male. In fishes, locomotor activity correlates positively with ambient water temperature (Castonguay and Cyr 1998; Forsatkar et al. 2016). In both experiments, females were allowed to choose between two stimulus males that were manipulated to differ in their apparent level or consistency of male behaviour after prior observation of the apparent behaviour. In two pioneering personality-mate choice studies using the rainbow krib, we tested female preferences for the level/consistency of aggression (Scherer et al. 2018) and boldness (Scherer et al. 2017b) of potential male mating partners in a correlative context. We found both these male personality traits to be linked with female choice. Regarding female choice for male aggression, females preferred consistent over inconsistent males, but the level of male aggression did not affect female preferences (Scherer et al. 2018). Further, females showed a dis-assortative preference for the level of male boldness and an assortative preference for the consistency of this behavioural trait (Scherer et al. 2017b). In the current study, we aimed to test for a potential causal link between the aforementioned two male personality traits and the previously observed female mating preference patterns (Scherer et al. 2017b; Scherer et al. 2018). However, the preliminary results from our latter two correlative studies provide only suggestive evidence for the direction of selection. We therefore considered here alternative selection trajectories, that is, in both mate choice experiments we tested for a directional selection for or against the level and consistency of male behaviour (apparent aggression, apparent boldness) as well as for an effect of behavioural (dis-) similarity.

EXPERIMENTAL PROCEDURES

Test fish and holding conditions

Test fish were sourced from the University of Hamburg and local suppliers. All individuals were held in family groups under standardised holding conditions (100 - 200 l tanks, 25 ± 1°C, aerated and filtered water, weekly water changes). Fish were fed with *Artemia* spp. once daily on five days per week in their holding tanks. During experimentation, individuals were kept individually in separate, smaller tanks (25 x 50 x 25 cm) and fed daily to maintain constant conditions between trials. The latter tanks were equipped with an immersion heater, an internal filter and half a clay pot (4 x 8 x 8 cm) as shelter. Fish were uniquely marked on their dorsal side with Visible Implant Elastomers (VIEs, VIE-Northwest Marine Technology, Shaw Island, WA, USA) of different colours for individual identification. VIEs do not affect mate choice in our study species (Schuett et al.

2017). Before experiments, all individuals were measured for their standard length using ImageJ (Schneider et al. 2012) (mean ± SE standard lengths in experiment 1: males = 4.69 ± 0.02 cm, females = 3.98 ± 0.08 cm; experiment 2: males = 5.38 ± 0.04 cm, females = 4.30 ± 0.03 cm). For all experimental trials, the water in test tanks was changed after every trial; water level was 10 cm. Unless otherwise stated, the water temperature in experimental tanks was maintained at 25 ± 1°C. During experimental trials, no humans were present in the experimental room to avoid disturbances and trials were video-recorded using an overhead video camera (Sony HDR-CX405). In both experiments, males were habituated to being in a clear Plexiglas cylinder (diameter = 8.0 cm) twice for 10 min, once on two consecutive days before the mate choice trials. Individuals became readily accustomed to these cylinders and did not show any behavioural signs of distress whilst in the cylinders. Whenever possible, blinded methods were used.

Experiment 1: Female choice for male apparent aggression

In experiment 1 (February - May 2017), we assessed female mating preference for the apparent level (N = 48 preference assessments) and apparent consistency (N = 48 preference assessments) of male aggression (see '*Mate choice trials*' below). Before the mate choice trials, all males (N = 96) and females (N = 48) were tested for their natural aggressive behaviour twice with 48 h (± 15 min) between the two tests to determine the average level and consistency of behaviour (see '*Aggression test* below'). We tested for repeatability of aggression and for sex differences in the level and consistency of behaviour. Two days elapsed between the last aggression tests and the beginning of mate choice trials.

Aggression test

Male and female aggression was quantified separately and indirectly as the mean distance of approach (cm) towards a computer-animated, same-sex conspecific opponent, as outlined in Scherer et al. (2017a). Briefly, we introduced two focal individuals, matched for sex and body length, each into one of the two adjacent test tanks (visually isolated from each other) that were aligned to face a computer monitor on their shorter axis (Figure 1a, left panel, set-up with a grey background). After a 10-min acclimation period, focal individuals were exposed to a computer-animated and unfamiliar same-sex, same-size opponent (N_{males} = 9, $N_{females}$ = 7; size difference between the opponent and focal individuals < 3 mm) for a test period of 11 min. The simulated opponent was animated to swim back and forth horizontally along the width of a white computer screen (see Scherer et al. 2017a for details).

For all trials, the mean distance to the animated opponent was assessed for 10 min (we did not track the first minute of a video) using the tracking software Ethovision XT 11 (Noldus, Wageningen, The Netherlands). For each individual, we averaged the two mean distances to the opponent as a proxy measure of each fish's individual level of aggressiveness. Further, we assessed the behavioural consistency of each individual as inconsistency, that is, as the absolute value of the difference between its mean distance of approach towards the animated opponent in the repeated aggression tests (Scherer et al. 2018; Scherer and Schuett 2018).

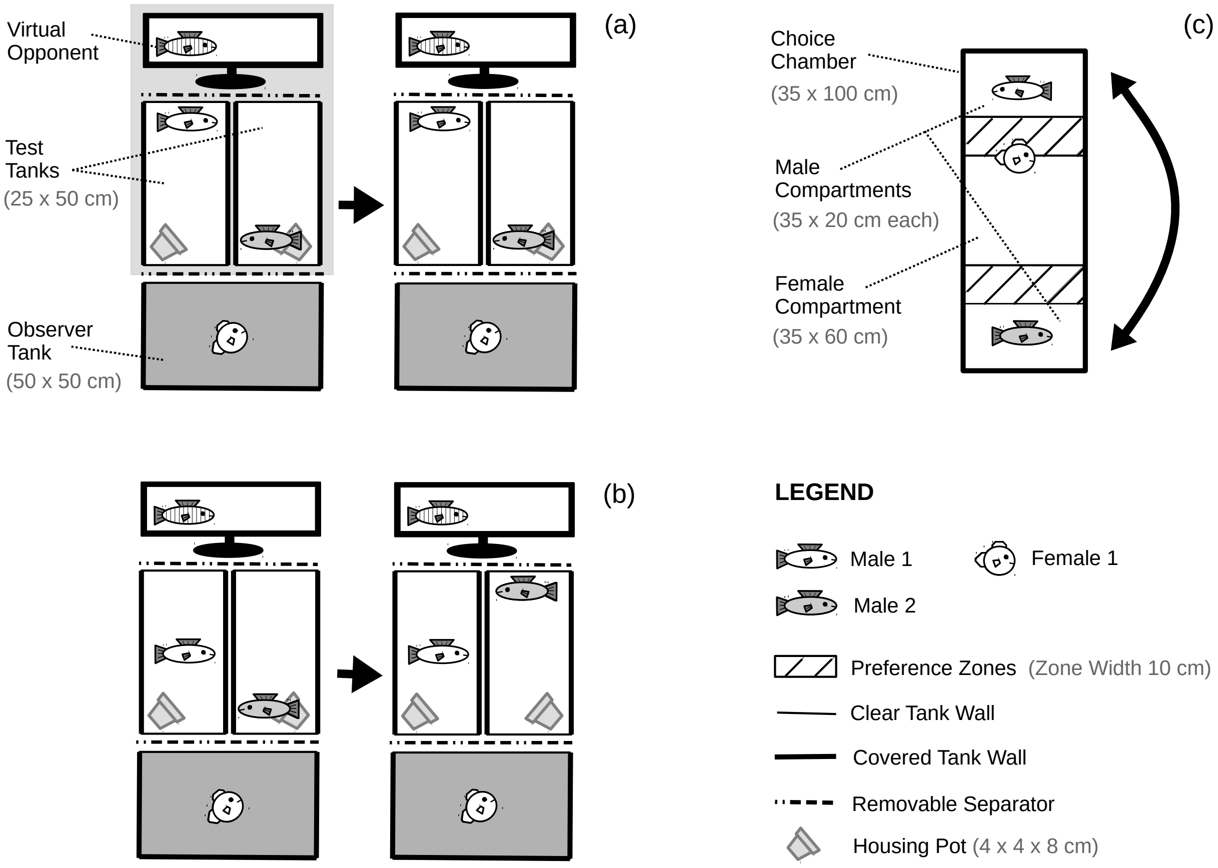

Figure 1. Top-view schematic of the experimental set-up for the (a-b) observation and subsequent (c) choice phase of mate choice trials. (a) The grey underlay (left panel) indicates the set-up for the aggression test. During two periods of the observation phase, we manipulated either (a) the level or (b) the consistency of male apparent aggression (mean distance towards an animated opponent). Throughout the observation and choice phase of mate choice trials, the paired males and the female observer (cylinder diameter 20 cm, placed in the centre of her tank) were placed in clear Plexiglas cylinders (not shown) so that their locations could be manipulated (as shown in panels a, b, c), with the exception that the female could freely swim within the test arena during the choice phase (c). During the aggression test, individuals were not fixed in their position. Arrows indicate (a-b) the direction of manipulation between the first and second observation periods within a mate choice trial or (c) individual males that were switched in their position between recording periods. Grey tank background indicates black tank surrounding (including a black lid). (a-b) During acclimatisation periods, removable separators visually separated the female observer tank, the stimulus males' tanks, and the computer monitor from each other. (c) During recordings, the compartments of the mate choice arena were physically separated (clear Plexiglas), during the acclimatisation they were additionally visually separated.

Mate choice trials

A mate choice trial consisted of an initial observation phase, followed immediately by a choice phase (Figure1). During the observation phase, a female was allowed to observe two stimulus males concurrently, with the males either differing in their apparent level of aggression (high vs. low) with consistency held constant (both fish consistent; Figure 1a) or differing in their apparent consistency of aggression (consistent vs. inconsistent; Figure 1b) with level of aggression held constant (intermediate aggression level for both males). Males were made to appear highly or less aggressive (or alternatively consistent or inconsistent) by manipulating their distance to an animated opponent moving on a computer monitor screen (Scherer et al. 2017a). The spatial position of each stimulus male was standardised by introducing them into separate clear Plexiglas cylinders (diameter = 8.0 cm) that were placed on the bottom of their test tanks either close to (4 cm), intermediate to (24 cm) or far from (44 cm) the animated opponent so as to simulate high, intermediate or low aggression level in the stimulus male, respectively. Consistency was manipulated by changing (inconsistent aggression) or maintaining (consistent aggression) the distance to the animated opponent between two periods of the observation phase as follows.

Following 10 min of acclimatisation to the experimental tanks (Figure 1a, b), we started the observation phase (22 min), which consisted of two consecutive 11-min periods. After the first observation period, we either changed or maintained the positions of the paired stimulus males according to their respective manipulation and allowed the fish to acclimatize for another 5 min. When testing female preference for the apparent level of male aggression, both males differed in their apparent level of aggression but showed the same apparent behavioural consistency. During both observation periods, one of the paired stimulus males was placed in close proximity to the virtual opponent (apparent high-aggression male) and the other one in further away from the opponent (the apparent low-aggression male) (Figure 1a). Conversely, when testing female preference for consistency, we altered the position of one of the two stimulus males relative to the animated opponent between the two observation periods (thus simulating inconsistency in his aggression level), while keeping the position of the other male (we sham-changed the position; thus simulating consistency in his aggression level) (Figure 1b). We placed the apparently inconsistent male close to the opponent during one observation period and far from the opponent during the other observation period (in randomised order). The apparently consistent male was placed at an intermediate distance from the opponent during both observation periods (Figure 1b). Thus, both stimulus males showed on average the same apparent level of aggression, but differed in their apparent behavioural consistency.

We also carried out control mate choice trials (for both level and consistency of male aggression, respectively) in a similar manner to that described above, except that the computer screen monitor did not display a virtual conspecific opponent but only a static white background during the observation phase (Figure 1a, b). The control trials were used to discount the possibility that differences in the manipulated distances between the observer female and the stimulus males per se could account for any subsequent female preference for either stimulus male. Hence, there were four different treatments for the observation phase: level, level control, consistency, and consistency control. Each female was tested for her mating preference four times, once in each treatment (resulting in N = 192 mate choice trials), with 48 h between consecutive mate choice trials and test order randomised. A difference in female preference between trials with the presence of a virtual opponent (level and consistency treatment) vs. absence of such opponent (level control and consistency control treatment) would validate that female preference is related to a male's distance to an opponent (i.e. apparent aggression) and not simply to male spatial position per se. However, such an effect was expected only if there was an effect of male apparent aggression on female mating preference in trials with a virtual opponent present during the observation phase. As this was not the case (see '*Results*'), an analysis of the control trials would be redundant and not informative. For completeness, we nonetheless present an analysis of the results for control trials in the Supplemental Material 1.

Immediately following the observation phase, the paired stimulus males (in their respective cylinders) and the observer female were transferred to a dichotomous mate-choice arena with the female in a central compartment (Figure 1c) to test for the female's mating preference (e.g. Dechaume-Moncharmont et al. 2011; Thünken et al. 2007). After a 10-min acclimatisation period, the free-swimming female was allowed to choose between the two stimulus males (constrained in cylinders) during a 22-min mate choice phase that was divided into two recording periods of 11 min each. In-between these two recording periods, we switched the two stimulus males in their position (followed by another 5 min of acclimatisation) to control for any potential female side bias (Poschadel et al. 2009; Scherer et al. 2017b).

Using Ethovision XT 11, we quantified female association time (time spent within 10 cm of either male compartment, hereafter preference zone; Figure 1c) for the two males over both recording periods (videos were analysed for 10 min, no tracking of the first minute) as a proxy for her mating preference (Jeswiet and Godin 2011). Female preference for a particular male was calculated as her total association time with that male divided by her total association time spent with both males (e.g. Poschadel et al. 2009; Schlupp et al. 1999; Schlüter et al. 1998). Females that showed an obvious side bias (i.e. spent > 80% of total association time in a particular preference zone over both recording periods) were excluded from statistical analyses (e.g. Dosen and Montgomerie 2004; Hoysak and Godin 2007; Kniel et al. 2015; Poschadel et al. 2009; Schlupp et al. 1999; Schlüter et al. 1998; Williams and Mendelson 2010). The numbers of females exhibiting such side bias were 15 (level), 5 (consistency), 8 (level control), and 8 (consistency control) for each treatment, respectively.

For each mate choice trial, the focal female was unfamiliar with the stimulus males (i.e. she had not seen them before). Stimulus males were not used more than once per day. We matched paired stimulus males for family, body size (standard length difference < 5%; mean ± SE = 0.216 ± 0.011 cm), natural aggression level (male difference in their distance to virtual opponent; mean ± SE = 1.42 ± 0.12 cm) and natural consistency of aggression (male difference in their consistency in distance to virtual opponent; mean ± SE = 1.559 ± 0.138 cm).

Experiment 2: Female choice for male apparent boldness

In experiment 2 (February - April 2018), we tested for an effect of the apparent level (N = 60 preference assessments) and apparent consistency (N = 60 preference assessments) of male boldness on female preference. Before mate choice trials, we tested all males (N = 71) and females (N = 60) for their boldness level twice, with 48 h (± 15 min) elapsed between tests (see '*Boldness test*' below). We tested for repeatability of boldness and for a sex difference in the level and consistency of behaviour. We started mate choice trials three days after the boldness typing was completed.

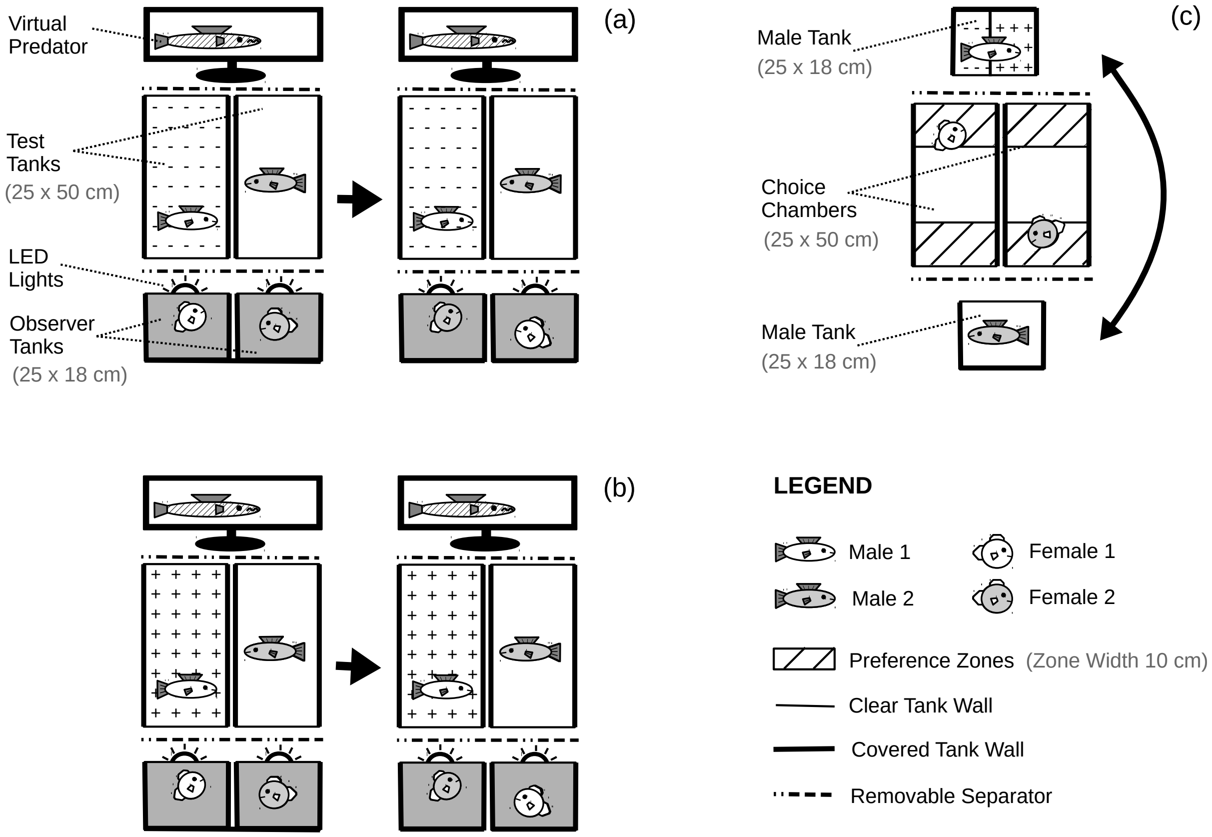

Figure 2. Top-view schematic of the experimental set-up for (a, b) the observation and subsequent (c) choice phase of mate choice trials in experiment 2. (a, b): The observation phase set-up was also used for boldness tests. The level and consistency of male boldness was manipulated using low (- -), medium (blank tank background) or high (+ +) water temperature. Arrows indicate that females were switched in their position between the first and second observation periods within a mate choice trial. Grey tank background indicates black tank surrounding (including a black lid). (a, b) During acclimatisation periods, the female observer tanks, the stimulus male tanks, and the computer monitor were visually separated from each other using removable separators. During the two observation periods, these separators were removed to allow full vision. (c) During the two test periods, males were kept in clear Plexiglas cylinders (diameter = 8 cm), positioned in the centre of their respective tanks, ensuring they remained visible to both females throughout the test phase. (c) During recordings, the tanks of the mate choice arena were physically separated (clear Plexiglas), during the acclimatisation they were additionally visually separated.

Boldness test

Male and female boldness was assessed as activity under simulated predation risk (total distance moved in cm, hereafter: APR) using animated individuals of *Parachanna obscura* (N = 4, mean ± SE standard length = 19.3 ± 0.3 cm), a naturally occurring sympatric fish predator of *P. pulcher* (Scherer et al. 2017a; Scherer et al. 2017b). Boldness tests and the subsequent calculation of the average level and inconsistency of behaviour were performed as described in the above *Aggression test* in '*Experiment 1: Female choice for male apparent aggression*'. Here, we used a 6 min test period and tracked individuals for 5 min (no tracking of the first minute). For all individuals, the boldness tests were carried out using a virtual predator specimen that focal fish had not seen before. Different to the above protocol, individuals were transferred to the test tanks without their housing pot. Further, we here aligned two observer tanks behind the test tanks (Figure 2a, b). We included observer tanks in order to perform the boldness tests and the observation phase of mate choice trials under the exact same conditions, minimizing effects that may interfere with the prediction of male behaviour exhibited during the observation phase of mate choice trials. During mate choice trials, observer tanks allowed the test females to view the apparent boldness of the stimulus males (see '*Mate choice trials*' below). During the boldness test, we introduced dummy observers (i.e. opposite-sex individuals, which were not further used in this experiment) into the observer tanks. Observer conspicuousness was reduced using reflecting lighting (LED lights; I-SY-TL5P01) and black plastic surrounding of the observer tanks (see Figure 2a, b).

Mate choice trials

Similar to the above experiment 1, females could choose between two paired stimulus males after prior observation of apparent male behaviour (Figure 2). During the observation, paired stimulus males were manipulated to appear either shy or bold to an observer female by placing them in tanks of different ambient water temperatures, whilst viewing a virtual fish predator moving on a nearby computer screen (Figure 2a, b). We used three different temperature treatments: low (21 ± 1°C), medium (25 ± 1°C) and high (29 ± 1°C). We created an apparent difference in male level of APR by keeping one of the paired stimulus males in medium water temperature (apparent moderate APR) and the other one in either low water temperature (apparent low APR) (Figure 2a) or high water temperature (apparent high APR) (Figure 2b). To test female preference for apparent consistency of male boldness, we performed a second mate choice trial using the same pair of males (48 h ± 15 min between repeated tests). During second mate choice trials, the apparent low-level (or high-level) male was now kept in high (or low) water temperature (apparent high APR) making it appear inconsistent, while the male being previously kept in medium water temperature was again concurrently presented in the same (medium) temperature treatment making it appear consistent. Testing female preference for male apparent inconsistency relies on the assumption that fish can remember individual conspecifics and their behaviour for at least two days. Fishes can identify and recognize con- and hetero-specifics for several weeks (reviewed in Bshary et al. 2001). Further, fishes remember social information and subsequently use this information to make reproductive decisions (Doutrelant and McGregor 2000; Ophir and Galef 2003; Schlupp et al. 1994; Witte and Godin 2010).

For efficiency of time, we tested two females simultaneously for their mating preferences (Figure 2). During the observation, each of the two females could only view one male at a time, we therefore divided the observation phase into two periods (6 min each) with the female observer tanks being switched in their position in between observation periods (Figure 2a, b) (10 min of acclimatisation before the first observation period, another 2 min of acclimatisation after female tanks were switched). Different to the above experiment 1, we did not change male treatments in between the two observation periods of a single mate choice trial (Figure 2a, b) (behavioural consistency was manipulated by performing a second mate choice trial, see above).

Similar to the mating preference test in experiment 1, the dichotomous choice test (Figure 2c) was performed with two test periods of 11 min, with the males being switched in their position between test periods; initial acclimatisation was 10 min, and acclimatisation before the second test period was 5 min. We assessed female preference and female side bias from the association time spent with the two males, as described for experiment 1. Side-biased females were excluded from preference analyses (excluded trials: N_{level} = 3, $N_{consistency}$= 4; resulting in N_{level} = 17 and $N_{consistency}$= 34 remaining trials).

Male treatment temperatures were induced in their individual housing tank 2 days prior to a mate choice trial (using submerged heaters), ensuring sufficient acclimation time (0.17°C change/hour) to the new temperature regime. Males did not show any signs of distress in response to temperature changes induced. To ensure that temperatures remained constant throughout experimental trials, all experimental tanks were covered externally with polystyrene (apart from tank sides needed to see through; see Figure 1). The room temperature was set to 20.0 °C using air conditioning. The water temperature in the female tanks (housing and experimental tanks) was maintained at 25 ± 1°C (equivalent to male medium temperature treatment). Male pairs were matched as closely as possible for natural inconsistency (mean ± SE; inconsistency = 193.28 ± 18.25 cm, within-pair difference in inconsistency = 107.44 ± 15.99 cm) and natural level (mean ± SE; average APR for all males = 684.21 ± 41.67 cm, within-pair difference of APR = 112.27 ± 18.93 cm moved) in APR. For male pair formation, we did not use the males showing the highest inconsistency values during boldness tests (N = 11) in order to efficiently manipulate male behaviour. Therefore, the number of males tested for boldness (N_{males} = 71) was higher than the number of males used to form male pairs (N_{pairs} = 30, N_{males} = 60). Males were further matched for size as closely as possible (mean ± SE difference in standard length = 0.11 ± 0.02 cm) and for family.

Our manipulations during the observation phase were effective in manipulating behavioural inconsistency: the inconsistent male showed significantly higher inconsistency than the consistent male in a pair (see Supplemental Material 2 and Supplemental Material 3 for method validation). Further, apparently consistent and inconsistent males did not differ in their apparent level of APR (see Supplemental Material 2 and Supplemental Material 3). For our manipulation of the behavioural level, we could confirm that males in the high temperature treatment showed higher APR compared to males in the medium temperature treatment. However, the low and medium temperature treatment males did not differ in their level of apparent APR. Therefore, we restricted the analysis of female preference for the apparent level to mate choice trials where males in the high vs. medium temperature treatment were presented (during the first mate choice trial); that is, all first trials containing low vs. medium temperature treatments were excluded (N = 30 preference assessments were excluded; resulting in N = 30 remaining preference assessments). Further, we removed all mate choice trials from the data set where the behavioural manipulation via ambient water temperature was not successful; i.e. in some mate choice trials, the apparently bold male showed a higher level of APR than the apparently shy male (excluded trials: N = 10 out of 30 trials; N = 20 remaining trials) or the apparently consistent male showed higher inconsistency than the apparently inconsistent male (excluded trials: N = 22 out of 60 trials; N = 38 remaining trials).

The apparent level of male APR (assessed as outlined in *Boldness Test*) exhibited during the first test period of the observation phase highly correlated with their apparent level during the second test period of the observation phase (linear mixed-effect model with male APR of the first test period as dependent variable, male APR of the second test period as fixed effect, and male ID as well as mate choice trial number (first or second) as random terms; $\chi^2_1 = 56.918$, $P < 0.0001$, intercept ± SE = 333.278 cm, coefficient ± SE = 0.720 ± 0.083 cm; $N = 120$ test periods of 60 mate choice trials). However, males were significantly more active (exhibited higher APR values) during the first test period of the observation phase compared to the second test period (linear mixed-effect model with male APR as dependent variable, test period (first or second) as fixed effect, and male ID and mate choice trial number (first or second) as random terms; $\chi^2_1 = 7.086$, $P = 0.0008$, intercept ± SE = 1051.01 cm, coefficient ± SE = -87.66 ± 32.61 cm; $N = 240$ test periods of 60 mate choice trials). Due to this behavioural difference, we did not use the average male APR over both test periods, but kept these two scores of an observation phase separately for analyses.

We calculated male apparent inconsistency (absolute difference in apparent APR between first and second mate choice trial) from the female's perspective. That is, we calculated two different scores of male apparent inconsistency, one score for each female of a particular mate choice trial. Each score was based on the very behaviour the female could observe (a female could only observe one male during the first test period and the other one during the second test period). For each male, we thus calculated one score of apparent inconsistency based on the two first observation phase test periods of each mate choice trial and the other score based on the two second observation phase test periods (the order in which a female could observe a male was consistent between the two mate choice trials).

DATA ANALYSES

General details

We performed all data analyses using R version 3.4.1 (R Core Team 2017). If not stated otherwise, LMMs (linear mixed-effects models) were fitted using the *lme4* package (Bates et al. 2014). Models were step-wise simplified using backward model selection starting with the least significant terms. Model assumptions were tested visually using residual and normal q-q plots. For fixed effects, we calculated partial R^2 with CL (confidence level) using the *r2glmm* package (Jaeger 2016). For insignificant fixed effects, R^2 was based on the model before the term was dropped. For all preference analyses, female mating preference was *arcsine square-root* transformed for normality. Predictor variables were z-transformed for standardisation using the *GenABEL* package (GenABEL project developers 2013). We checked for collinearity between predictors using Spearman rank correlations (Dormann et al. 2013).

Personality assessments in experiments 1 and 2

To test for consistent personality differences among individuals, repeatability of aggression and boldness was estimated for males (aggression: N = 96; boldness: N = 71) and females (aggression: N = 48; boldness: N = 60) separately by fitting

LMMs with ID as random term using the *rptR* package (Stoffel et al. 2017) (bootstrapping runs = 1000; permutations = 1000). We further tested for a sex difference in the level of natural aggression and boldness by fitting one LMM to each of the two behaviours (aggression: N = 288 of 144 individuals; boldness: N = 262 measurements of 131 individuals; two measurements per individual). We included sex as fixed term and ID as random term in the model. To test for a sex difference in inconsistency, we fitted an LM (linear model) per behaviour to male and female inconsistency (one inconsistency measure per individual: aggression: N = 144; boldness: N = 131) with sex as predictor variable.

Experiment 1: Female choice for male apparent aggression

We tested for a directional female preference for either apparent high-aggression or apparently consistent males by testing for a deviation from random choice (female preference = 0.50) using a null model approach. For each of the two opponent treatments (the level and consistency treatment) separately, we ran a null model with female preference for either the apparent high-aggression male (N = 33 mate choice trials) or the apparently consistent male (N = 43 mate choice trials) as the response variable. As random terms, we included male mate choice trial number and female mate choice trial number. A directional preference would be indicated if the observed mean with 95% CI (confidence interval) does not include 0.50.

To test whether male-female behavioural (dis-) similarity had an effect on female preference, we first calculated relative similarity in the level and consistency of aggression between the female and the two paired stimulus males (Scherer et al. 2017b; Scherer and Schuett 2018). More specifically, we subtracted the similarity (absolute value of the difference in average level or consistency, respectively) between the female and the respective high-aggression/consistent male of a pair from the similarity between the female and the low-aggression/inconsistent male. Thus, for positive values of relative similarity, the female's similarity with the high-aggression/consistent male was higher than the female's similarity with the low-aggression/inconsistent male and *vice versa*. We calculated relative similarity (for level and consistency, respectively) for both male natural behaviour and male apparent behaviour, resulting in four measures of male-female behavioural similarity; namely, relative similarity in the apparent level of aggression, relative similarity in the apparent consistency of aggression, relative similarity in the natural level of aggression, and relative similarity in the natural consistency of aggression.

For the level treatment, we fitted an LMM on female preference for the apparent high-aggression male (N = 33), with relative similarity in apparent level of aggression as fixed effect. We further included natural level of aggression and relative similarity in natural consistency as covariates. We did not include relative similarity in apparent consistency because this would be the same value for all individuals. As random terms, we included male and female mate choice trial number. For the consistency treatment, we fitted an LMM with female preference for apparently consistent males (N = 43) as the response variable and included relative similarity in apparent consistency as fixed effect and relative similarity in natural consistency and relative similarity in the natural aggression level as covariates. Again, we used the same random terms as above (male and female mate choice trial number).

Experiment 2: Female choice for male apparent boldness

We assessed female preference for the level of apparent male boldness using the respective first mate choice trial only. The second mate choice trial was not used to test female preference for the apparent level of behaviour to avoid an interference with previously received information gained during the first mate choice trial. Female preference assessed during the second mate choice test was used to test for an effect of male apparent inconsistency (difference in apparent APR between the first and second mate choice trial) on female mate choice.

We tested for a directional female preference for the apparently bold or apparently consistent male by fitting a null model (please see '*Experiment 1: Female choice for male apparent aggression*') to the data for each of these two target groups. As random term, we included male pair ID (each pair was used to simultaneously test two females).

To test whether female preference was affected by (dis-) similarity in the apparent level of behaviour, we fitted an LMM on female preference for the apparently bold male. As fixed effects, we included in the model relative similarity in the apparent and in the natural level of boldness and the relative similarity in the natural consistency of boldness. Also, we included the difference in the apparent level of aggression between the paired stimulus males as fixed effect to control for the behavioural contrast. We did not include relative similarity in apparent consistency because female preference for the apparent level was assessed prior to the consistency manipulation. As random term, we included male pair ID. For details on the calculation of relative similarities, see '*Experiment 1: Female choice for male apparent aggression*'. To test for an effect of (dis-) similarity in apparent consistency on female preference, we fitted a similar LMM. The dependent variable was female preference for the apparently consistent male and, as fixed effects, we included relative similarity in the apparent consistency, relative similarity in the natural consistency, relative similarity in the apparent level (average over the first and second mate choice trial), relative similarity in the natural level of boldness, and male difference in apparent consistency. Again, we included male pair ID as random term.

RESULTS

Experiment 1: Female choice for male apparent aggression

The natural levels of male and female aggressive behaviour were highly repeatable (males: R ± SE = 0.710 ± 0.050, CI [0.598, 0.795], N = 96 males; females: R ± SE = 0.684 ± 0.078, CI [0.511, 0.808], N = 48; Table 1). Further, males and females were equally aggressive, but males tended to be more consistent in their level of aggression compared to females (Table 1). We found no deviation from random female preference for either the level or consistency of male apparent aggression (Figure 3a; for statistics see Supplemental Material 4). Similarly, we found no effect of relative similarity in the level and consistency of apparent or natural aggression on female preference (Figure 3b, c; for statistics see Supplemental Material 5). However, there was a strong trend of female preference decreasing with increasing relative similarity

Table 1. Results for sex differences in the level (LMMs) and inconsistency (LMs) of aggression (mean distance to an animated same-sex and same-size opponent) and boldness (activity under simulated predation risk) in experiment 1 and 2. Significant effects highlighted in bold.

Experiment	Trait		N (trials)	Estimate ± SE (cm)	Test statistic	df	P-value	R^2 [CL]
1	Aggression	Level	288	Males: 13.49 ± 1.36 Females: 15.61 ± 1.11	$\chi^2 = 2.415$	1	0.120	0.014 [0.000, 0.053]
		Inconsistency	144	Males: 4.63 ± 0.72 Females: 5.94 ± 0.59	F = 3.313	1	0.071	0.023 [0.000, 0.094]
2	Boldness	Level	262	Males: 682.73 ± 56.32 Females: 411.88 ± 41.47	$\chi^2 = 21.296$	1	**< 0.0001**	0.129 [0.064, 0.210]
		Inconsistency	131	Males: 426.39 ± 62.52 Females: 227.55 ± 33.75	F = 1.877	1	0.173	0.014 0.000, 0.081]

in natural consistency of aggression (for statistics see Supplemental Material 5).

Experiment 2: Female choice for male apparent boldness

Male and female APR levels were highly repeatable (males: R ± SE = 0.637 ± 0.071, CI [0.470, 0.752], N = 71; females: R ± SE = 0.743 ± 0.060, CI [0.605, 0.840], N = 60; Table 1). Males were more active than females when the animated predator was present, though the sexes did not differ in behavioural consistency (Table 1). Females did not show an overall directional preference for the apparent level or consistency of male boldness (Figure 4a; Supplemental Material 4). Female preference for the apparently bold male significantly increased with within-male pair difference in the apparent level, such that the larger the difference in male apparent boldness, the greater was the female preference for the bold-appearing male (Figure 5; Supplemental Material 6). The difference in male apparent consistency did not affect female preference (Supplemental Material 6) and there was no effect of relative similarity in apparent/natural boldness (level and consistency) on female preference (Figure 4b-c; Supplemental Material 6).

DISCUSSION

In the present study, we found no effect of apparent male aggression on female choice. Similarly, apparent male boldness did not affect female choice, though female preference for the apparent bold male increased the larger the difference in the apparent boldness level between paired stimulus males. Further, there was no effect of natural male aggression/boldness on female choice in the two experiments. In experiment 1, female preference tended to decrease with increasing relative similarity in natural consistency of aggression. Males and females showed stable personality variation in both natural aggression and boldness. The sexes were equally aggressive, but males tended to be more consistent in their level of aggression compared to females. Also, males were more active than females, in the presence of

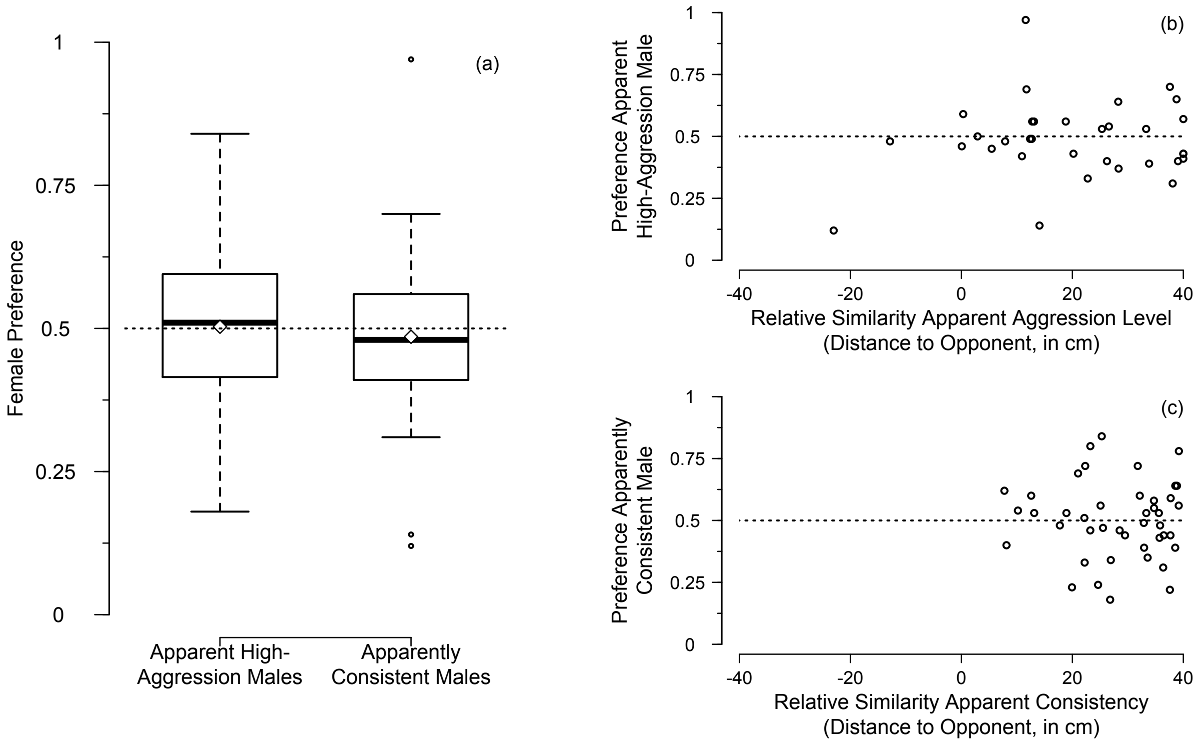

Figure 3. No female preference for apparent high-aggression or apparently consistent males (a) in experiment 1. Further, female preference was not affected by relative similarity in the apparent level (b) or consistency (c) of aggression. (a-c) Dotted line indicates random female choice. (a) Boxplot with 1.5 interquartile ranges, medians (-) and means (◊).

the animated predator, but the sexes did not differ in their consistency of boldness.

The absence of a direct effect of apparent behaviour in both experiments may be explained by at least two possible reasons. First, there may not be a causal relationship between the two behavioural traits and female choice. This further implies that the previously found association of natural male aggression (Scherer et al. 2018) and boldness (Scherer et al. 2017b) with female mating preference in the rainbow krib were caused by a correlation of the behavioural traits with other traits that are important during mate choice, such as colouration (Godin and Dugatkin 1996; Mafli et al. 2011). However, rainbow kribs provide extensive parental care and both aggression and boldness generally affect the style and amount of care given by parents in many species (reviewed in Chira 2014). In the rainbow krib, territorial breeding pairs guard their fry (direct care) and protect them from con- and heterospecific intruders (parental defence) for several weeks. Considering that such parental activities are crucial to the reproductive success of (bi-) parental species (e.g. Clutton-Brock 1991), it seems unlikely that there is no causal link between either of the two behavioural traits and mate choice in our model species.

Second, there may be a causal link between the two behavioural traits and female choice but our behavioural manipulations were not effective in revealing it. That is, in each of the two experiments presented here, we aimed to manipulate apparent male behaviour by addressing a single variable. In experiment 1, apparent male aggression was manipulated by fixing a male's average

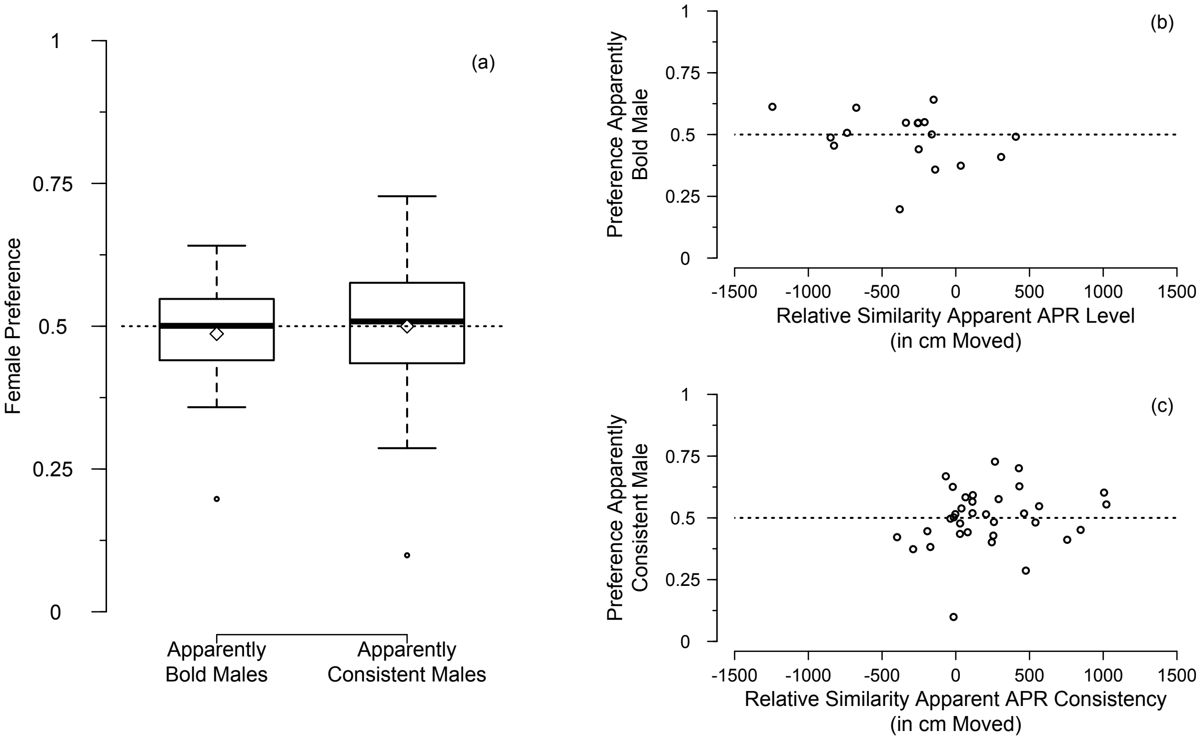

Figure 4. No female preference for apparently bold or consistent males (a) in experiment 2. Further, female preference was not affected by relative similarity in the apparent level (b) or consistency (c) of boldness. (a-c) Dotted line indicates random female choice. (a) Boxplot with 1.5 interquartile ranges, medians (-) and means (◊).

distance to an opponent. In experiment 2, apparent male boldness was manipulated using a gradient in ambient water temperature. Natural male aggression and boldness could still be expressed via fin postures or body colouration. Fish widely use their fins as a communication channel: aggressiveness is mediated using fin displays (e.g. Riebli et al. 2011) while clamped fins signal anxiety or discomfort (U. Scherer, personal observation). Rainbow kribs can quickly change their body colouration (U. Scherer, personal observation). The expression of aggression is usually associated with a strong boost in colourful body pigmentation, but when encountering a potential threat the fish can become very pale or dark in their overall colour appearance (U. Scherer, personal observation). However, the paired stimulus males used for a mate choice trial were always matched for natural behaviour (and family) as closely as possible. Hence, we would not expect much difference in fin postures or colouration between the two paired stimulus males, which in turn does not leave much room for mate choice based on natural male behaviour. In addition, a discrepancy in apparent and natural male behaviour may provide the female with conflicting information that counterbalance each other, resolving into random choice with regard to male behaviour.

In experiment 2, we found an effect of within-male pair contrast in the apparent level of boldness on female preference (the larger the difference the higher female preference for the apparently bold male), supporting the possibility that there is a causal link between boldness and female choice. The direction of effect indicates a general female preference for bolder males,

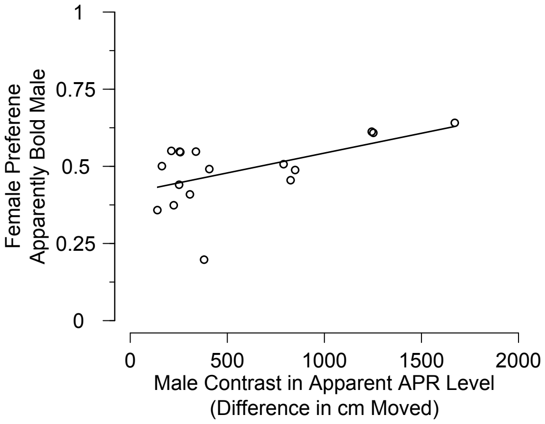

Figure 5. Effect of the within-male pair difference in the apparent APR level on female preference.

which may have several adaptive advantages. A higher level of boldness is associated with increased reproductive success (in the zebrafish, *Danio rerio*, and in the largemouth bass, *Micropterus salmoides*; Ariyomo and Watt 2012; Ballew et al. 2017), faster decision making (in three-spined sticklebacks, *Gasterosteus aculeatus*; Mamuneas et al. 2015), higher foraging success (in guppies, *Poecilia reticulata*; Dyer et al. 2008), larger body size (*Brachyrhaphis episcopi*; Brown et al. 2007), and a higher amount of parental provisioning in terms of fin digging (in the convict cichlid, *Archocentrus nigrofasciatus*; Zworykin and Budaev 2000). However, a directional preference seemingly conflicts with the result of our correlative mate choice study (Scherer et al. 2017b) where we found a dis-assortative female preference for male boldness (i.e. females preferred males of a dis-similar level of boldness). This discrepancy in the direction of effect found may be attributed to the fact that, in the present study, males were generally bolder compared to females. Thus, females were often more similar to the apparently shy male than to the apparently bold male (indicated by negative relative similarity values). In a mate choice trial with such a similarity distribution, a female would be expected to prefer the bold male irrespectively of whether the underlying preference pattern is of dis-assortative and directional nature. Thus, female preference for bolder males in the present study does not exclude dis-assortment. Possibly, there was not enough variation in relative behavioural similarity to detect dis-assortment (we often obtained negative values for relative similarity in the apparent behavioural level). Our result also indicates that the apparent behavioural contrast created between the paired stimulus males was not large enough to provoke a response in female preference (at least for part of the paired stimulus males). As mentioned above, possibly conflicting information between apparent and natural male boldness may necessitate a larger difference in the apparent behaviour in order to overpower other signals.

Notably, natural male aggression/boldness also did not affect female preference, though both traits have been shown to be important in mate choice of female rainbow kribs (Scherer et al. 2017b; Scherer et al. 2018). This is not surprising since we matched paired stimulus males for their natural level and consistency of behaviour (see above). The difference in their natural behaviour was rather low and should not further affect female preference here. However, in experiment 1, male-female similarity in natural consistency tended to affect female preference, which indicates that natural male behaviour could at least party be sensed by females (e.g. through body colouration or fin postures, see above).

Male and female natural aggressiveness did not differ from each other, which is consistent with the species' biology: both sexes are territorial. However, males tended to show higher behavioural consistency. Typically, in cichlids, it can be observed that males do a greater proportion of parental defence behaviours than females (which in turn provide more direct care) (Itzkowitz 1984; Lavery and Reebs 1994; McKaye and Murry 2008). Thus, male aggressiveness might be better conserved than female aggressiveness due to its higher relevance during parental care (reviewed in Schuett et al. 2010). Further, males showed higher activity levels when being exposed to an animated predator compared to females. This behavioural difference may be caused by a morphological difference between the sexes: male rainbow kribs are generally larger than females and activity was positively correlated with body size (U. Scherer, J.-G. J. Godin and W. Schuett, unpublished data).

Taken together, we did not find a direct effect of apparent male behaviour on female choice, but our data suggest that the contrast in apparent male behaviour either was not large enough or was not sufficiently distinct from natural male behaviour (at least in experiment 2) to reveal such an effect if one exists. Future studies may follow up on our behavioural manipulation via temperature gradient using a more refined methodology. This would be very interesting as we did not exploit the full range of our species' temperature tolerance, especially for mate choice trials testing for an effect of the apparent behavioural level wherein we only used a relatively low between-treatment contrast (which allowed us to manipulate behavioural consistency in subsequent mate choice trials). Furthermore, future directions may involve digital methods that provide a powerful tool to specifically manipulate behaviour. For example, Gierszewski et al. (2018) provide a software to create 3D fish animations where, among others, the swimming pattern of individual fish can be customized. Finally, we want to emphasize that the usage of behavioural manipulations is essential to answering current hypotheses regarding the evolution of stable personality variation. Although the precise manipulation of specific behavioural traits is not trivial, there are several promising options that are worth exploring.

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CHAPTER 4 - SUPPLEMENTAL MATERIAL 1

Validation of behavioural manipulations in experiment 1: Comparison of female preference in the treatment vs. control condition

A difference in female preference between opponent (level and consistency treatment) and respective no-opponent control trials (level control and consistency control treatment) would validate that female preference depended on a male's apparent distance towards the opponent and not on male position independent of an opponent's presence. We tested for a difference in female preference in the level and level control treatment by fitting an LMM to female preference for the apparent high-aggression male as the response variable (N = 73) with treatment (level and level control) as fixed effect. We further included female ID, male ID, male mate choice trial number, and female mate choice trial number as random effects. However, a significant difference would only be expected for a directional female preference for/against male apparent aggression. If female preference depends on behavioural (dis-) similarity, we would expect the treatment effect to depend on female behaviour. Therefore, we further included an interaction between the treatment and female aggression level into the above model. Please note, we were only interested in the interaction term (and the main effect 'treatment') and thus did not test for an effect of female aggression level alone. For the manipulation of behavioural consistency, we fitted an LMM to female preference for the apparent consistent male as the response variable (N = 83). Similarly to the above model, we included the treatment (consistency and consistency control) and female inconsistency in interaction with the treatment as fixed effects. We used the same random effects as above. There was no difference in female preference for male apparent aggression between opponent and respective no-opponent control trials (Table 1).

Table 1. Results of LMMs testing for an effect of relative similarities (rS) on female preference for male apparent aggression in experiment 1. Female preference was arcsine square-root transformed for analysis, intercepts and estimates are not back-transformed.

Response	Fixed effect	N (trials)	Intercept ± SE	Estimate ± SE	χ^2	df	P-value	R^2 [CL]
Preference apparent high aggression male	Treatment	73		Level control: 0.802 ± 0.030 Level: 0.777 ± 0.040	0.470	1	0.493	0.006 [0.000, 0.092]
	Treatment : female level		0.801 ± 0.030	-0.022 ± 0.037	0.342	1	0.559	0.005 [0.000, 0.087]
Preference apparent consistent male	Treatment	83		Consistency control: 0.792 ± 0.033 Consistency: 0.795 ± 0.037	0.009	1	0.923	0.000 [0.000, 0.060]
	Treatment : female consistency		0.795 ± 0.036	0.021 ± 0.033	0.438	1	0.508	0.004 [0.000, 0.076]

CHAPTER 4 - SUPPLEMENTAL MATERIAL 2

Validation of behavioural manipulations in experiment 2: Effect of the ambient water temperature on individual activity under simulated predation risk

In order to verify our behavioural manipulations via ambient water temperature, we tested for an effect of treatment temperatures on male apparent boldness (measured as activity under simulated predation risk, hereafter APR). We expected (a) male apparent level of behaviour to increase with increasing water temperature and (b) male apparent inconsistency of be higher for males that were exposed to different treatment temperatures (inconsistency treatment) compared to males that were kept in the same temperature regime throughout (consistency treatment). Further, (c) we expected the average behavioural level between males in the consistency vs. inconsistency treatment not to differ from each other.

(a) Validation for the manipulation of male apparent level of boldness

To test for an effect of the treatment temperature on male apparent level of boldness, we fitted an LMM to male APR shown during the observation phases of mate choice trials (N = 120 observation phase video recordings, obtained from 60 paired stimulus males in 30 mate choice trials). Please note that we obtained two measures of male APR per mate choice trial, one for each of the two recording periods (please see main text). We only included data obtained during the first mate choice trial because behavioural data obtained during the second mate choice test were not part of the assessment of female preference for male behavioural level. We included the treatment (low, medium or high water temperature; N = 30, 60, and 30 male videos, respectively) as fixed effect and the observation phase recording period (first or second) and male ID as a random effect in the model. For pairwise post-hoc group comparisons (P-value correction using the false discovery rate after Benjamin and Hochberg (1995)), we used the "*multcomp*" package (Hothorn et al. 2008). We found the level of male APR to be significantly affected by the test condition (LMM; χ^2 = 11.954, P = 0.003; N = 120) (Table 1; Supplemental material 3): male APR in the high-temperature treatment was significantly higher compared to the low- and medium-temperature treatments, which were not significantly different from each other.

(b) Validation for the manipulation of male behavioural inconsistency

To test the manipulation of male apparent inconsistency, we fitted an LMM to male inconsistency assessed during the observation phases of mate choice trials (N = 120 inconsistency measurements obtained from 60 males during the observation phases of 30 mate choice trials). We calculated two inconsistencies per male, each one based on either the two first or the two second recording periods of mate choice trials. These two values for male apparent inconsistency are meaningful because they reflect the female's perspective (a female could

Table 1. Results for post-hoc pairwise group comparisons of male level and inconsistency of APR shown in the different temperature treatments. Estimates represent the difference between the two treatments being compared.

Personality component	Pairwise comparison	N (videos)	Estimate ± SE (cm)	z-Value	P-value
Behavioural level	Low - medium temperature	120	121.70 ± 82.88	1.468	0.142
	Low - high temperature		-341.21 ± 95.70	-3.565	0.001
	Medium - high temperature		-219.52 ±+ 82.88	-2.649	0.012
Behavioural inconsistency	Consistency - inconsistency treatment	120	144.09 ± 57.39	2.511	0.012

observe a particular male either during the two first or during the two second observation phase recording periods). We included the treatment (inconsistency treatment: N = 60, consistency treatment: N = 60) as fixed effect and male ID as random effect in the model. We found male inconsistency to be significantly affected by the treatment (LMM; $\chi^2 = 5.994$, P = 0.014; N = 120) (Table 1; Supplemental Material 3). Males of the inconsistency treatment were significantly more inconsistent compared to males of the consistency treatment.

(c) Difference in the behavioural level between consistent and inconsistent males

We further tested for a difference in the apparent APR level between apparent consistent and inconsistent males. To do this, we fitted an LMM to the apparent APR level of both consistent and inconsistent males ($N_{consistent} = 30$ males, $N_{inconsistent} = 30$ males, $N_{total} = 240$ measurements, each male was scored four times: two mate choice trials, each one including a first and second observation phase recording period). We included male consistency treatment (consistent or inconsistent) as a fixed effect and male ID, observation phase recording period (first or second) and trial number (first or second mate choice trial) as random effects in the model. Apparent consistent and inconsistent males did not differ in their apparent APR (LMM; consistency treatment estimate ± SE = 893.21 ± 39.16 cm, inconsistency treatment estimate ± SE = 945.81 ± 95.38 cm, $\chi^2 = 0.895$, P = 0.344; N = 240).

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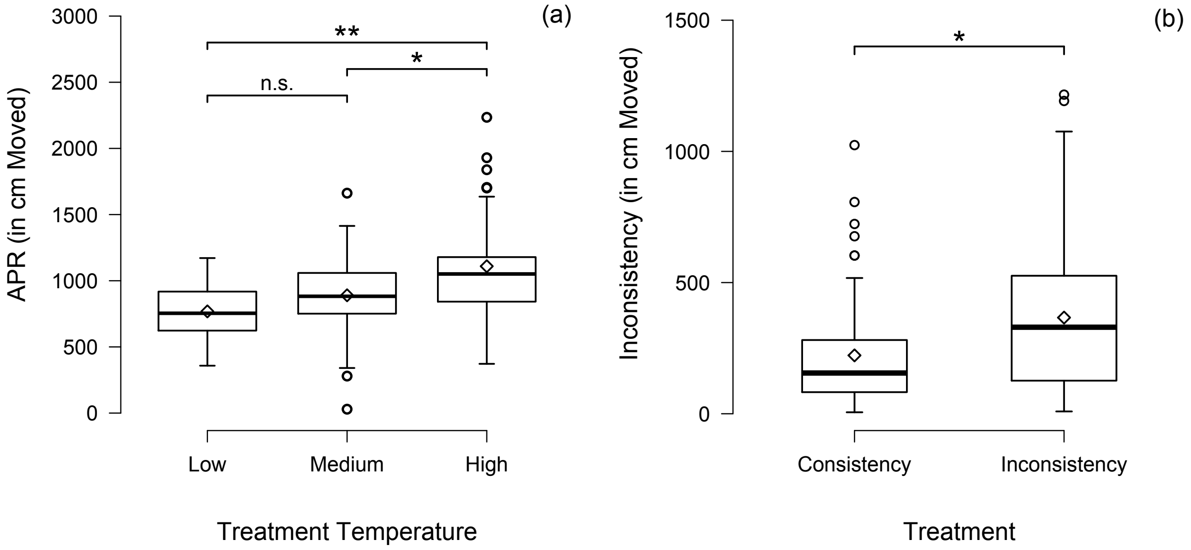
CHAPTER 4 - SUPPLEMENTAL MATERIAL 3

Validation of behavioural manipulations in experiment 1: Comparison of female preference in the treatment vs. control condition



Supplemental Material 3. Graphical illustration for the method validation in experiment 2 (see Supplemental Material 2 for the statistical analysis). Effect of the temperature treatments on the (a) level and (b) inconsistency of male APR. Significances for pairwise comparisons: "n.s." (not significant), "*" ($P < 0.05$), "**" ($P < 0.01$). Boxes with median (-) and mean (◊), whiskers represent 1.5 interquartile ranges.

CHAPTER 4 - SUPPLEMENTAL MATERIAL 4

Supplemental Material 4. Results for LMMs to test for a deviation from female random choice for the level and consistency of male apparent aggression (mean distance to an animated same-sex and same-size opponent) and boldness (activity under simulated predation risk) in experiment 1 and 2.

Experiment	Response	N (trials)	Female preference arcsine-square root transformed		Female preference back-transformed	
			Intercept ± SE	95 % CI	Intercept	95 % CI
1	Preference apparent high-aggression male	33	0.772 ± 0.031	[0.711, 0.865]	0.487 ± 0.001	[0.415, 0.579]
	Preference apparently consistent male (aggression)	43	0.789 ± 0.027	[0.721, 0.859]	0.503 ± 0.001	[0.435, 0.573]
2	Preference apparently bold male	17	0.771 ± 0.027	[0.715, 0.826]	0.485 ± 0.485	[0.430, 0.541]
	Preference apparently consistent male (boldness)	34	0.784 ± 0.022	[0.741, 0.827]	0.498 ± 0.001	[0.455, 0.542]

CHAPTER 4 - SUPPLEMENTAL MATERIAL 5

Supplemental Material 5. Results of LMMs testing for an effect of relative similarities (rS) on female preference for male apparent aggression in experiment 1. Female preference data were arcsine square-root transformed for analysis; intercepts and estimates are not back-transformed.

Response	Fixed effect	N (trials)	Intercept ± SE	Estimate ± SE	χ^2	df	P-value	R^2 [CL]
Preference apparent high aggression male	rS apparent level	33	0.777 ± 0.035	0.017 ± 0.031	0.288	1	0.591	0.036 [0.000, 0.246]
	rS natural level		0.777 ± 0.035	-0.006 ± 0.033	0.028	1	0.867	0.000 [0.001, 0.151]
	rS natural consistency		0.776 ± 0.034	0.034 ± 0.030	1.053	1	0.305	0.036 [0.000, 0.246]
Preference apparent consistent male (aggression)	rS apparent consistency	43	0.788 ± 0.024	-0.004 ± 0.024	0.031	1	0.861	0.001 [0.000, 0.117]
	rS natural consistency		0.788 ± 0.025	-0.047 ± 0.023	3.763	1	0.052	0.084 [0.001, 0.289]
	rS natural level		0.788 ± 0.024	0.037 ± 0.023	2.358	1	0.125	0.057 [0.000, 0.248]

CHAPTER 4 - SUPPLEMENTAL MATERIAL 6

Supplemental Material 6. Results of LMMs testing for an effect of relative similarities (rS) and male behavioural difference on female preferences for male apparent boldness in experiment 2. Female preference was arcsine square-root transformed for analysis, intercepts and estimates are not back-transformed. Significant effects highlighted in bold.

Response	Fixed effect	N (trials)	Intercept ± SE	Estimate ± SE	χ^2	df	P-value	R^2 [CL]
Preference apparent bold male	rS apparent level	17	0.771 ± 0.022	0.009 ± 0.029	0.101	1	0.751	0.006 [0.000, 0.296]
	rS natural level		0.771 ± 0.022	-0.001 ± 0.024	0.000	1	0.981	0.000 [0.000, 0.277]
	Difference apparent level		**0.771 ± 0.023**	**0.061 ± 0.023**	**5.638**	**1**	**0.018**	**0.295** **[0.025, 0.638]**
	rS natural consistency		0.771 ± 0.023	0.014 ± 0.023	0.337	1	0.562	0.021 [0.000, 0.333]
Preference apparent consistent male (boldness)	rS apparent consistency	34	0.784 ± 0.021	0.025 ± 0.022	1.288	1	0.256	0.038 0.000, 0.246]
	rS natural consistency		0.784 ± 0.021	-0.009 ± 0.023	0.153	1	0.696	0.000 [0.000, 0.161]
	Difference apparent consistency		0.784 ± 0.021	-0.042 ± 0.034	1.481	1	0.224	0.044 [0.000, 0.256]
	rS apparent level		0.784 ± 0.021	0.017 ± 0.023	0.525	1	0.469	0.016 [0.000, 0.195]
	rS natural level		0.784 ± 0.021	0.003 ± 0.023	0.015	1	0.902	0.000 [0.000, 0.145]

CHAPTER 5

No male mate choice for female boldness in a bi-parental West African cichlid, the rainbow krib (*Pelvicachromis pulcher*)

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ABSTRACT

Background. In many species, males have a lower reproductive investment than females and are therefore assumed to increase their fitness with a high number of matings rather than by being choosy. However, in bi-parental species, also males heavily invest into reproduction. Here, reproductive success largely depends on costly parental care; with style and amount of parental effort in several cases being associated with personality differences (i.e., consistent between-individual differences in behaviour). Nonetheless, very little is known about the effect of personality differences on (male) mate choice in bi-parental species.

Methods. In the present study, we tested male mate choice for the level and consistency of female boldness in the rainbow krib, *Pelviachromis pulcher*, a bi-parental and territorial West African cichlid. Individual boldness was assumed to indicate parental quality because it affects parental defence behaviour. For all males and females, boldness was assessed twice as the activity under simulated predation risk. Mate choice trials were conducted in two steps. First, we let a male observe two females expressing their boldness. Then, the male could choose between these two females in a standard mate choice test.

Results. We tested for a male preference for behavioural (dis-) similarity vs. a directional preference for boldness but our data support the absence of effects of male and/or female boldness (level and consistency) on male mating preference.

Discussion. Our results suggest female personality differences in boldness may not be selected for via male mate choice.

INTRODUCTION

Ever since Darwin, female mate choice has received extensive attention in sexual selection studies though male mate choice has long been overlooked (Arnaud and Haubruge 1998; Herdman et al. 2004). Males were assumed not to be choosy because of their low reproductive investment: the production of tiny sperm is less costly than the production of large oocytes allowing males to increase their fitness through a high number of matings rather than through choosiness (Bateman 1948; Kokko and Jennions 2003; Trivers 1972). However, male investment into reproduction is not as low as previously presumed; the production of sperm, especially when produced in a large amount, can actually be quite costly (Caballero-Mendieta and Cordero 2013; Olsson et al. 1997; Wedell et al. 2002). Furthermore, characteristics of the mating system can lead to an equal or even heavily male-biased reproductive investment, such as in bi-parental and sex-role reversed species (Cantoni and Brown 1997; Gross and Sargent 1985; Svensson 1988). In bi-parental species, both the male and the female parent provide intensive offspring care, which can be extremely costly (Marconato et al. 1993; Royle et al. 2012; Steinhart 2004). Under such increased costs of reproduction (e.g., time, energy and resources) not only females but also males are expected to be choosy (Bonduriansky 2001; Wong and Jennions 2003).

Male mating preferences have largely been tested for female traits that indicate fecundity (Bonduriansky 2001; Edward and Chapman 2011; Wang et al. 2017b); for instance body size (Olsson 1993), weight (Welke et al. 2012), fatness (Bonduriansky and Brooks 1998) or colouration (Amundsen and Forsgren 2001). Little is known about male mating preference for consistent differences in behavioural traits (e.g., aggression, boldness and explorative tendency), also referred to as personality differences, coping styles or temperaments (Schuett et al. 2010). To the best of our knowledge, the relatively few studies examining mate choice for personalities mainly consider female but not male mate choice (Kralj-Fišer et al. 2013; Montiglio et al. 2016; Scherer et al. 2017b; Schuett et al. 2011b; Teyssier et al. 2014; but see: Laubu et al. 2017). Male mate choice for personality traits is especially interesting in bi-parental species because (I) female behaviour can directly affect reproductive success through amount and style of parental care (reviewed in Chira 2014). For example, female exploratory behaviour increased the number of fledglings in blue tits, *Cyanistes caeruleus* (Mutzel et al. 2013) and aggressive Ural owl females, *Strix uralensis*, raised more offspring (Kontiainen et al. 2009). Further, (II) due to the mutual provision of care also the interplay between male and female personality has the potential to affect reproductive success (David et al. 2015; Laubu et al. 2016; Schuett et al. 2011a). Behavioural similarity in the level and consistency of exploratory behaviour positively affected fledgling condition of breeding pairs in the zebra finch, *Taeniopygia guttata* (Schuett et al. 2011a). In the convict cichlid, *Amatitlania siquia*, pairs that achieved post-pairing similarity on the proactive-reactive continuum could increase the number of their offspring (Laubu et al. 2016).

In the present study, we tested male mating preference for female boldness (probability to engage into risky behaviour; Wilson et al. 1994) in a territorial and bi-parental West African cichlid species, the rainbow krib, *Pelvicachromis pulcher*. Bi-parental cichlids commonly show a division of labour with specific sex roles during offspring care (e.g. Itzkowitz 1984; Lavery and Reebs 1994; McKaye and Murry 2008): typically, males do a greater proportion of the territory defence (vigilance behaviours, attacking intruders) females provide more direct offspring care (egg oxygenation, keeping the brood together, guidance to feeding grounds). Accordingly, one could hypothesize females to show a directional preference for male boldness (indicating high parental quality). In contrast, males could be expected to show no preference for female boldness because the benefit of a high behavioural level in female boldness during direct offspring care might be rather low. However, we previously tested female preference for male boldness in this species (Scherer et al. 2017b) and found a dis-assortative preference for the behavioural level and an assortative preference for the consistency of male boldness. Most importantly, (dis-) assortment indicates mutual mate choice because it results from a joint assessment process (Johnstone 1997). Thus, not only females but also males might choose their mate on the basis of its boldness in the rainbow krib. Such a preference pattern may ease parental care coordination through a facilitation of labour division with the bold parent performing territory defence and the shy parent providing direct offspring care. That is, roles might be based on individual behavioural predisposition rather than on the sex (Scherer et al. 2017b). Here, we used an experimental design similar to our female choice study testing for the male perspective: males were allowed to choose between two females that differed in their level and consistency of boldness (activity under simulated predation risk). Prior to mate choice, males were allowed to eavesdrop on female boldness. We measured individual boldness twice to determine behavioural consistency at the individual and population level. We hypothesized to find the same pattern as in our female choice study (Scherer et al. 2017b): consistent personality differences in both sexes and a mating preference for a dis-similar level and similar consistency of boldness (II). Alternatively, we considered female behaviour itself to be important (I): we tested for a general male preference for a high level and high consistency of female boldness. A high level of boldness could indicate high parental effort, while behavioural consistency could indicate the reliability of the trait and, therefore, the quality of the signal (Royle et al. 2010).

MATERIAL & METHODS

Study animals and holding conditions

All fish were kept at the Universität Hamburg (100 x 50 x 25 cm tanks, 26 ± 1°C water temperature, aerated and filtered water, weekly water changes, 12:12 h light:dark). Male *P. Pulcher* originated from the university breeding stock but due to a heavily skewed sex ratio females were largely bought as juveniles from external suppliers. Fish were held in shoals of approx. Forty individuals matched for sex and origin (university stock: matched for family; external suppliers: matched for supplier and batch). Fish were fed 5 days a week with live *Artemia spp.*

For the duration of experimental trials fish were transferred to individual housing tanks (25 x 50 x 25 cm; same holding conditions as above) and were fed 7 days a week ensuring equal conditions between successive trials. On experimentation days, fish were fed after the observations. All fish were measured for their standard length (males: mean ± SE = 5.03 ± 0.08 cm; females: mean ± SE = 3.97

± 0.04 cm) using ImageJ (Schneider et al. 2012) 5 days before experimental trials and were marked for individual identification using VIE tags (visible implant elastomers; VIE-Northwest Marine Technology, Shaw Island, WA, USA) four days before experimental trials. Such VIEs do not affect mate choic e in *P. Pulcher* (Schuett et al. 2017). After VIE tagging, all individuals resumed to normal behaviour without any signs of distress within less than 24 h.

General outline

Experimental trials were conducted during July and August 2017. Our work was approved by the German *'*Behörde für Gesundheit und Verbraucherschutz Hamburg*'* (permission number 52/16). We used a similar experimental set up and procedure as described in Scherer et al. (2017b). In order to assess the level and consistency of boldness, all males (*N* = 44) and females (*N* = 44) were tested for their boldness twice (please see *'Boldness test'*) with 3 days in between; successive trials were performed on the same time of day (±15 min). We always boldness typed two same-sex individuals simultaneously (with no visual contact between test fish). During female boldness tests, males were allowed to observe female behaviour. Male mating preference for the two females was tested directly after the female boldness test in a standard binary choice test (please see *'Mate choice trials'*). Such binary choice tests are a standard procedure being appropriate to predict mating preferences in cichlid fishes from the time spent near potential mates (Dechaume-Moncharmont et al. 2011; Scherer et al. 2017b; Thünken et al. 2007). Importantly, male choice was assessed after and not during predator exposure reducing potential effects of male anti-predator behaviour on male mate choice. Empirical studies have shown that fish observe (and remember) conspecific behaviour, and that they later use such information during their own social interactions with the previously observed individual (Bierbach et al. 2013; Doutrelant and McGregor 2000; Scherer et al. 2017b; Schlupp et al. 1994; Witte and Godin 2010). Male preference was assessed for each male once (*N* = 44). Each female dyad (*N* = 22) was used for two mate choice trials, once after each boldness test. We performed a complete water change in all experimental tanks before each boldness test/mate choice trial.

Boldness test

Boldness was measured as the individual activity under simulated predation risk (hereafter APR; Scherer et al. 2017a; Scherer et al. 2017b) via exposing individuals to a video animated photograph of a naturally occurring predator, the African obscure snakehead, *Parachanna obscura* (*N* = 4, mean ± SE standard length = 16.11 ± 0.38 cm). Predator specimen were animated to swim back and forth in front of a white background using PowerPoint (1 cm/sec) (Scherer et al. 2017a; Scherer et al. 2017b). Rainbow kribs decrease their activity in the presence of such animated predators compared to predator free control trials (Scherer et al. 2017a). Further, this response is comparable to the individual response towards a live *P. obscura* specimen (Scherer et al. 2017a).

To begin a boldness test, we introduced two same sex individuals into two neighbouring test tanks without visual contact (Figure 1a). For boldness tests of males, simultaneously tested males were randomly chosen but for boldness tests of females, simultaneously tested females were

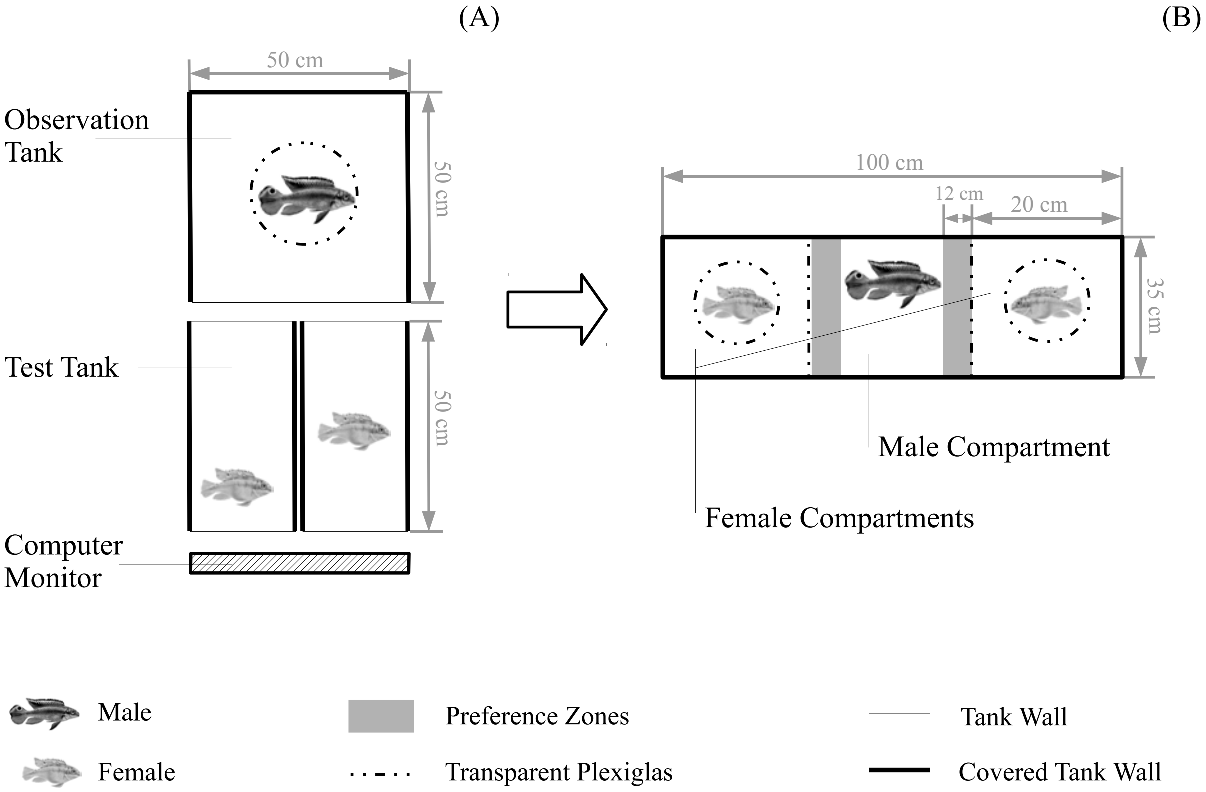

Figure 1. Experimental set-up for behavioural tests. Set-up for (A) the boldness test and for female boldness tests (B) the subsequent mate choice test. Water level for all tanks was 10 cm. Fish are not to scale.

matched for origin and standard length (size difference <5%; mean ± SE = 0.03 ± 0.01 cm). After an acclimation of 10 min, both test fish were allowed visual access to a computer monitor (UltraSharp U2412M 61 cm (24″); Dell, Round Rock, TX, USA) on one end of the two tanks through removal of a white separator. During this test period (duration = 11 min), we presented a randomly chosen animation of an unfamiliar predator specimen to both test fish. Also, we removed another white separator at the back of the two tanks for the duration of the test period allowing an observer fish (acclimated for 10 min) full view to both test fish and the predator animation (Figure 1a). For female boldness tests, we randomly chose a male observer not being related (non-sibling and non-familiar) to the females for further assessment of male mating preference (please see 'Mate choice trials'). For male boldness tests, we introduced a randomly chosen dummy female that was not part of this study. The observer fish was hidden in a cylinder (diameter = 20 cm), which was coated with one-way mirror foil. The usage of the cylinder ensured that both test fish were visible to the observer during the test period while the one-way foil reduced visibility of the observer to test fish (avoiding an impact of the observer on test fish behaviour). Observers did not show signs of distress when being kept in the cylinder. The observer tank was covered with black plastic plates, including a black plate covering the top to further decrease visibility of the observer to test fish. The sides of boldness test tanks were covered with white plastic plates to avoid disturbances and visual contact between test fish. Test periods were video-recorded from an above camera. After male boldness tests, test fish were returned to their individual housing tank. After female boldness tests, female test fish and the male observer were directly transferred to a mate choice chamber for assessing male mating preference (please see ‘Mate choice trials’).

Individual APR was assessed from the videos for all males and females as the total distance moved (cm) during 10 min (starting 1 min after the video start) using the animal tracking software Ethovision XT 11 (Noldus, Wageningen, The Netherlands). For all preference analyses, we used female APR of the boldness test that was observed by the respective observing male. For males, the individual behavioural level was assessed as the average APR of both boldness tests. Behavioural consistency was measured as inconsistency: the absolute value of the difference in the APR between the two boldness tests (Scherer et al. 2017b). Due to an error in three male boldness tests (each trial including two simultaneously tested males) we had to remove six males from the data set. The two females of each boldness test were classified into bold (mean ± SE APR = 1,037.27 ± 113.24 cm moved) and shy (mean ± SE APR = 577.18 ± 79.26 cm moved), depending on their level of boldness relative to each other; and into consistent (mean ± SE inconsistency = 268.5 ± 40.5 cm) and inconsistent (mean ± SE inconsistency = 565.0 ± 60.2 cm), depending on their inconsistency relative to each other. Bold and shy females significantly differed in their level of behaviour (mean ± SE within-dyad difference in APR = 196.2 ± 34.0 cm moved; average over both female boldness tests used) (linear mixed-effect model with female behavioural level (APR in cm) as dependent variable, female level classification as fixed effect, and female ID as well as female dyad ID as random effects; χ^2_1 =20.670, $P < 0.0001$, coefficient ± SE = 450.6 ± 85.9 cm moved; N = 88 measures of 44 females in 22 dyads, each female tested twice). Likewise, consistent and inconsistent females significantly differed in their behavioural consistency (mean ± SE within-dyad difference in inconsistency = 296.6 ± 42.9 cm) (linear mixed-effect model with female inconsistency as dependent variable, female consistency classification as predictor variable, and female dyad ID as random effect; χ^2_1 =16.434, $P < 0.0001$, coefficient ± SE = 296.6 ± 60.0 cm; N = 22 female dyads). Importantly, the behavioural classification into bold and shy (or consistent and inconsistent) was based on the behavioural contrast between the two females of a dyad and does not represent a global classification.

Mate choice trials

To begin a choice test, we transferred the two females and the observer male from the female boldness test tanks to the mate choice chamber (Figure 1b): the male was transferred to the male compartment in the middle and the two females were randomly assigned to the two female compartments of the choice chamber. All fish were allowed to acclimate for 10 min without visual contact (removable white separators) followed by a 12 min test period with full visual contact between the three compartments (separators removed). Thereafter, we repeated this test period with the females being switched between the two female compartments controlling for a potential male side bias. All fish were allowed to acclimate without visual contact for 5 min before starting the second test period (duration = 12 min) with full vision. During the whole duration of mate choice trials, females were kept in Plexiglas cylinders (inner diameter = 7.4 cm) to control for general female locomotor activity. Prior to mate choice trials, we habituated females to the cylinders: we kept them in the cylinder for 45 min per day, on three consecutive days (starting 5 days before experimental trials, no cylinder training during the two days before the start of experimental trials). The mate choice chamber was surrounded with white plastic plates. Both test periods were video-recorded from above.

Male preference was assessed from the videos using Ethovision XT 11. We tracked the association time (sec); i.e., the amount of time spent near the two female compartments (within a zone-width of 10 cm, hereafter preference zone; Figure 1b) during both test periods. Male preference for each female was then calculated over both test periods setting the total association time for one female into relation to the total association time for both females. This results into a preference score ranging from 0 (no time spent with a female) to 1 (100% of the total time spent with a female). Further, we calculated male side bias over the two test periods as the total amount of time spent in the left preference zone set into relation to the total amount of time spent in both preference zones (Scherer et al. 2017b). We *a priori* decided a male to be side-biased, when it spent more than 80% of the total association time in just one preference zone, regardless which female was there. Side-biased preference data were excluded from the analyses (e.g. Scherer et al. 2017b; Schlupp et al. 1999), (*N* = 3 excluded mate choice trials).

Data analyses

Data were analysed in R version 3.4.0 (R Core Team 2017). All data used for analyses are provided as supplemental information (Supplemental Material 1 and Supplemental Material 2) To assess behavioural consistency on population level, we calculated normal and adjusted (corrected for trial number) repeatabilities for male (*N* = 76 trials of 38 males) and female (*N* = 88 trials of 44 females) APR with 1,000 bootstrapping runs and 1,000 permutations using the *rptR*-package (Stoffel et al. 2017). Adjusted repeatabilities were calculated taking account for potential effects of habituation to the stimulus by adding the test trial number as fixed term (Bell et al. 2009; Nakagawa and Schielzeth 2010). Also, we tested for an effect of the boldness test trial number on APR in both sexes using paired *t*-tests.

In the present study, we tested for a linear function describing the relationship between male preference and female quality. Visual data inspection did not suggest a non-linear relationship. However, preference functions can also be shaped non-linearly (Reinhold and Schielzeth 2015; Wiegmann et al. 2013). We tested for a directional male preference for a high level or high consistency of female boldness by running two linear mixed-effects models (LMMs) on male mating preference. As response variable, we used either male preference for bold females (*N* = 35) or for consistent females (*N* = 35), respectively. Female ID and female dyad ID were included as random effects but no fixed effects were included (aka null model). Deviation from random choice would be revealed when the 95% confidence interval (CI) of the intercept does not include 50% (Scherer et al. 2017b). In a different mate choice study, we found female rainbow kribs to prefer males that show a combination of high behavioural consistency and high level of aggression (Scherer et al. 2018). Therefore, we also tested males for a mating preference for females showing both high level and high consistency of boldness (*N* = 18) through running a third null model, again, only including female ID and female dyad ID as random effects.

We tested for a male preference for behavioural (dis-) similarity by fitting an LMM on male preference for bold females (*N* = 35). We included relative similarity in the behavioural level and relative similarity in the behavioural consistency as fixed effects and female ID as well as female dyad ID as random effects. Following Scherer et al. (2017b), we calculated relative similarity as the male's similarity with the shy female minus the male's similarity with the bold female (for the level and consistency of behaviour, respectively). Similarity in the level and consistency of APR was calculated as the absolute value of the difference between the male and each of the two females, respectively. Relative similarity for the behavioural level was assessed using female behaviour shown during the respective male observation and average male behaviour shown over both boldness tests. Positive values of relative similarity indicate the male's similarity with the bold female is higher than its similarity with the shy female, vice versa, negative values show the male's similarity with the shy female is higher. Because male APR was strongly affected by the boldness test trial number (please see 'Results') we calculated two additional versions of relative similarity for the behavioural level; one version using male APR measured during the first boldness test, and another version using male APR measured during the second boldness test (again, we used female APR that was observed by the respective male, not the average female APR). We performed the above described model three times; all models were identical but contained different versions of relative similarity for the behavioural level (calculated using male APR assessed either during the first-, the second- or both boldness tests). Prior to analyses, male preference score was arcsine-square root-transformed for normality of residuals and predictor variables (relative similarity in the behavioural level and in behavioural consistency) were z-transformed for standardisation. We report partial R^2 with 95% confidence levels (CL), calculated using the *r2glmm*-package (Jaeger 2016), and estimates for all predictor variables. For insignificant predictors we report test statistics derived from the latest model incorporating the term (backward model selection). Model assumptions were visually checked. For an example code of our preference analyses please see (Scherer et al. 2018).

Differences in the behavioural contrast between the two females of a dyad (that is how much the females differed in their level and consistency of behaviour, respectively) are inherent in our experimental design because female dyads were only matched for size but formed randomly in regard to their behaviour. We tested for an effect of female behavioural contrast on male mate choice by fitting an LMM on male choosiness (absolute value of the difference in male strength of preference for the two females of a dyad) (*N* = 35). We included female within-dyad contrast in the behavioural level as well as female within-dyad contrast in behavioural consistency as fixed effects and female dyad ID as random effect. Female within-dyad contrast in the behavioural level did not affect male choosiness (LMM: χ^2_1=1.059, *P* = 0.303, coefficient ± SE (standardised) = −0.051 ± 0.048; *R*^{2} = 0.032, 95% CL [0.000–0.229]; *N* = 35). However, male choosiness increased with increasing female within-dyad contrast in behavioural consistency (LMM: χ^2_1=5.703, *P* = 0.017, coefficient ±SE (standardised) = 0.137 ± 0.054; *R*^{2} = 0.202, 95% CL [0.027–0.451]; *N* = 35). Also, we tested whether male choosiness (*N* = 35) was affected by the relative similarity in the level (male average APR used for calculation) and consistency of boldness by fitting another LMM on male choosiness, including relative similarity in the behavioural level (absolute value) as well as relative similarity in the behavioural consistency (absolute value) as fixed effects and female dyad ID as random effect. We did not detect any effects of relative similarity in the level (LMM: χ^2_1=1.441χ^2_1=1.441, P = 0.230, coefficient ± SE (standardised) = −0.063 ± 0.047; R^2 = 0.042, 95% CL [0.000–0.250]; N = 35) or consistency (LMM: χ^2_1=2.114, P = 0.146, coefficient ± SE (standardised) = 0.078 ± 0.051; R^2 = 0.067, 95% CL [0.000–0.291]; N = 35) of boldness on male choosiness.

Even though there was not much suggestive evidence for the behavioural contrast within dyads affecting male choosiness, we performed all preference analyses (testing for a directional preference and testing for male choice based on (dis-) similarity) with the full data set and with a smaller data set where the trials with low behavioural contrast were removed. For the directional preference analyses, we removed all preference data derived from mate choice trials where female within-dyad behavioural contrast in the level (N = 15 trials removed) or consistency (N = 17 trials removed) was less than 200 cm moved. When testing for male preference for high level and high consistency females we used the sum of the behavioural contrast in level and consistency as threshold (again 200 cm moved; N = 24 trials removed). Similarly, for our preference analysis regarding mate choice for (dis-) similarity, we removed all mate choice trials with relative similarity in level and consistency (absolute values added up; N = 11 trials removed) being less than 200 cm moved. The threshold of 200 cm was chosen to ensure a minimum behavioural contrast without decreasing N (and the statistical power) too much (please note, we obtained qualitatively the same results when other thresholds were chosen).

RESULTS

We found female (LMM: R = 0.673, SE = 0.090, 95% CI [0.448–0.808], N = 44) but not male APR (LMM: R = 0.000, SE = 0.088, 95% CI [0.000–0.273], N = 38) to be repeatable over the two boldness tests. However, when controlling for the trial number, both females (LMM: R = 0.707, SE = 0.082, 95% CI [0.515–0.837], N = 44) and males (LMM: R = 0.338, SE = 0.137, CI = [0.086–0.590], N = 38) were significantly repeatable in their boldness. Male boldness significantly increased from the first (mean ± SE APR = 498.2 ± 57.8 cm moved) to the second (mean ± SE APR = 1265.8 ± 89.4 cm moved) boldness test (paired t-test: t_{37} = −8.861, P < 0.0001, N = 38; Figure 2a). Although less pronounced, also female boldness increased from the first (mean ± SE APR = 703 ± 86.8 cm moved) to the second (mean ± SE APR = 911.4 ± 116.3 cm) boldness test (paired t-test: t_{43} = −2.650, P= 0.011, N = 44; Figure 2b).

Male preference for bold females did not show a deviation from random choice (mean preference: 0.497; 95% CI [0.432–0.562], N = 35) (Figure 3a). Although male choosiness increased with increasing behavioural contrast in female consistency (please see ‘Data analyses’), male preference for consistent females did not deviate from random choice (mean preference: 0.519; 95% CI [0.446–0.593], N = 35) (Figure 3b). Likewise, male preference for females that were both bold and consistent did not deviate from random choice (mean preference: 0.478; 95% CI [0.409–0.548], N = 35). Furthermore, we did not detect any effects of relative similarity in the level or consistency of APR on male mating preference for bold females (Table

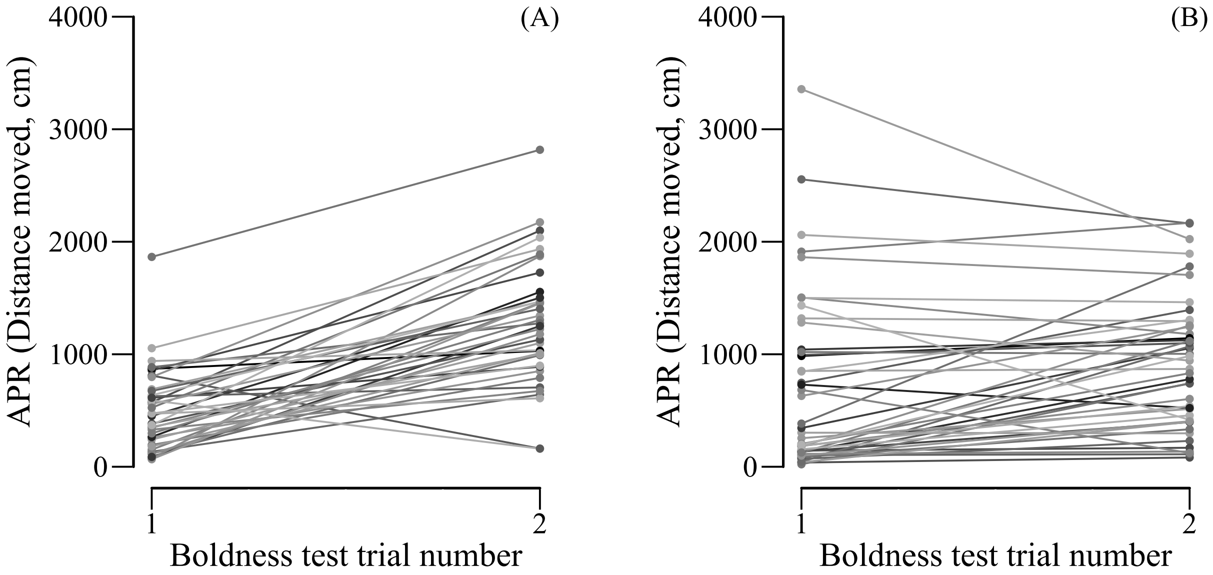

Figure 2. (A) Male and (B) female APR (activity under simulated predation risk) over two boldness tests.

1, Figure 4). Also, when performing our preference analyses considering the effect of the boldness test trial number on male APR, and using a smaller data set where mate choice trials with a low behavioural contrast in absolute or relative female behaviour were removed, we did not detect significant effects of female boldness on male mate choice (Table 1).

DISCUSSION

In the present study, we did not detect any effects of the level or consistency of female boldness on male mating preference. Females showed stable personality differences in our measure of boldness (with and without controlling for the trial number). Male boldness was only repeatable when

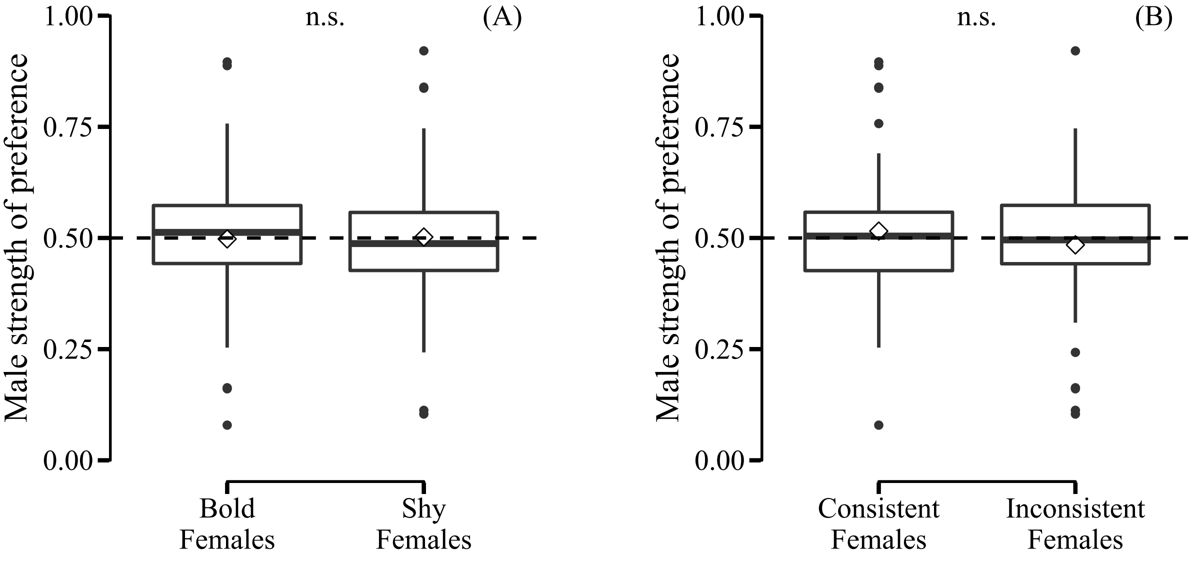

Figure 3. Male preference for the (A) level and (B) consistency of female boldness. Boldness was measured as APR (activity under simulated predation risk; in cm). Boxplots with 1.5 interquartile ranges, mean (◊) and medians (-); n.s. D non-significant. No deviation from random choice (male strength of preference = 0.50, dashed line) detected

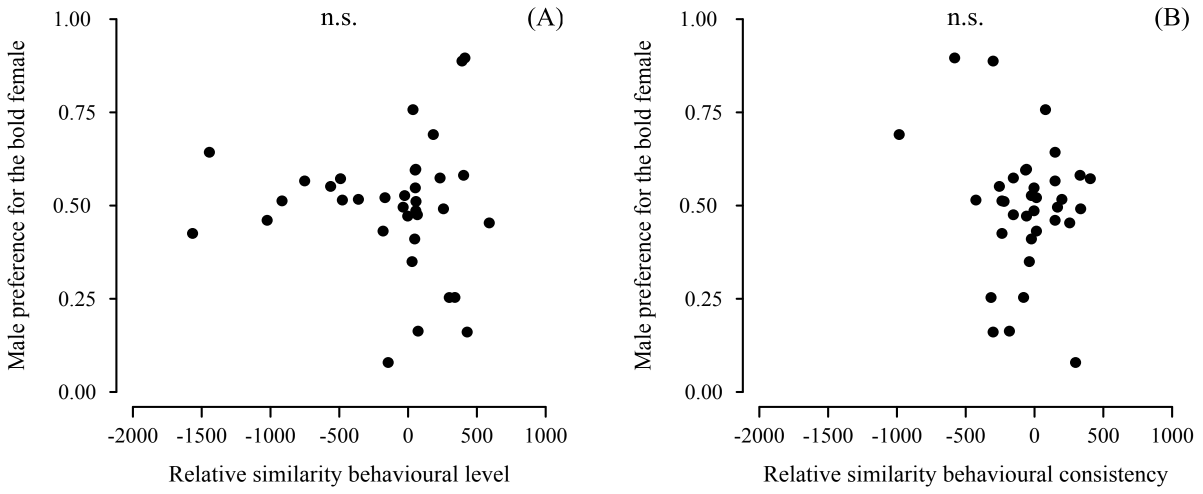

Figure 4. Male preference for the relative similarity in the (A) level and (B) consistency of female boldness. Boldness was measured as APR (activity under simulated predation risk; in cm). Relative similarity in average male APR and female APR observed directly before mate choice. Positive values of relative similarity indicate the male's similarity with the respective bold female of a female dyad was higher than its similarity with the respective shy female. Vice versa: negative values indicate the male was more similar to the shy female than to the bold female. No significant effects detected (n.s., non-significant).

controlling for the trial number. In both sexes, the level of boldness increased with the number of times being tested.

To the best of our knowledge, this is the first study testing for male mate choice in *P. pulcher*. Therefore, there is no empirical evidence for the existence of male mate choice in our target species. We had expected male mate choice in *P. pulcher* because there is strong empirical evidence for male choice in a closely related sister species with very similar breeding ecology, *P. taeniatus*. Male *P. Taeniatus* choose their mate based on relatedness (Thünken et al. 2011), colouration (Baldauf et al. 2011) and ornamentation (Baldauf et al. 2010). Other recent studies found no support for male mate choice in bi-parental species, namely the convict cichlid, *Amatitlania siquia* (Laubu et al. 2017) and the zebra finch, *Taeniopygia guttata* (Wang et al. 2017a; Wang et al. 2017b).

In our study, a biological explanation for the lack of male choice could be the existence of typical sex roles during parental care with the male engaging into parental defence behaviours and the female providing direct care. Under this constellation, the effect of female boldness on the performance of maternal care duties may be rather low and might therefore not be very important during mate choice. But, in three-spined sticklebacks, *Gasterosteus aculeatus*, boldness and sociability are negatively correlated (Jolles et al. 2015); possibly suggesting that boldness might also indirectly affect maternal care. Further, a strict parental role allocation defined solely by the sex would not be in line with the female preference for male boldness: female rainbow kribs preferred males of a dis-similar level of boldness indicating parental roles are rather determined by the mate’s personality than by the sex (Scherer et al. 2017b). That is, the shy individual would perform a greater proportion of the direct care while the bold individual would specialize on defence behaviours.

Beside the above biological explanation for the lack of male preference for boldness in our study there are several potentially confounding effects that might have

Table 1. Summary of LMM analyses of male choice for (dis-)similarity in boldness. Boldness was measured as APR (activity under simulated predation risk; in cm). All LMMs had female ID and female dyad ID as random effects. Models were based either on the full data set or a reduced data set where all mate choice trials with relative similarity in the level and consistency of behaviour smaller than 200 cm moved (absolute value of the sum) were removed.

Data set	Male behavioural level	Dependent variable	Fixed effects	Estimate ± SE	χ^2	*P*	R^2 [CL]	N
Full data set	First boldness test	Male preference	Relative similarity level	-0.037 ± 0.032	1.311	0.252	0.038 [0.241, 0.146]	35
			Relative similarity consistency	-0.041 ± 0.032	1.618	0.203	0.046 [0.000, 0.257]	
	Second boldness test	Male preference	Relative similarity level	0.012 ± 0.032	0.139	0.709	0.004 [0.000, 0.155]	35
			Relative similarity consistency	-0.041 ± 0.032	1.618	0.203	0.046 [0.000, 0.257]	
	Mean	Male preference	Relative similarity level	-0.007 ± 0.032	0.059	0.808	0.002 [0.000, 0.146]	35
			Relative similarity consistency	-0.041 ± 0.032	1.618	0.203	0.046 [0.000, 0.257]	
Low behavioural contrast removed	First boldness test	Male preference	Relative similarity level	0.0103 ± 0.033	0.095	0.757	0.003 [0.000, 0.320]	15
			Relative similarity consistency	-0.078 ± 0.041	3.247	0.072	0.163 [0.001, 0.556]	
	Second boldness test	Male preference	Relative similarity level	-0.004 ± 0.032	0.016	0.901	0.001 [0.000, 0.314]	15
			Relative similarity consistency	-0.015 ± 0.032	0.235	0.628	0.017 [0.000, 0.355]	
	Mean	Male preference	Relative similarity level	-0.016 ± 0.045	0.127	0.722	0.005 [0.000, 0.219]	24
			Relative similarity consistency	-0.056 ± 0.044	1.529	0.216	0.064 [0.000, 0.346]	

affected our results. First, fish were kept in same-sex family groups. Such holding conditions are required in order to avoid territorial and aggressive behaviour as well as individual differences in reproductive experience. However, these holding conditions led to a heavily biased male sex ratio possibly causing a decline in choosiness. That is because any skew increases direct costs of mate sampling, intra-sexual competition and the risk of ending up unmated for the sex in greater number (Dechaume-Moncharmont et al. 2016; Kokko and Mappes 2005). A biased sex ratio can cause a lack of experience needed to discriminate between potential mates (Bailey and Zuk 2008; Dukas 2005; Hebets 2003; Rosenqvist and Houde 1997). Both male inexperience and the male-biased sex ratio in stock tanks might have caused the lack of male discrimination between potential mates in the present study. On the other hand, similarly inexperienced females kept under identical holding conditions did show mating preferences for boldness in our female mate choice study (Scherer et al. 2017b).

Second, females were paired up to dyads randomly in regard to their behaviour. This resulted in female dyads being differently contrasted in their level and consistency of boldness, including very poorly contrasted female dyads. However, a removal of poorly contrasted female dyads from the data set did not affect the result of our preference analyses.

Third, in the present study, male repeatability of boldness was unexpectedly low and was only present when accounting for the trial number. Former measurements of male boldness in this species (Scherer et al. 2017b) revealed much higher behavioural stability suggesting a possible noise (e.g., caused by the strong increase of male boldness from the first to the second boldness test) in male behavioural data of this study. If male preference for female boldness is related to male boldness (as expected) a noise in male personality assessment could mask a potential preference for (dis-) similar females.

The increase in the level of male and female boldness with the number of times being tested may indicate habituation to the stimulus (Bell et al. 2009; Nakagawa and Schielzeth 2010). That is, individuals might get less sensitive to the predator stimulus with time because they have learned from former experiences that it does not pose a threat to them. We emphasize caution in repeatedly using a behavioural assay to measure personality traits. For instance, boldness can hardly be tested over and over again using the same stimulus and procedure without confounding the assessment with habituation. This poses an issue that is important, yet difficult to tackle. Effects of habituation are hard to get rid of; but could be reduced, for example, by modifying the stimulus used between successive measurements and controlling for the number of times being tested in between-individual comparisons.

CONCLUSIONS

Comparing our results to our female mate choice study for boldness (Scherer et al. 2017b) we discover two main differences. First, male behavioural repeatability strongly decreased in the present study compared to our female choice study. Although we are not certain about the reason for the low male repeatability this might be (at least partly) attributed to a follow-up effect of behavioural habituation to the stimulus. Second, while female mate choice was affected by an interplay between male and female behaviour, we did not detect any effects of female boldness on male choice. Sexual selection might act differently on male and female boldness because boldness may affect male (territory defence) but not female (direct offspring care) parental care behaviour. On the other hand, (dis-) assortment shown by the females indicates mutual mate assessment (Johnstone 1997). The causality in male–female preference mismatch remains unclear. Therefore, further research is needed to test how the interplay between parental personalities and offspring care is linked to an individual's fitness in order to shed light on the driving evolutionary mechanisms that form stable personality variation in bi-parental species.

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CHAPTER 6

Personality differences, parental care behaviour and reproductive success in the bi-parental rainbow krib (*Pelvicachromis pulcher*)

Ulrike Scherer and Wiebke Schuett

ABSTRACT

Although the existence of personality difference is well supported we have yet to fully understand the mechanisms that shape the distinct polymorphism we can observe. Recently, sexual selection has been proposed to play a major role in generating and maintaining stable personality variation. In the proposed framework, parental care has been suggested as a likely key element mediating between personality differences and reproductive success in (bi-) parental species. In the present study, we tested how personality differences (level and consistency of boldness), parental care (activity in the presence of an intruder, time spent near the intruder and time spent attending the brood; level and consistency of behaviour, respectively), and reproductive success (likelihood to reproduce, number - and size of offspring) are interconnected in a bi-parental cichlid. Breeding pairs were formed either assortatively and/or dis-assotatively with respect to both their pre-determined level and inconsistency of boldness. Pairs that were formed dis-assortatively with respect to their pre-determined level of boldness had a higher number of offspring but pairs that showed assortment during parental care had larger offspring indicating a reproductive trade-off. Pre-determined personality (level and consistency) did not predict parental care. We propose, pairs might get similar during the breeding mediating between the two trade-offs. However, positive assortment for few but large offspring and dis-assortment for many but small offspring may represent distinct investment strategies allowing individuals to adapt to varying environmental pressures. We found no effect of behavioural consistencies in parental care behaviours on reproductive success. Implications of our results are discussed.

INTRODUCTION

Personality differences (consistent differences in individual behaviour) are widely spread throughout the animal kingdom (reviewed in: Bell et al. 2009; Gosling 2001; Kralj-Fišer and Schuett 2014; Stamps 2007). Broadly, they comprise boldness, exploration, activity, aggressiveness, and sociability (Réale et al. 2007). But although the existence of personality differences is empirically well supported (Bell et al. 2009), we are still trying to understand their adaptive significance. Conceptual frameworks cover several explanations including physiological constraints of flexibility (Bell and Aubin-Horth 2010; Wolf and McNamara 2012), social advantages of behavioural consistency (Dall et al. 2004; McNamara et al. 2009; Wolf and McNamara 2012), state-dependent feedback loops and life-history trade-offs (McNamara and Houston 1996; Schuett et al. 2015), fluctuating selection (Dingemanse et al. 2004; Dingemanse and Réale 2005), and frequency-dependent selection (Roff 1998; Wolf and McNamara 2012). Behavioural traits cannot only affect survival but also reproductive success (reviewed in Smith and Blumstein 2008). Thus, sexual selection may play a major role in understanding why individuals consistently differ in their behaviour; although the potential importance of sexual selection has long been overlooked (Schuett et al. 2010).

A first conceptual framework of how sexual selection may generate and maintain distinct behavioural variation was proposed by Schuett et al. (2010). As a potential key element linking personality differences to reproductive success the authors suggest parental care behaviour: parental personality differences affect offspring care behaviour, which in turns affects reproductive success (Schuett et al. 2010). Parental care, i.e. the investment into offspring after fertilisation (Gross and Sargent 1985), has most commonly been described in mammals and birds; but it can be observed in many taxa, including fishes, insects, amphibians, reptiles, and spiders (Royle et al. 2014). Depending on the species and their offspring's demands, parental activities comprise various behaviours (e.g. nest building, brooding, egg/offspring attendance, or the provision of food (Smiseth et al. 2012)), but as different as they are, all parental care behaviours are meant to increase reproductive success via improving offspring growth and/or survival (Royle et al. 2014). For example, in burying beetles, *Nicrophorus vespilloides*, parental efforts in food provisioning (carcass manipulation and regurgitation) increased both offspring growth and survival (Eggert et al. 1998). Thus, in care giving species, parental care is a strong driver of individual fitness. In turn, amount and style of care provided can be affected by personality differences (reviewed in Chira 2014).

Personality differences may affect parental care behaviour (and, ultimately, reproductive success) in a directional manner or via effects of the behavioural compatibility between mates (Schuett et al. 2010). Directional effects result from a correlation between the behavioural expression and parental care quality, e.g. the more aggressive the parents are the more effort they are expected to put into defending the offspring, and as a result, the more offspring are expected to survive. In western bluebirds, *Sialia mexicana*, high aggression males put more effort into defending the nest (Duckworth 2006) and in Tengmalm's owls, male nest defence predicted offspring survival (Hakkarainen and Korpimäki 1994). Alternatively, behavioural compatibility between the two individuals of a breeding pair may increase reproductive success through better cooperation and coordination of parental behaviours (Schuett et al. 2010). For example, in birds, positive assortment has been reported to increase the synchronisation of feeding rates, which in turn increased reproductive success (Mariette and Griffith 2012; van Rooij and Griffith 2013). However, also dis-assortment may be beneficial. For example, if there are different parental activities (e.g. direct care vs. defence behaviours) it may facilitate the specialisation into distinct roles reducing stress and conflict and easing the efficient coordination of care (Royle et al. 2010; Scherer et al. 2017b; Schuett et al. 2010).

Not only the behavioural level (magnitude in the average level of the expression) but also the consistency of behaviour (variance or range of the expression) varies between individuals and may have far-reaching fitness effects itself (Dingemanse et al. 2010; Ioannou and Dall 2016; Schuett et al. 2010; Stamps et al. 2012). Similar to the above mentioned effects of the behavioural level, behavioural consistency could affect parental care (and reproductive success) in a directional manner (e.g. if constant engagement into offspring protection is beneficial) or via compatibility (e.g. it may ease the negotiation over the amount of care provided: flexible negotiation vs. fixed bids) (Royle et al. 2010; Schuett et al. 2010).

However, we know only very little about the effects of personality differences (level and consistency of behaviour) on parental care behaviour and reproductive success. Particularly, potential effects of between-individual differences in behavioural consistency have rarely been considered (but see Schuett et al. 2011). Also, we lack studies that apply a more holistic approach testing the interconnection between personality differences, parental care, and reproductive success; instead of focussing on just a partial aspect, i.e. on the effect of personality differences on parental care or on the effect of parental care on reproductive success.

Here, we present a laboratory study where we tested the interplay between personality differences, parental care behaviour and reproductive success in the rainbow krib, *Pelvicachromis pulcher*, a bi-parental and territorial West African cichlid. We assessed personality differences in aggression (time spent near an animated opponent matched for size and sex, Scherer et al. 2017a) and boldness (activity under simulated predation risk, Scherer et al. 2017a). We then created breeding pairs that varied in their behavioural contrast regarding the level and consistency of boldness. Aggression and boldness are thought to affect parental care behaviour in this species because both traits affect how individuals react to potential brood predators (con- and heterospecific) (Scherer et al. 2017b; Scherer et al. 2018). For the pairing, we concentrated on boldness (and not aggression) as this behaviour seemed of higher relevance during previous mate choice studies (Scherer et al. 2017b; Scherer et al. 2018). During the breeding we assessed the level and consistency of parental care in the presence of a computer-animated intruder (male conspecific, female conspecific, predator) as the activity in the presence of the intruder (boldness), amount of time spent near the intruder (aggression), and time spent attending the brood. Reproductive success was assessed as the likelihood of successful reproduction, and the number and size of offspring produced. As outlined above, we followed up two main hypotheses: we expected pre-determined personality differences, parental care, and reproductive success to be interconnected either by directional effects or by effects of behavioural compatibility (assortment vs. dis-assortment), i.e. for each of the two selection trajectories (directional vs. compatibility) we expected (I) pre-determined personality differences to predict parental care behaviour, (II) parental care behaviour to predict reproductive success, and, consequently, (III) personality differences to predict reproductive success. As a basic assumption underlying our investigation we expected individuals to show stable personality variation.

METHODS

Study animals and holding conditions

Rainbow kribs are territorial cave breeders that care for their fry for several weeks, as can be observed in their sister species *P. taeniatus* (Thünken et al. 2010). Breeding pairs perform different parental activities including direct care (keeping the brood together, guiding them to feeding grounds, cleaning of eggs) and offspring defence (territory patrolling, protection from con- and heterospecific intruders) (U. Scherer, personal observation).

Test fish were obtained from a house breed at the University of Hamburg (males) and local suppliers (females). Fish were maintained in same-sex groups matched for family (males) or origin (females), respectively. Holding conditions were standardized; all fish were kept in 100l (100 x 50 x 25 cm) and 200l tanks (100 x 50 x 50 cm), maintained at 25±1°C via air conditioning, and a 12:12 hours light:dark period (sun rise 6.30am, sun set 6.30pm). Tanks were endowed with a layer of sand (thickness approx. 1 cm), an internal filter and one or two plastic plants (approx. 10 x 20 cm). Water changes were done once a week and fish were fed with live *Artemia* spp. daily. For breeding pairs that had eggs of fry in their tank we increased the number of water changes to three times a week and reduced the amount of water being changed to approx. 30% (no water changes before parental care tests). Four days before the experiment started, individuals were transferred to individual housing tanks (25L, 50 x 50 x 25 cm, same holding conditions as above) and measured for their standard length using ImageJ (Schneider et al. 2012) (mean ± SE standard length, males: 5.42 ± 0.05 cm, females: 4.39 ± 0.04 cm). Individuals used in the experiment carried unique VIEs for individual identification (Schuett et al. 2017).

Personality tests

Before the breeding, all males (N = 54) and females (N = 54) were personality typed twice, each for their aggressive behaviour and their boldness. The first aggression and boldness tests were performed with 24 h ± 15 min elapsed between tests (test order was randomised) and three days later the two behavioural tests were repeated in the same scheme.

Aggression tests were performed following Scherer et al. (2017a). In short, individual test fish were exposed to a computer-animated, same-sex, same-size opponent that was presented on a nearby computer screen (Figure 1a) for 11 min. The opponent (a photograph) was animated to swim back and forth in front of a white background (see Scherer et al. 2017a for details on animation production). We assessed individual aggressiveness as the total amount of time spent within 12 cm distance to the animated opponent (hereafter 'interaction zone') from videos (tracking software: Ethovision XT 11, Noldus, Wageningen, The Netherlands; tracking duration 10 min; no tracking of the first minute). For all trials, we used conspecific specimen that the test fish had not seen before (N_{males} = 9, $N_{females}$ = 7; size difference between the opponent and test fish ≤ 2 mm).

Boldness tests were performed in the same manner as the aggression tests and are described in detail elsewhere (Scherer et al. 2017a). Different to the above protocol for the aggression test, we presented an animated predator specimen (*Parachanna obscura*, a naturally sympatric occurring predator of *P. pulcher*) on the computer screen. Further, we here assessed individual activity (our measure of boldness) as the total number of squares visited

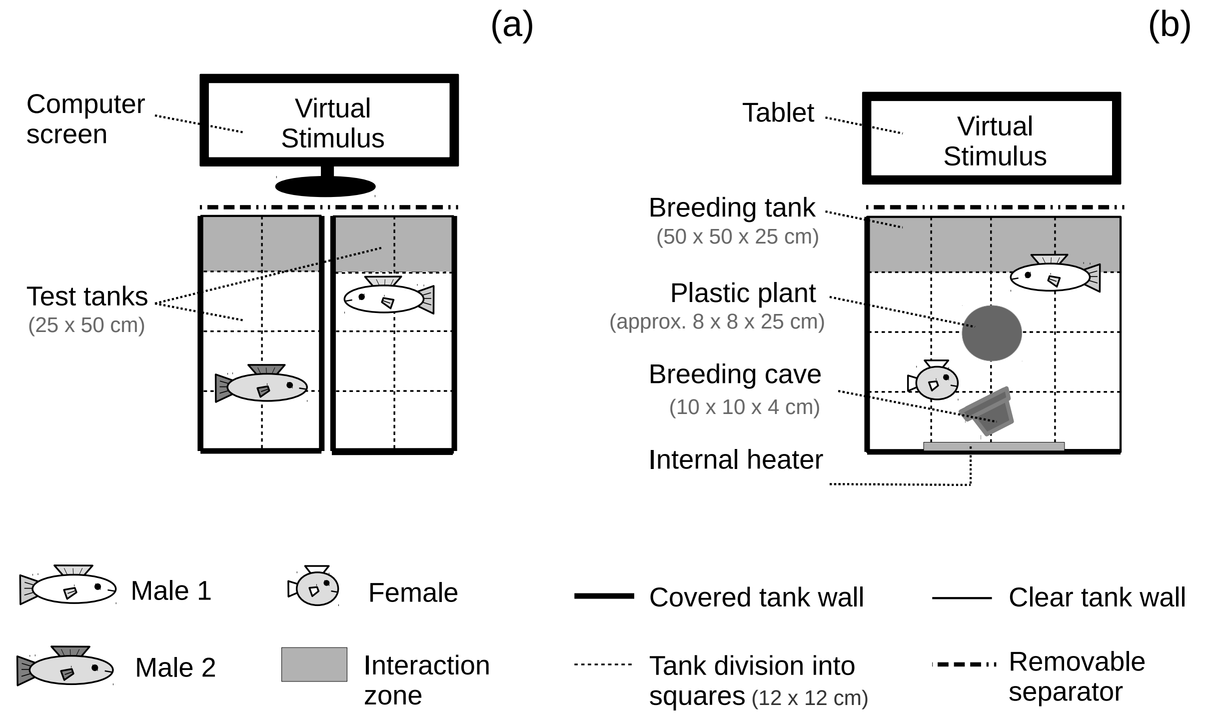


Figure 1. (a) Experimental set up for the personality typing: two identical and visually separated tests tanks that were aligned to face a computer screen (Dell, UltraSharp U2412M, 1.920 × 1.200 pixel, 61-cm screen, USA, Round Rock) showing either an animated conspecific opponent (aggression test) or a predator (predator test). Before the start of a trial, test fish were allowed to acclimate for 10 min without visual access to the computer screen. (b) Experimental set up for parental care tests: parental behaviours were tested in the breeding tank by introducing a tablet (Surftab Theatre, 13.3" Full-HD-IPS display; Trekstar, Bensheim, Germany) at the side of the tank (opposing the breeding cave) showing an animated intruder (male, female, or predator). (a) Test fish were video-recorded from above (Sony HDR-CX405). (b) The video camera was positioned in the front of the breeding tank in approx. 50 cm distance to the breeding tank and 30 min before the start of a trial.

(including revisits) (test tanks were divided into 8 squares each measuring 12x12 cm squares, Figure 1a). For all trials, we used predator specimen the test fish had not seen before ($N_{predators}$ = 4, mean ± SE standard length = 19.3 ± 0.3 cm).

Pairing & breeding experiment

Within four days following the personality assessment, we set up breeding pairs (N = 54), which were created to vary regarding their behavioural contrast in the average level (mean ± SE contrast in the level: 38.5 ± 4.5 squares visited) and consistency (mean ± SE contrast in inconsistency: 15.3 ± 2.3 squares visited) of boldness. Male-female behavioural contrast in the average level or inconsistency, respectively, was calculated as the absolute value of the difference in the behaviour of the two individuals of a breeding pair. We calculated the average level of boldness for each individual as the mean activity over the two boldness tests (mean ± SE in the number of squares visited: males = 63.56 ± 4.26, females = 41.07 ± 3.64). Behavioural consistency was calculated as inconsistency: the absolute value of the difference in activity between the two boldness tests (Scherer et al. 2017b; Scherer and Schuett 2018) (mean ± SE in the number of squares visited: males = 22.32 ± 2.21, females = 19.91 ± 2.21).

To initiate breeding, we introduced the male and the female of a pre-determined breeding pair into a breeding tank (Figure 1b), equipped with half a clay pot as breeding cave, a plastic plant, a layer of sand, and an internal heater (all in a standardised position). We checked the breeding cave for eggs using a small dentist mirror (diameter = 3 cm) on a daily basis. Breeding pairs that did not successfully spawn within 21 days were transferred back into their home tanks and were not further used in this experiment. Breeding pairs that did produce fry were allowed to raise their brood for 30 days (spawning = day 1). We assessed parental care behaviour as outlined below. For each breeding pair, we assessed reproductive success as a binary yes or no (used to obtain the success rate, i.e. the probability of successful reproduction). Further, for breeding pairs that did reproduce (N = 20), we counted the number of fry produced (mean ± SE number of fry per brood = 68.7 ± 9.4) and measured the fry for standard length (mean ± SE standard length = 1.56 ± 0.03 cm) using ImageJ at the end of the breeding period (on day 30).

Parental care tests

During the breeding period, we quantified parental care behaviour (level and consistency of behaviour) as the individual activity in the presence of an animated intruder, time spent near the intruder and time spent attending the brood. We used three different intruder types: a conspecific male, a conspecific female, and a predator. Each intruder type was used twice, i.e. each breeding pair was tested for its parental care behaviour six times with three days elapsed between successive trials. We randomised the testing order for the first time an intruder type was used (first, second and third parental care test) and then repeated parental care tests in the same order (e.g. male-female-predator, which was repeated in the same order). We started our parental care observations on day 10 of the breeding period, i.e. one day after the fry became free-swimming: fertilised eggs took three days to develop into wrigglers (free embryos), which stayed in the breeding cave for approx. another five days (own observations).

To start a parental care test, we introduced a tablet on a side of the breeding tank (Figure 1b) showing one of three above named intruder types for 11 min. We video-recorded the breeding pair's response and manually assessed the total amount of time spent within 12 cm distance to the animated intruder (interaction zone, see Figure 1b) and the activity for each parent from the videos (duration of video analysis was 10 min, starting 1 min after the start of the video). Similar to the procedure in the boldness tests (see above), activity was assessed as the total number of squares visited (including revisits). Therefore, the breeding tank was divided into 16 squares each measuring 12x12 cm squares (Figure 1b) using markings alongside the vertical tank walls. Further, male and female brood attendance behaviour was quantified from a single-frame analysis (21 frames: every 30 sec for 10 min, starting 1 min after the video start) where we scored whether the individual was within one standard length distance to the brood (approx. 6 cm, or half a square), if so, this was scored as brood attendance (Thünken et al. 2010). An individual's time spent attending the brood was then calculated as the number of frames where the individual was attending the brood in relation to all frames analysed. For each of the three parental behaviours (time spent near the intruder, activity in the presence of the intruder, and time spent attending the brood), we calculated the average level and inconsistency (absolute value of the range in the behavioural level) of behaviour for each of the three intruder types separately.

We used the same animations that we used for the personality tests but always presented an unfamiliar specimen to focal fish. Male intruder sizes were matched to the male's standard length and, similarly, female intruder sizes were matched to the female's standard length (size difference ≤ 2 mm). Predator sizes were identical to the ones used in the boldness test. All videos were analysed by the same observer. At the time of video analysis, the observer was not aware of parental fish's pre-determined boldness scores.

Our measures of parental care behaviour (level and inconsistency of behaviour) did not differ between intruder types (Table 1). Therefore, we calculated the average level and inconsistency (absolute value of the range in the behavioural level) for all behaviours (activity in the presence of an intruder, time spent near the intruder, and time spent attending the brood) over the six parental care tests, i.e. pooled for intruder type.

DATA ANALYSES

General details

Data analyses were performed in R version 3.5.3 (R Core Team 2019). LMMs (linear mixed-effects models) and GLMMs (generalized linear mixed-effects models) were fitted via stepwise-backward model selection using the *lme4*-package (Bates et al. 2014). For insignificant fixed effects, we report test statistics from the last model incorporating the term. Model assumptions were verified visually via residual and normal q-q plots. Individual time spent attending the brood (proportional data) was arcsine square-root transformed for normality when being used as dependent variable. Female size did neither affect offspring size (Spearman's rank correlation, Rho = -0.286, S = 1709.9, P-value = 0.222) nor did it affect offspring number (Spearman's rank correlation, Rho = 0.082, S =1221, P-value = 0.731). For analyses, we therefore did not correct offspring size and - number for female size. As pre-determined aggression was not repeatable (see below) we focussed on boldness as a measure of pre-determined personality differences.

Behavioural repeatabilities

We tested for behavioural repeatability in boldness (activity under simulated predation risk) and aggression (time spent near the animated opponent) for males and females separately ($N_{individuals}$ = 54 and N_{trials} = 108 for males and females, respectively) using the *rptR*-package (Stoffel et al. 2017) (bootstrapping runs = 1000; permutations = 1000). Further, we assessed the repeatability of male ($N_{individuals}$ = 20) and female ($N_{individuals}$ = 20) parental behaviours: activity in the presence of an animated intruder, the amount of time spent near this intruder, and the time spent attending the brood in the presence of the animated intruder. Parental behaviours were not affected by intruder type (see above), we therefore assessed repeatabilities using the data obtained during all parental care tests, i.e. pooled for intruder type (N_{trials} = 120 for males and females, respectively). However, we adjusted repeatabilities of parental care for intruder type, and for test series (1-6; to account for habituation (Scherer and Schuett 2018)).

Directional effects

(I) Pre-determined personality differences and parental care behaviour

For each individual, we tested for whether pre-determined average level of boldness

Table 1. Results of LMMs (linear mixed-effects models) testing for an effect of the intruder type (male, female, predator) on the average behavioural level and inconsistency of the three parental target behaviours (calculated for each of the three intruder type separately). All models included individual ID and family (males) or origin (females) as random effects. The intruder type did not affect parental care behaviours (significance level = 0.05).

	Dependent variable	Predictor variable		N		df	Estimate ± SE	χ^2	P-value
				Individuals	Data points				
Males	Activity in the presence of an intruder (parental care test)	Intruder type	Female	20	60	2	93.300 ± 9.278	0.871	0.647
			Male				85.975 ± 7.325		
			Predator				87.875 ± 8.100		
	Time spent near the intruder (parental care test)	Intruder type	Female	20	60	2	345.675 ± 36.410	4.883	0.087
			Male				337.850 ± 22.337		
			Predator				298.200 ± 22.337		
	Time spent attending the brood (parental care test)	Intruder type	Female	20	60	2	0.927 ± 0.044	1.798	0.407
			Male				-0.005 ± 0.049		
			Predator				0.055 ± 0.049		
	Inconsistency in activity in the presence of an intruder (parental care test)	Intruder type	Female	20	60	2	55.800 ± 10.150	3.402	0.183
			Male				31.350 ± 13.360		
			Predator				48.750 ± 13.360		
	Inconsistency in the time spent near the intruder (parental care test)	Intruder type	Female	20	60	2	221.950 ± 39.350	1.101	0.577
			Male				223.700 ± 48.360		
			Predator				178.600 ± 48.360		
	Inconsistency in the time spent attending the brood (parental care test)	Intruder type	Female	20	60	2	0.551 ± 0.061	1.653	0.438
			Male				0.442 ± 0.085		
			Predator				0.494 ± 0.085		
Females	Activity in the presence of an intruder (parental care test)	Intruder type	Female	20	60	2	61.850 ± 4.015	0.357	0.837
			Male				60.375 ± 4.887		
			Predator				60.400 ± 4.887		
	Time spent near the intruder (parental care test)	Intruder type	Female	20	60	2	271.950 ± 31.896	1.691	0.429
			Male				274.475 ± 25.917		
			Predator				243.800 ± 25.917		
	Time spent attending the brood (parental care test)	Intruder type	Female	20	60	2	1.111 ± 0.041	2.381	0.304
			Male				1.128 ± 0.048		
			Predator				1.056 ± 0.048		
	Inconsistency in activity in the presence of an intruder (parental care test)	Intruder type	Female	20	60	2	31.328 ± 6.988	0.619	0.734
			Male				26.978 ± 5.509		
			Predator				29.028 ± 5.509		
	Inconsistency in the time spent near the intruder (parental care test)	Intruder type	Female	20	60	2	226.500 ± 38.280	0.087	0.957
			Male				213.950 ± 49.290		
			Predator				226.600 ± 49.290		
	Inconsistency in the time spent attending the brood (parental care test)	Intruder type	Female	20	60	2	0.363 ± 0.056	0.542	0.763
			Male				0.417 ± 0.079		
			Predator				0.370 ± 0.079		

predicted the level of behaviour in the three parental behaviours (activity in the presence of an animated intruder, time spent near the intruder, and time spent attending the brood) separately by fitting an LMM on each of the three parental behaviours (average level of behaviour pooled for intruder type, $N_{individuals} = 20$, $N_{data\ points} = 20$, for all models) including the average activity shown during the boldness tests as predictor variable. We did separate analyses for males and females. As random terms, we included pair ID and individual family (for males) or origin (for females). Similarly, we tested whether pre-determined inconsistency of boldness predicted inconsistency in the three parental behaviours: we fitted an LMM to the inconsistency of each of the three parental behaviours (pooled for intruder type, $N_{individuals} = 20$, $N_{data\ points} = 20$, for all models), including the inconsistency shown during the boldness tests as predictor variable. Again, we did the analyses for males and females separately and used the same random terms as above.

(II) Parental care behaviour and reproductive success

We tested for a directional effect of the level and inconsistency of the three parental behaviours on reproductive success by fitting three LMMs (each model including the measures of one of the three parental behaviour as predictor variables) on each of the two measures of reproductive success (number and size of offspring), respectively (six models in total, $N_{breeding\ pairs} = 20$, $N_{data\ points} = 20$, for all models). As predictor variables, each model contained male level, female level, an interaction between male and female level, male inconsistency, female inconsistency, and an interaction between male and female inconsistency. For each of the six models, male/female level and inconsistency values were based on one of the three parental behaviours (activity in the presence of an animated intruder, time spent near the intruder, or time spent attending the brood, respectively). As above, we included male family and female origin as random terms. Please note, although we primarily aimed to test for directional effects using the above analysis we also including the interaction term between male and female behaviour considering compatibility effects. As individual behavioural expression itself is not included when performing our tests for compatibility effects (only male-female behavioural contrast is considered there, see below) we regarded an inclusion of the interaction term here to be of additional informative value.

(III) Pre-determined personality differences and reproductive success

We tested for a directional effect of pre-determined boldness on reproductive success by fitting a GLMM on the success rate (probability of successful reproduction) (N = 54 breeding pairs) and two more LMMs, one on offspring number (N = 20 successful breeding pairs) and one on offspring size (N = 20 successful breeding pairs). Offspring size was averaged for each brood. The three models incorporated male average level, female average level, male inconsistency, and female inconsistency of pre-determined boldness as predictor variables. We also considered male and female behaviour to have an interdependent effect and therefore further included an interaction between male and female behavioural level, and between male and female inconsistency as fixed effects (as explained above). As random terms, we included male family and female origin.

Compatibility effects

(I) Pre-determined personality differences and parental care behaviour

We tested whether a breeding pair's behavioural contrast in pre-determined boldness predicts their contrast in parental behaviours by fitting an LMM on each of the six behavioural contrasts (contrast in the level and inconsistency, respectively, for each of the three parental behaviours), resulting into six LMs ($N_{\text{breeding pairs}} = 20$, $N_{\text{data points}} = 20$, for all models). As predictor variables, we included the contrast in the pre-determined level of boldness and the sex of the bold individual as predictor variable (for the three models on the contrast of the behavioural level), or we included the contrast in pre-determined inconsistency of boldness and the sex of the consistent individual as predictor variables (for the three models on the contrast of behavioural inconsistency). Within-pair behavioural contrasts were assessed as the absolute value of the difference in the level/inconsistency between male and female behaviour (see also above). Individuals were classified into bold or consistent based on a within-pair comparison of the average level/inconsistency shown during the boldness tests. Please note, the sex was only included as a covariate accounting for sex differences but was not of further interest here and is therefore not discussed. We included male family and female origin as random terms.

(II) Parental care behaviour and reproductive success

We tested for an effect of the within-pair behavioural contrast in parental care behaviours on offspring number and - size by running six LMMs, including three models that were fit on offspring number and another three models that were fit on offspring size (one model for each of the three parental behaviours, respectively). We included the contrast in the behavioural level, contrast in inconsistency and an interaction between these two contrasts as predictor variables. We used the same random terms as above.

(III) Pre-determined personality differences and reproductive success

We tested for an effect of a breeding pair's behavioural contrast in pre-determined boldness on reproductive success by fitting a GLMM on the success rate (N = 54 breeding pairs) and two LMMs, one on average offspring size and one on offspring number (N = 20 successful breeding pairs, respectively). We included the contrast in the level, contrast in inconsistency, and an interaction between the contrast in the level and the contrast in inconsistency as fixed effects. As above, we included male family and female origin as random terms in all models.

Correlation of behaviours and offspring measures

We tested for potential correlations between the levels and inconsistencies of the behavioural measures obtained during the boldness test and parental care test. However, correlation coefficients were all ≤ 0.80, we therefore did not consider our measures to be redundant (Dormann et al. 2013). See Supplemental Material 1 for Spearman rank correlation based correlation matrices; matrices were calculated using the *PerformanceAnalytics*-package (Peterson and Carl 2018). Offspring size and number were negatively correlated with each other (Spearman's rank correlation, Rho = -0.626, S = 2162.6, P-value = 0.003).

RESULTS

Please note, we conducted multiple tests (N = 45) on a single data set and therefore present and discuss our results on an adjusted significance level of 0.001 (Bonferroni correction). However, we are aware that this is a very conservative p-value correction (Streiner and Norman 2011) and therefore also acknowledge our findings on a standard significance level of 0.05 in this *Results* section but do not discuss these findings in avoidance of false positives.

Behavioural repeatabilities

Male and female boldness, measured as activity under simulated predation risk before the breeding, was highly repeatable (Table 2). However, male and female aggressiveness, measured indirectly as the time spent near an animated opponent before the breeding, was not repeatable (Table 2). The three parental behaviours observed during the breeding were repeatable (Table 2).

Directional effects

(I) Pre-determined personality differences and parental care behaviour

Male and female parental behaviours (activity in the presence of an intruder, time spent near the intruder, and time spent attending the brood; level and inconsistency, respectively) did not correlate with the pre-determined average level/inconsistency of boldness (Table 3, Figure 2a-b). However, on a significance level of 0.05, male average level of boldness tended to positively correlate with its time spent attending the brood (Table 3, Supplemental Material 2a).

(II) Parental care behaviour and reproductive success

We found no directional effects of male and female level and inconsistency in the three parental behaviours on offspring number and - size (Table 4, Figure 2a-b). However, on a significance level of 0.05, the of

Table 2. Repeatability of male and female aggression and boldness shown before the breeding, and parental behaviours shown during the breeding. Significant repeatabilities are highlighted in bold.

Context	Behaviour	Sex	N		Repeatability	
			Individuals	Data points	R	CI
Aggression test	Time spent near the opponent	Males	54	108	0.212	[0.000, 0.409]
		Females	54	108	0.000	[0.000, 0.000]
Boldness test	Activity in the presence of a predator	Males	54	108	**0.629**	**[0.458, 0.759]**
		Females	54	108	**0.594**	**[0.409, 0.731]**
Parental care tests	Time spent near an intruder	Males	20	120	**0.498**	**[0.278, 0.685]**
		Females	20	120	**0.349**	**[0.148, 0.565]**
	Activity in the presence of an intruder	Males	20	120	**0.347**	**[0.146, 0.558]**
		Females	20	120	**0.178**	**[0.018, 0.378]**
	Time spent attending the brood	Males	20	120	**0.211**	**[0.040, 0.424]**
		Females	20	120	**0.212**	**[0.037, 0.411]**

offspring produced by a breeding pair positively correlated with female activity in the presence of an intruder during parental care tests and tended to increase with male average time spent attending the brood (Table 4, Supplemental Material 2a-b). Further, on a significance level of 0.05, the number of offspring was predicted by an interaction of male and female inconsistency in the time spent attending the brood (Table 4): while female inconsistency was positively associated with the number of offspring male behaviour had a rather low effect (Supplemental Material 3). And the interaction of male and female inconsistency in the time spent near the intruder strongly tended to affect offspring size (Table 4, Supplemental Figure 2a-b).

(III) Pre-determined personality differences and reproductive success

The number of offspring produced significantly increased with the level of pre-determined male boldness (Table 5, Figure 2a). There were no other directional effects of pre-determined boldness on reproductive success (Table 5, Figure 2a-b). However, we found further effects on a significance level of 0.05: a breeding pair's likelihood to reproduce strongly tended to decrease with increasing male level of pre-determined boldness though the number of offspring produced significantly increased with the level of pre-determined male boldness (Table 5, Supplemental Material 2a).

Compatibility effects

(I) Pre-determined personality differences and parental care behaviour

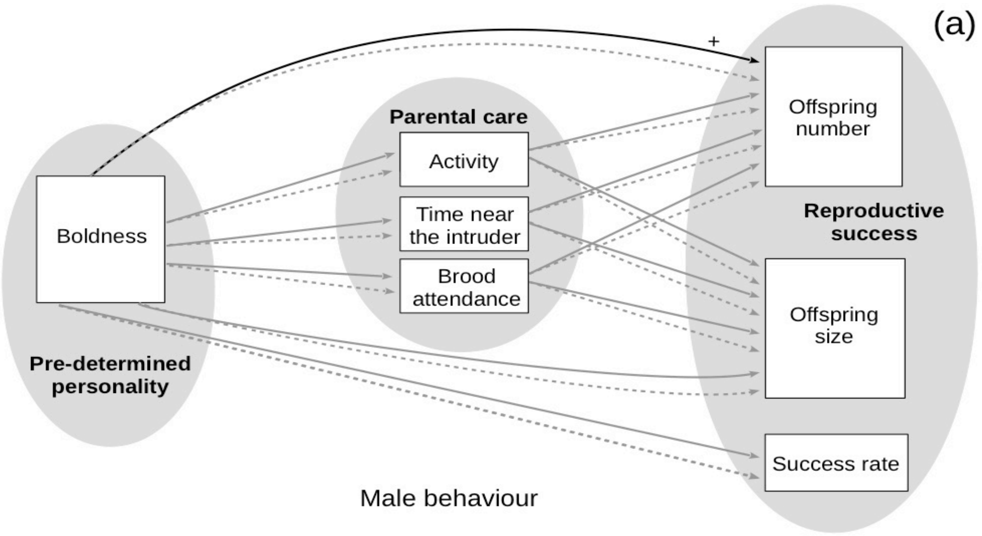
Male-female behavioural contrast in pre-determined boldness (level and consistency) did not predict the behavioural contrast in their parental behaviours (Table 6, Figure 2c). Considering the unadjusted significance level of 0.05: Breeding pairs that showed a higher contrast in pre-determined level of boldness also showed a higher contrast in their time spent attending the brood during parental care tests (Table 6, Supplemental Material 2c).

(II) Parental care behaviour and reproductive success

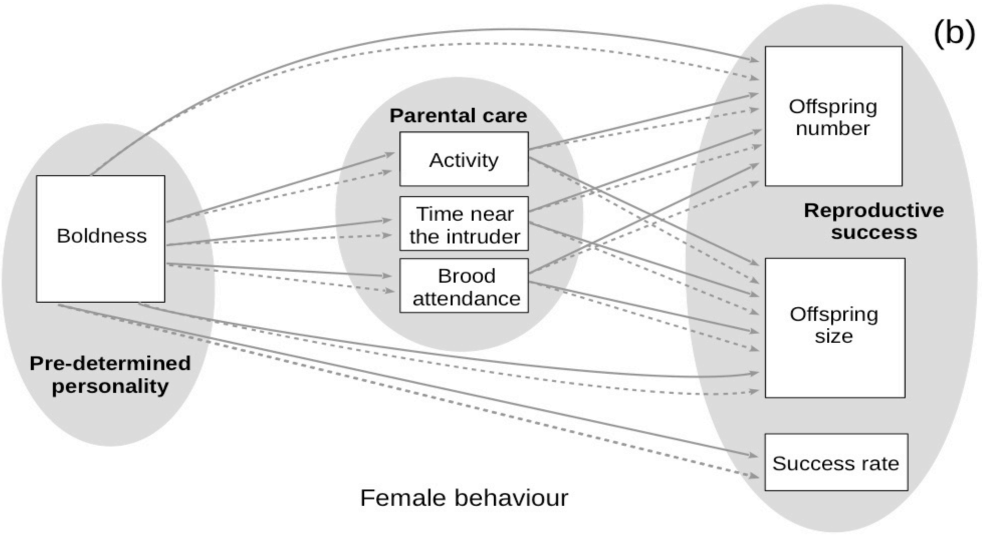
Offspring size significantly decreased with male-female contrast in their activity in the presence of an intruder and with their contrast in the time spent near the intruder, i.e. parents that behaved more similarly (regarding the level of these two behaviours) produced larger offspring (Table 7, Figure 2c). Other parental behaviours (level and inconsistency, respectively) did not affect reproductive success (Table 7, Figure 2c). On a significance level of 0.05, we found further effects: the number of offspring produced was affected by the parental contrast in the time spent near the intruder (level and inconsistency) (Table 7, Supplemental Material 2c): the number of fry produced was higher for pairs that were dis-similar regarding the level of behaviour and similar regarding their inconsistency (Figure 3). Offspring size was significantly affected by the parental contrasts in the level of all three parental behaviours (Table 7, Supplemental Material 2c): the average offspring size of a brood decreased with increasing parental contrast in their activity in the presence of an intruder and with increasing time spent near the intruder. The contrast in the time spent attending the brood positively affected offspring size (Table 7, Supplemental Material 2c). However, the level-inconsistency interaction of the activity in the presence of an intruder and time spent near the intruder tended to predict the number of offspring (Table 7). Further, the number offspring tended to increase

Table 3. Results of LMMs testing for a correlation between boldness shown before the breeding and parental behaviours during the breeding period (level and inconsistency, respectively). No significant effects (adjusted significance level = 0.001).

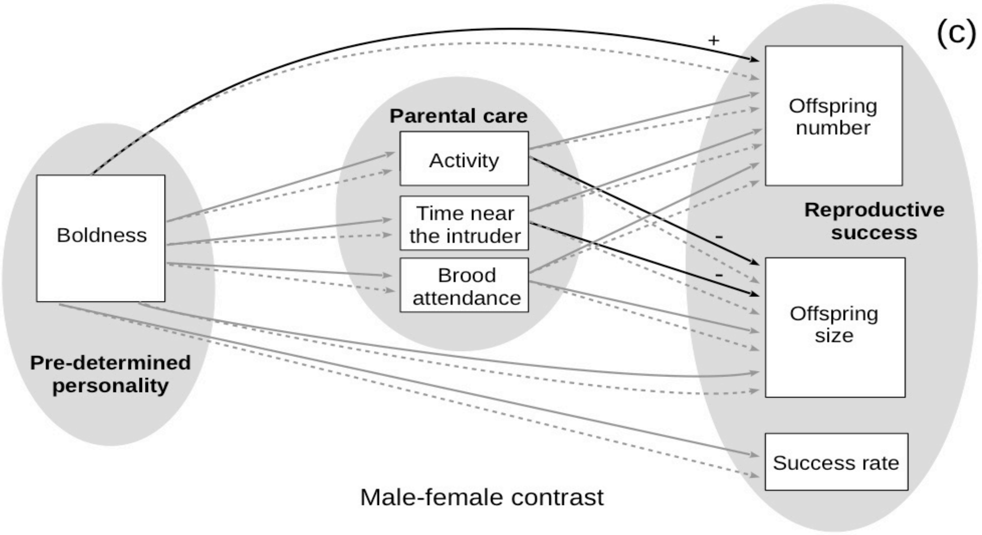
		Dependent variable	Predictor variable	N		Intercept ± SE	Estimate ± SE	df	χ^2	P-value
				Individuals	Data points					
Males	Level	Activity in the presence of an intruder (parental care test)	Average activity in the presence of a predator (boldness test)	20	20	100.691 ± 19.395	-0.206 ± 0.313	1	0.428	0.513
		Time spent near the intruder (parental care test)	Average activity in the presence of a predator (boldness test)	20	20	230.568 ± 79.864	1.711 ± 1.290	1	1.686	0.194
		Time spent attending the brood (parental care test)	Average activity in the presence of a predator (boldness test)	20	20	0.809 ± 0.074	0.002 ± 0.002	1	3.456	0.063
	Inconsistency	Activity in the presence of an intruder (parental care test)	Inconsistency in activity in the presence of a predator (boldness test)	20	20	113.493 ± 25.994	-0.598 ± 0.897	1	0.419	0.517
		Time spent near the intruder (parental care test)	Inconsistency in activity in the presence of a predator (boldness test)	20	20	295.095 ± 62.908	2.842 ± 2.139	1	1.602	0.206
		Time spent attending the brood (parental care test)	Inconsistency in activity in the presence of a predator (boldness test)	20	20	0.591 ± 0.083	-0.002 ± 0.003	1	0.438	0.508
Females	Level	Activity in the presence of an intruder (parental care test)	Average activity in the presence of a predator (boldness test)	20	20	68.702 ± 5.273	-0.221 ± 0.146	1	2.174	0.140
		Time spent near the intruder (parental care test)	Average activity in the presence of a predator (boldness test)	20	20	301.020 ± 54.021	-1.213 ± 1.496	1	0.647	0.421
		Time spent attending the brood (parental care test)	Average activity in the presence of a predator (boldness test)	20	20	1.072 ± 0.056	0.001 ± 0.001	1	0.086	0.771
	Inconsistency	Activity in the presence of an intruder (parental care test)	Inconsistency in activity in the presence of a predator (boldness test)	20	20	46.717 ± 9.213	0.428 ± 0.397	1	1.129	0.288
		Time spent near the intruder (parental care test)	Inconsistency in activity in the presence of a predator (boldness test)	20	20	351.804 ± 49.835	2.221 ± 2.148	1	1.042	0.307
		Time spent attending the brood (parental care test)	Inconsistency in activity in the presence of a predator (boldness test)	20	20	0.420 ± 0.062	-0.001 ± 0.003	1	0.106	0.745



Male behaviour



Female behaviour



Male-female contrast

Figure 2. Graphical illustrations for the results of (G)LMMs testing for directional effects of (a) male and (b) female behaviour, and (c) male-female behavioural contrasts on reproductive success. Effects shown for the level (solid lines) and inconsistency (dashed lines) (in the contrast) of the behaviour. Insignificant effects indicated by grey lines. For significant effects (adjusted significance level of 0.001) the direction of effect is indicated by "+" (positive) or "-" (negative).

Table 4. Results of LMMs testing for a directional effect of male and female level and inconsistency in the three parental behaviours on the number and size of offspring produced. Significant effects are highlighted in bold (adjusted significance level = 0.001).

Dependent variable	Predictor variable		N		Intercept ± SE	Estimate ± SE	df	χ^2	P-value
			Breeding pairs	Data points					
Number of offspring	Activity in the presence of an intruder (parental care test)	Male level	20	20	11.480 ± 36.421	0.161 ± 0.198	1	0.543	0.461
		Female level			7.083 ± 35.131	1.029 ± 0.465	1	4.237	0.040
		Male level : female level			-85.931 ± 71.604	-0.021 ± 0.013	1	2.044	0.153
		Male inconsistency			18.652 ± 35.592	-0.131 ± 0.098	1	1.609	0.205
		Female inconsistency			12.906 ± 36.437	0.158 ± 0.221	1	0.471	0.493
		Male inconsistency : female inconsistency			-111.100 ± 72.480	14.620 ± 13.132	1	1.180	0.277
	Time spent near the intruder (parental care test)	Male level	20	20	-5.143 ± 30.242	-0.025 ± 0.042	1	0.345	0.557
		Female level			-5.148 ± 30.238	0.002 ± 0.056	1	0.002	0.965
		Male level : female level			-12.580 ± 32.940	0.000 ± 0.000	1	0.265	0.607
		Male inconsistency			-	-	-	-	-
		Female inconsistency			-	-	-	-	-
		Male inconsistency : female inconsistency			5.148 ± 30.238	-0.001 ± 0.000	1	4.338	0.037
	Time spent attending the brood (parental care test)	Male level	20	20	22.150 ± 34.550	80.450 ± 45.250	1	2.928	0.087
		Female level			21.760 ± 92.370	18.060 ± 83.640	1	0.047	0.829
		Male level : female level			113.800 ± 352.460	184.150 ± 679.660	1	0.071	0.789
		Male inconsistency			36.710 ± 61.000	14.230 ± 34.040	1	0.171	0.679
		Female inconsistency			52.280 ± 47.740	-40.060 ± 45.140	1	0.741	0.391
		Male inconsistency : female inconsistency			125.190 ± 405.120	-11.170 ± 212.480	1	0.003	0.961
Offspring size	Activity in the presence of an intruder (parental care test)	Male level	20	20	1.487 ± 0.136	-0.000 ± 0.001	1	0.086	0.769
		Female level			1.475 ± 0.132	0.001 ± 0.002	1	0.282	0.596
		Male level : female level			1.891 ± 0.286	0.000 ± 0.000	1	2.364	0.124
		Male inconsistency			1.541 ± 0.047	0.000 ± 0.000	1	0.446	0.504
		Female inconsistency			1.475 ± 0.131	-0.001 ± 0.000	1	0.282	0.596
		Male inconsistency : female inconsistency			1.887 ± 0.282	0.000 ± 0.000	1	0.632	0.427
	Time spent near the intruder (parental care test)	Male level	20	20	1.592 ± 0.083	0.000 ± 0.000	1	0.272	0.602
		Female level			1.589 ± 0.083	0.000 ± 0.000	1	0.083	0.773
		Male level : female level			1.814 ± 0.135	0.000 ± 0.000	1	0.222	0.638
		Male inconsistency			1.600 ± 0.074	0.000 ± 0.000	1	0.461	0.497
		Female inconsistency			1.616 ± 0.071	0.000 ± 0.000	1	0.537	0.464
		Male inconsistency : female inconsistency			1.793 ± 0.128	0.000 ± 0.000	1	3.476	0.062
	Time spent attending the brood (parental care test)	Male level	20	20	1.356 ± 0.188	0.143 ± 0.209	1	0.461	0.497
		Female level			1.457 ± 0.242	-0.161 ± 0.246	1	0.425	0.515
		Male level : female level			1.163 ± 1.326	-0.511 ± 2.521	1	0.041	0.841
		Male inconsistency			1.476 ± 0.068	0.168 ± 0.117	1	1.949	0.163
		Female inconsistency			1.422 ± 0.367	0.024 ± 0.189	1	0.017	0.898
		Male inconsistency : female inconsistency			1.196 ± 1.507	-0.035 ± 0.776	1	0.002	0.964

Table 5. Results of (G)LMMs testing for an effect of pre-determined level/inconsistency of boldness on reproductive success. Significant effects are highlighted in bold (adjusted significance level = 0.001).

Dependent variable		Predictor variable	N		Intercept ± SE	Estimate ± SE	df	χ^2	P-value
			Breeding pairs	Data points					
Success rate	Activity in the presence of a predator (boldness test)	Male level	54	54	0.553 ± 1.156	-0.022 ± 0.012	1	3.702	0.054
		Female level			1.612 ± 1.313	-0.022 ± 0.017	1	1.941	0.164
		Male level : female level			3.028 ± 2.087	0.001 ± 0.001	1	0.931	0.335
		Male inconsistency			1.424 ± 1.366	0.011 ± 0.019	1	0.334	0.564
		Female inconsistency			1.424 ± 1.383	-0.011 ± 0.027	1	0.169	0.681
		Male inconsistency : female inconsistency			3.000 ± 2.099	-0.000 ± 0.001	1	0.016	0.898
Number of offspring	Activity in the presence of a predator (boldness test)	Male level	20	20	**29.618 ± 27.260**	**0.800 ± 0.193**	**1**	**10.160**	**0.001**
		Female level			35.698 ± 27.590	0.241 ± 0.254	1	0.812	0.368
		Male level : female level			8.351 ± 43.776	-0.012 ± 0.012	1	0.346	0.556
		Male inconsistency			34.039 ± 27.429	0.165 ± 0.304	1	0.292	0.589
		Female inconsistency			26.444 ± 26.191	0.433 ± 0.314	1	1.665	0.197
		Male inconsistency : female inconsistency			29.916 ± 28.106	-0.011 ± 0.021	1	0.295	0.587
Offspring size	Activity in the presence of a predator (boldness test)	Male level	20	20	1.567 ± 0.062	0.001 ± 0.001	1	0.296	0.587
		Female level			1.541 ± 0.088	0.000 ± 0.001	1	0.145	0.703
		Male level : female level			1.592 ± 0.177	0.000 ± 0.000	1	0.108	0.742
		Male inconsistency			1.591 ± 0.046	-0.001 ± 0.002	1	0.347	0.556
		Female inconsistency			1.541 ± 0.088	0.000 ± 0.002	1	0.044	0.834
		Male inconsistency : female inconsistency			0.000 ± 0.187	0.000 ± 0.000	1	0.000	0.979

Table 6. Results of LMMs testing for an effect of pre-determined behavioural contrast in boldness on the contrast in parental behaviours. As a covariate, the sex of the bold ID (individual) was included in the models. Significant effects are highlighted in bold (adjusted significance level = 0.001).

	Dependent variable	Predictor variable		N		Intercept ± SE	Estimate ± SE	df	χ^2	P-value
				Breeding pairs	Data points					
Level	Contrast in activity in the presence of an intruder (parental care test)	Contrast in average activity in the presence of a predator (boldness test)		20	20	-27.002 ± 15.320	-0.272 ± 0.319	1	0.715	0.398
		Sex of the bold ID	Male			26.180 ± 18.270		1	7.657	0.006
			Female			-29.610 ± 15.280				
	Contrast in the time spent near the intruder (parental care test)	Contrast in average activity in the presence of a predator (boldness test)		20	20	-67.597 ± 41.710	0.027 ± 0.869	1	0.001	0.975
		Sex of the bold ID	Male			62.340 ± 48.85		1	6.036	0.014
			Female			-67.330 ± 40.870				
	Contrast in the time spent attending the brood (parental care test)	Contrast in average activity in the presence of a predator (boldness test)		20	20	**0.099 ± 0.051**	**0.003 ± 0.001**	**1**	**4.899**	**0.027**
		Sex of the bold ID	Male			**-0.231 ± 0.067**		**1**	**15.786**	**<0.0001**
			Female			**0.099 ± 0.051**				
Inconsistency	Contrast in activity in the presence of an intruder (parental care test)	Contrast in the inconsistency in activity in the presence of a predator (boldness test)		20	20	9.631 ± 32.019	-0.025 ± 1.195	1	0.000	0.984
		Sex of the consistent ID	Male			5.432 ± 32.344		1	0.014	0.906
			Female			9.318 ± 28.264				
	Contrast in the time spent near the intruder (parental care test)	Contrast in the inconsistency in activity in the presence of a predator (boldness test)		20	20	-22.632 ± 45.874	-1.507 ± 2.110	1	0.504	0.478
		Sex of the consistent ID	Male			44.860 ± 61.220		1	1.967	0.161
			Female			-43.150 ± 36.220				
	Contrast in the time spent attending the brood (parental care test)	Contrast in the inconsistency in activity in the presence of a predator (boldness test)		20	20	0.091 ± 0.095	0.002 ± 0.004	1	0.198	0.657
		Sex of the consistent ID	Male			0.117 ± 0.075		1	2.205	0.138
			Female			-0.075 ± 0.126				

Table 7. Results of LMMs testing for an effect of the behavioural contrast in the level and inconsistency of parental behaviours on the number of offspring and offspring size. Significant effects are highlighted in bold (adjusted significance level = 0.05).

Dependent variable	Predictor variable		N		Intercept ± SE	Estimate ± SE	df	χ^2	P-value
			Breeding pairs	Data points					
Number of offspring	Activity in the presence of an intruder (parental care test)	Contrast in the level	20	20	72.956 ± 19.575	0.131 ± 0.155	1	0.674	0.412
		Contrast in inconsistency			70.611 ± 18.406	0.298 ± 0.182	1	2.197	0.138
		Contrast in the level : contrast in inconsistency			75.044 ± 18.522	-0.003 ± 0.001	1	35.958	0.058
	Time spent near the intruder (parental care test)	Contrast in the level	20	20	65.586 ± 24.366	0.098 ± 0.037	1	5.421	0.021
		Contrast in inconsistency			65.586 ± 24.366	-0.129 ± 0.033	1	9.417	0.002
		Contrast in the level : contrast in inconsistency			72.289 ± 20.566	0.000 ± 0.000	1	28.703	0.090
	Time spent attending the brood (parental care test)	Contrast in the level	20	20	70.830 ± 19.420	29.640 ± 42.490	1	0.472	0.492
		Contrast in inconsistency			70.190 ± 20.170	39.310 ± 21.360	1	28.968	0.089
		Contrast in the level : contrast in inconsistency			68.740 ± 20.060	-85.460 ± 121.600	1	0.477	0.490
Offspring size	Activity in the presence of an intruder (parental care test)	Contrast in the level	20	20	**1.579 ± 0.045**	**-0.002 ± 0.000**	**1**	**10.201**	**0.001**
		Contrast in inconsistency			1.579 ± 0.041	0.000 ± 0.000	1	0.413	0.520
		Contrast in the level : contrast in inconsistency			1.568 ± 0.004	0.000 ± 0.000	1	1.826	0.177
	Time spent near the intruder (parental care test)	Contrast in the level	20	20	**1.575 ± 0.036**	**-0.001 ± 0.000**	**1**	**14.088**	**0.0002**
		Contrast in inconsistency			1.575 ± 0.004	0.000 ± 0.000	1	0.036	0.850
		Contrast in the level : contrast in inconsistency			1.575 ± 0.004	0.000 ± 0.000	1	0.094	0.761
	Time spent attending the brood (parental care test)	Contrast in the level	20	20	1.577 ± 0.038	0.341 ± 0.115	1	5.532	0.019
		Contrast in inconsistency			1.578 ± 0.036	-0.071 ± 0.087	1	0.637	0.425
		Contrast in the level : contrast in inconsistency			1.576 ± 0.037	-0.084 ± 0.407	1	0.042	0.838

Table 8. Results of (G)LMMs testing for an effect of the within-pair pre-determined contrast in the level/inconsistency of boldness on reproductive success. No significant effects (adjusted significance level = 0.05).

Dependent variable	Predictor variable		N		Intercept ± SE	Estimate ± SE	df	χ^2	P-value
			Breeding pairs	Data points					
Success rate	Activity in the presence of a predator (boldness test)	Contrast in the level	54	54	-	-	-	-	-
		Contrast in inconsistency			-	-	-	-	-
		Contrast in the level : contrast in inconsistency			1.226 ± 1.293	0.002 ± 0.001	1	5.317	0.021
Number of offspring	Activity in the presence of a predator (boldness test)	Contrast in the level	20	20	59.520 ± 22.722	0.554 ± 0.186	1	6.507	0.011
		Contrast in inconsistency			58.301 ± 22.487	0.143 ± 0.436	1	0.102	0.751
		Contrast in the level : contrast in inconsistency			63.898 ± 24.578	0.020 ± 0.013	1	1.862	0.172
Offspring size	Activity in the presence of a predator (boldness test)	Contrast in the level	20	20	1.592 ± 0.041	0.000 ± 0.000	1	0.000	0.985
		Contrast in inconsistency			1.594 ± 0.033	-0.002 ± 0.002	1	1.236	0.266
		Contrast in the level : contrast in inconsistency			1.585 ± 0.047	0.000 ± 0.000	1	0.091	0.763

with increasing contrast in inconsistency of the time spent attending the brood (Table 7).

(III) Pre-determined personality differences and reproductive success

The number of offspring produced increased with increasing contrast in the level of pre-determined boldness (Table 8, Figure 2c). We found no other effect of the contrast in the level/inconsistency of pre-determined boldness on reproductive success (Table 8, Figure 2c). But, on a significance level of 0.05, a breeding pair's likelihood to reproduce was affected by the within-pair contrast in the level of boldness in interaction with the contrast in the inconsistency of boldness (Table 8, Supplemental Material 2c), i.e. the success rate of breeding pairs that were similar in their pre-determined inconsistency of boldness strongly increased with increasing similarity (low contrast) in their level of behaviour (Supplemental Material 4, continuous line and black data points). But breeding pairs that showed a higher behavioural contrast in inconsistency showed an increased likelihood to produce fry when being also behaviourally different in their level of behaviour (Supplemental Material 4, dashed line and data points with white filling).

DISCUSSION

Behavioural repeatabilities

In line with our basic assumption, male and female boldness (activity in the presence of an animated predator) shown before the breeding was highly repeatable. Also, the three behaviours recorded during the breeding (activity in the presence of an animated intruder, time spent near the intruder, and time spent attending the brood) showed significant repeatability. Male and female aggression (time spent near an animated conspecific) determined before the breeding was not repeatable. This is surprising given that we found high repeatability for both male and female aggressiveness in a different data set where we used the same protocol (U. Scherer and W. Schuett, submitted manuscript). We have experimentally and/or statistically excluded potential effects of day time, day of week, test series, disturbance level and hunger level (data not presented) but did not find a plausible explanation for this lack of stable personality variation in aggressiveness in the present study.

(I) Pre-determined personality differences and parental care behaviour

Unlike expected, male and female pre-determined boldness did not predict parental performance (neither directional nor compatibility effects found). Generally, a link between personality measures and parental care behaviour finds support in the literature (reviewed in Chira 2014). Possibly, we did not detect a link in the current study due to relatively long pairing period, that is, breeding pairs were allowed to spawn for up 3 weeks, and often successful spawnings occurred towards the end of this pairing period (approx. two to three weeks after the pairing). During this time breeding pairs might have archived post-pairing similarity in behaviour. Also, our parental care observations extended over several weeks and we averaged parental behaviours over the whole period giving much room for behavioural convergence. Behavioural convergence has been described in breeding pairs of the convict cichlid, *Amatitlania siquia* (Laubu et al. 2016). Here, dis-assortative breeding pairs (with respect to their behaviour on a proactive-reactive axis) that got similar with time increased their reproductive success. Such behavioural convergence might explain why parental behaviours obtained during the breeding period did not relate to pre-determined boldness in our study.

(II) Parental care behaviour and reproductive success

Offspring size decreased with increasing male-female contrast regarding their behavioural level in activity in the presence of an intruder and time spent near this intruder during the breeding. That is, behavioural assortment in these parental measures positively correlated with offspring size. Positive assortment for behaviour has often been reported to have reproductive advantages (Both et al. 2005; Dingemanse et al. 2004; Gabriel and Black 2011; Laubu et al. 2016; Schuett et al. 2011). A potential mechanism might be reduced sexual conflict over the amount of parental provisioning (Royle et al. 2010; Schuett et al. 2010). But also genetic advantages resulting from increased behavioural compatibility are possible (Schuett et al. 2010). Therefore, we encourage future studies to do cross-fostering experiments disentangling behavioural and genetic effects on reproductive success.

Further, (the behavioural contrast in) brood attendance behaviour did not affect reproductive success. In general, brood attendance increases reproductive success via offspring protection or guidance to feeding grounds. In this laboratory study, there was no actual thread to the offspring (as the intruder was not real) and all fish were fed *ad libitum*. Thus, the selective pressure that generates advantages of brood attendance behaviour may have been not present here. A similar rationale is valid with respect to the other two parental measures we obtained, activity in the presence of an intruder and time spent near this intruder: In nature, these behavioural measures (alone or the combination of male and female behaviour) reflect parental quality because they affect offspring survival via protection from con- and heterospecific brood predators. Thus, an experiment in a more naturalistic context (including an actual predatory threat) may provide further insights into how parental care performance may affect reproductive success in cichlids.

(III) Pre-determined personality differences and reproductive success

The number of offspring increased with the level of pre-determined male boldness and with male-female contrast in the level of pre-determined boldness. These two results are interconnected: male-female contrast of behaviour was positively correlated with the level of male boldness (analysis not presented). We set up breeding pairs that varied with regard to the sex of the bold individual but females were generally less bold compared to males (analysis not presented), i.e. for pairs where the female was the bold individual the contrast in behaviour was lower compared to pairs where the male was the bold individual. Given our data, it is difficult to distinguish between a directional effect vs. an effect of compatibility here. However, in a previous mate choice study, we found female rainbow kribs to show a dis-assortative mating preference, but no directional preference, for male level of boldness (Scherer et al. 2017b); indicating a reproductive benefit of dis-assortment. On the other hand, Ariyomo and Watt (2012) found a directional (and positive) effect of a high level of male boldness on egg fertilisation rates in zebrafish, *Danio rerio*. Hypothetically, an interaction between a directional effect and compatibility could be possible, e.g. higher reproductive success of breeding pairs that are of similar behaviour but only it this behaviour is highly expressed.

General discussion & conclusions

Most importantly, our results indicate a reproductive trade-off between many but small vs. few but large offspring as offspring size and – number were negatively correlated with each other. Dis-assortment in pre-determined boldness (activity in the presence of a predator) was associated with an increased number of offspring though positive assortment in the boldness-like parental measure (activity in the presence of an intruder) during the breeding was associated with increased offspring size. However, pre-determined personality did not predict parental care behaviour, which could be related to this trade-off. That is, dis-assortment might have initial advantages on the number of offspring produced but then breeding pairs may benefit from behaving more similarly during the breeding and may therefore behaviourally converge (please note, this is speculative). The existence of behavioural convergence as well as reproductive benefits resulting from it has been shown in another bi-parental cichlid (Laubu et al. 2016). Alternatively, dis-assortment for many but small and positive assortment for few but large offspring may represent distinct investment strategies that allow individuals to adapt to changing environmental pressures (Schrader and Travis 2012).

We did not detect any effects of behavioural consistency (for our hypotheses I-III). But in a previous female mate choice study, we found an assortative mating preference for the consistency of male boldness in this species (Scherer et al. 2017b) indicating a reproductive advantage of similar consistency in boldness. Considering that only twenty breeding pairs successfully reproduced and that we performed a relatively large number of single testings (accompanied by a (conservative) p-value correction) this negative result could be caused by a lack of statistical power. On a standard significance level (0.05), there were several interesting effects (including both the level and consistency of behaviours). Hence, we are confident that our data would benefit from advanced analytical methods that avoid this multiple testing problem.

We did not detect any directional effects (for our hypotheses I-III), which might indicate that the level of behaviour is not as important as behavioural compatibility or, alternatively, it may indicate methodological difficulties in testing for such directional effects. It would be informative to compare our study with field observations where breeding pairs have to cope with an actual con- and/or heterospecific brood predator. Here, the quality of parental behaviours might show a directional impact on offspring survival.

In summary, our results indicate an important reproductive trade-off between dis-assortment in personality and assortment in parental care that needs further investigation. With respect to this trade-off we speculate that behavioural convergence might occur within breeding pairs in our model species though this remains to be tested. Our data indicate further effects between (I) personality and parental care behaviour, (II) parental care and reproductive success, and (III) personality and reproductive success that were not significant on the adjusted significance level (0.001) but on a standard significance level (0.05) (or they tended to be significant on this standard significance level). Therefore, we believe that even more advanced statistical methods and/or a larger sample size might be valuable. Further, we found male-female compatibility to be more important than individual quality per se but we advocate behavioural observations including an actual predatory threat for a better resolution of these two direction of effects. Finally, we shall mention that this is a correlative study not allowing us to draw conclusions regarding causality. Thus, we encourage future studies to follow-up on this work. Further experimental work would especially be interesting with respect to disentangling the direction of effect between parental care and reproductive success (e.g. does the number of fry predict parental effort or does parental effort predict the number of fry?). In conclusion, our results suggest that sexual selection plays an important role in the evolution of personality differences in the rainbow krib and that the combination of parental personalities is important in determining their reproductive success though further work is needed to conclusively determining the general direction of selection.

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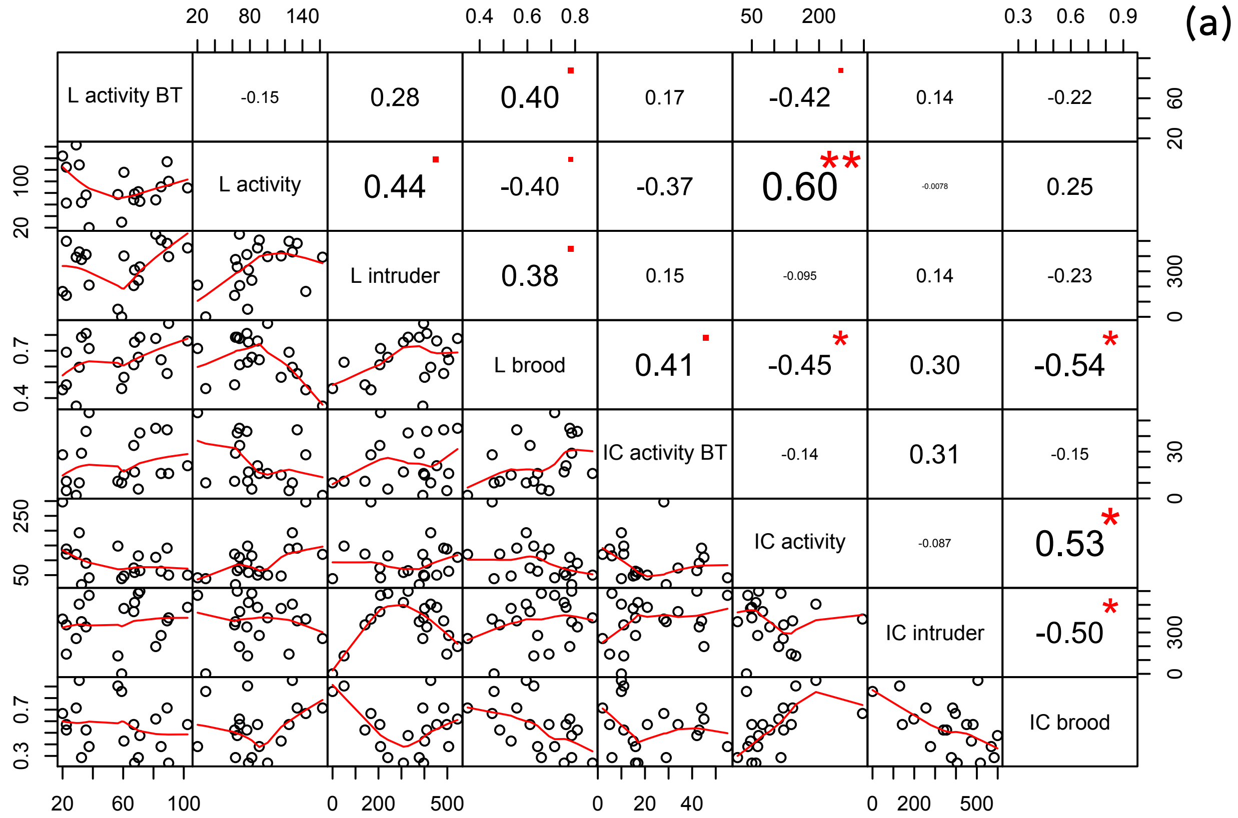
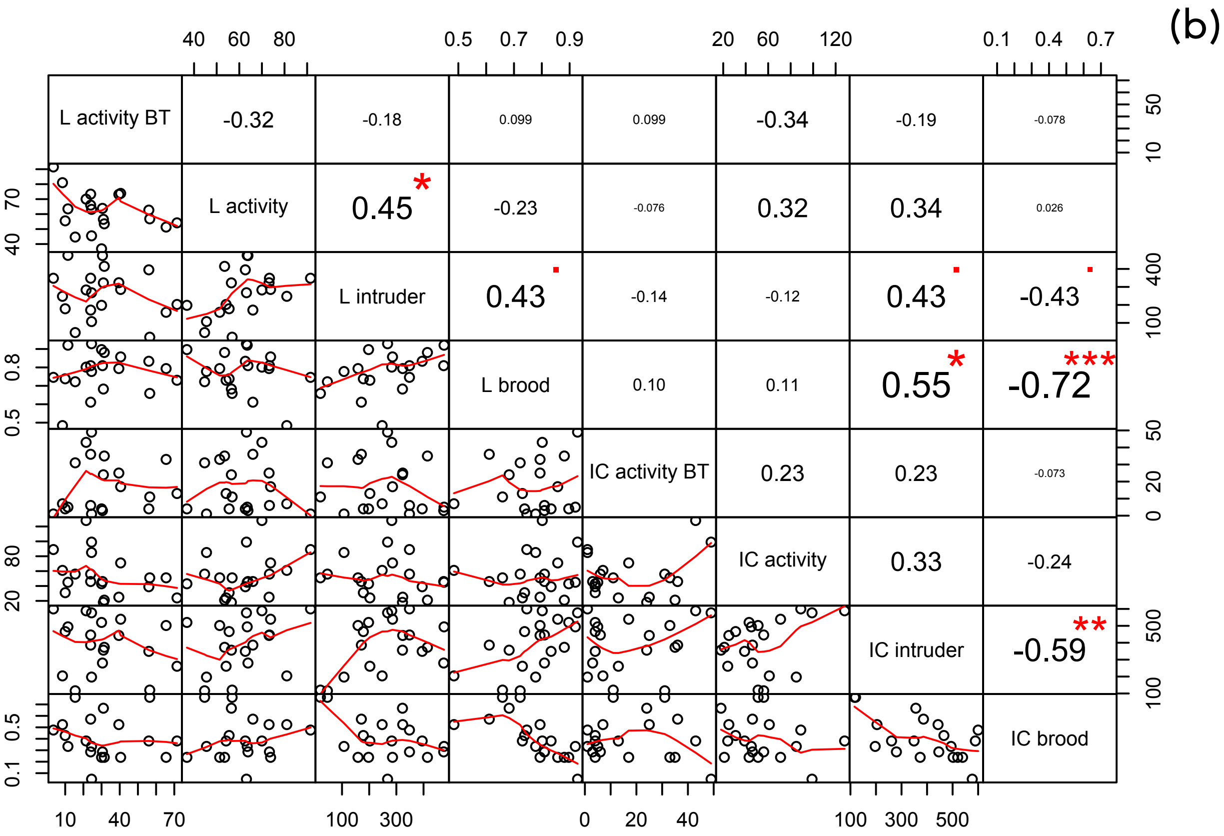
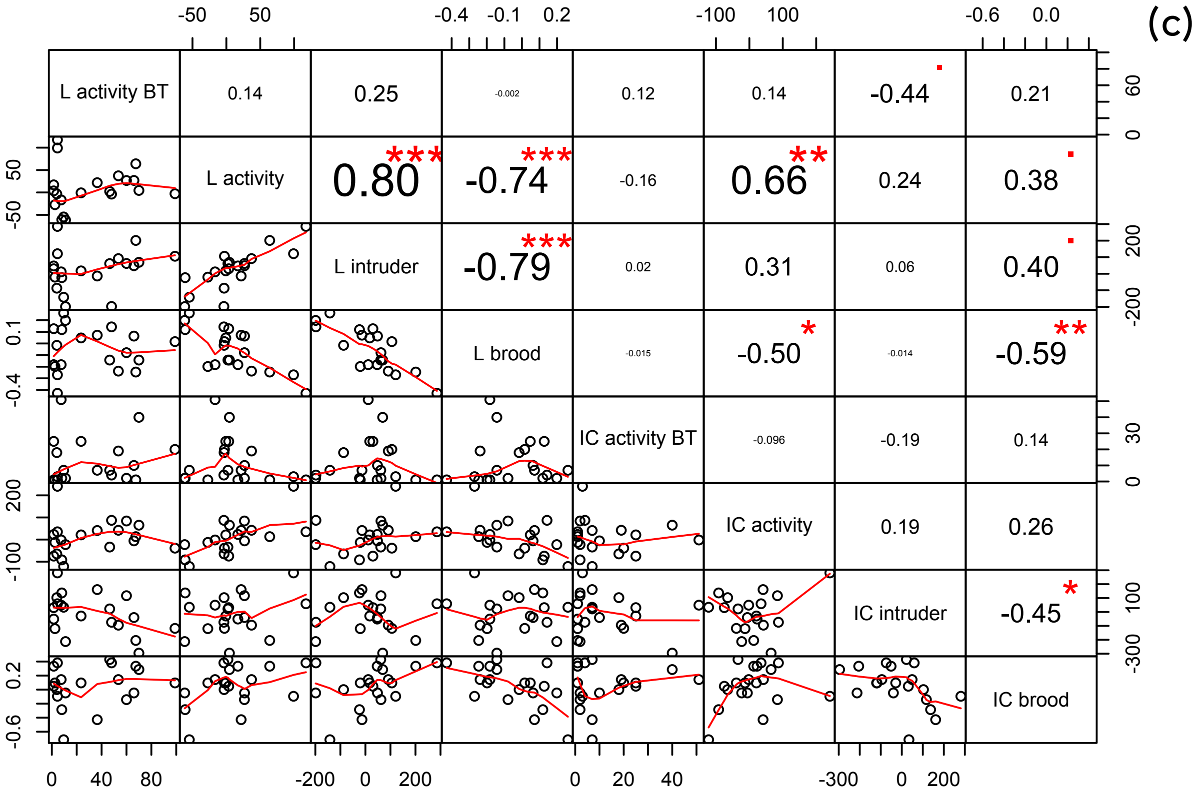
CHAPTER 6 - SUPPLEMENTAL MATERIAL 1



Supplemental Material 1. Correlation matrices for (a) male and (b) female behaviours and (c) male-female behavioural contrasts: L (level of behaviour), IC (inconsistency), BT (boldness test), activity (activity in the presence of the predator (BT) or intruder), intruder (time spent near the intruder), and brood (time spent attending the brood). P-values indicated as: . (0.10), * (0.05), ** (0.01), *** (0.001).

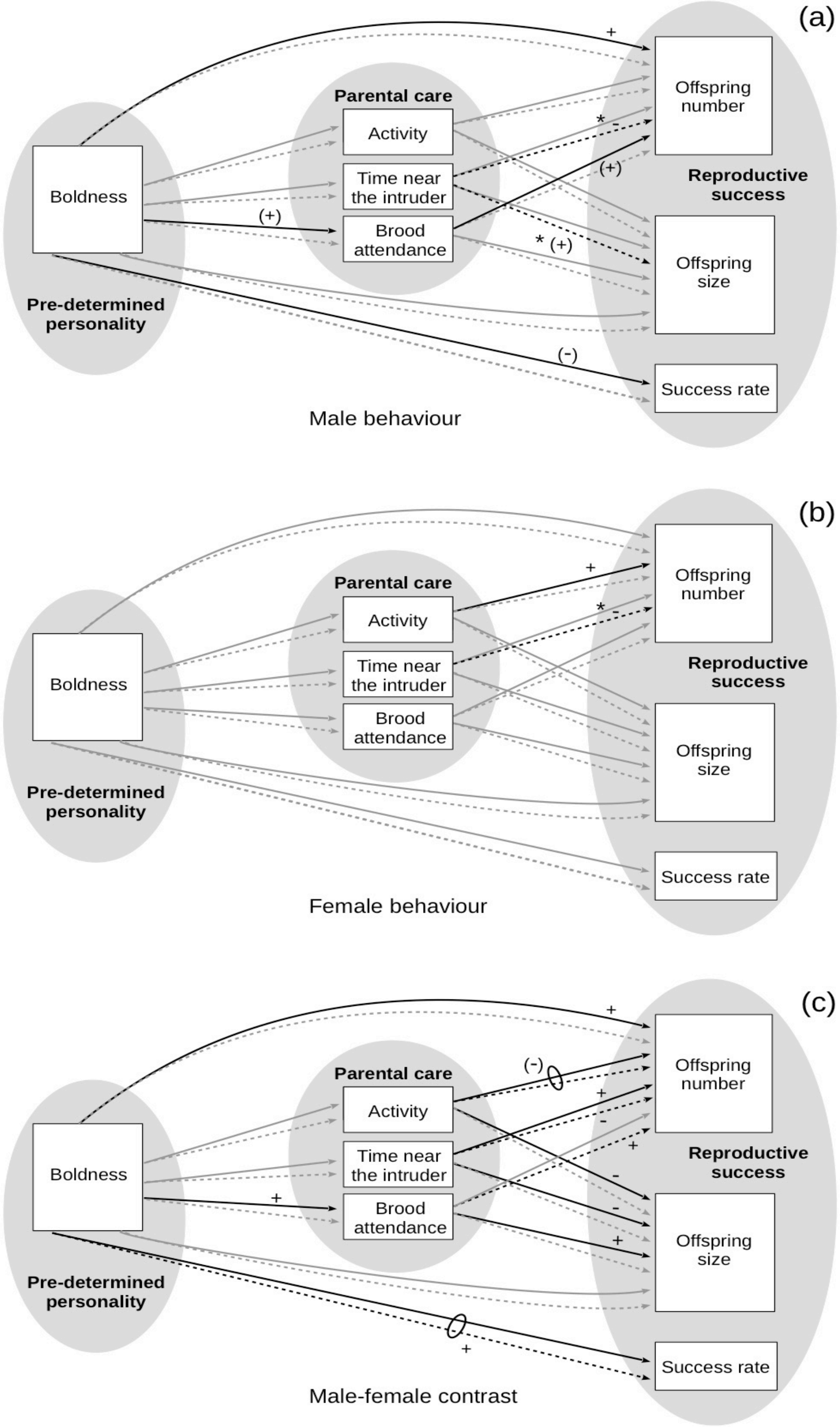


Supplemental Material 1. Continued.



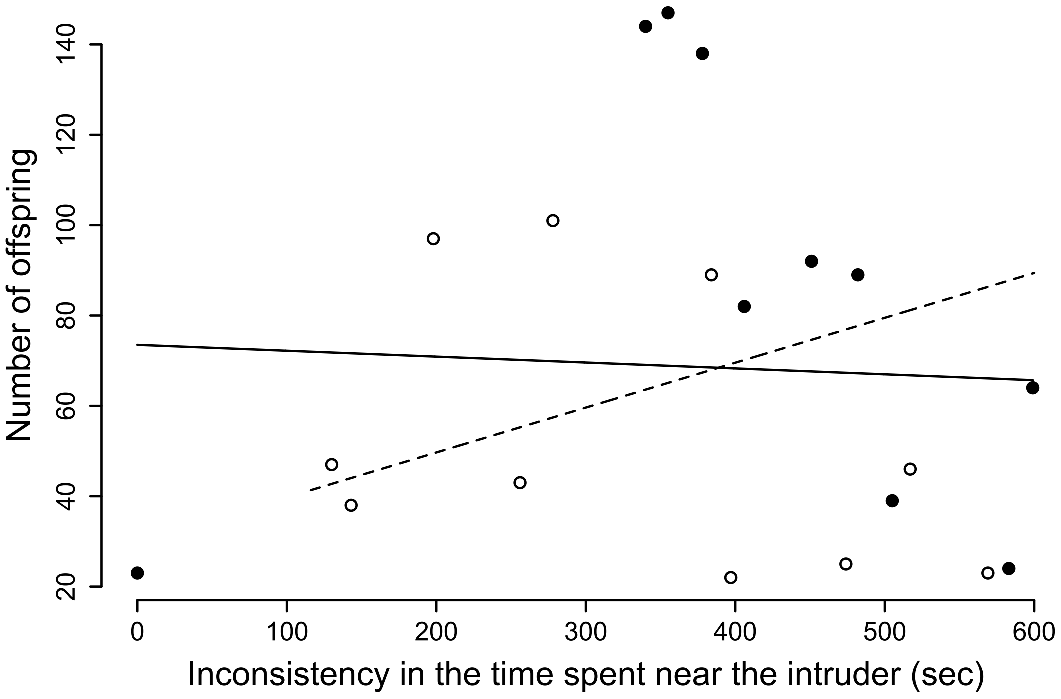
Supplemental Material 1. Continued.

CHAPTER 6 - SUPPLEMENTAL MATERIAL 2



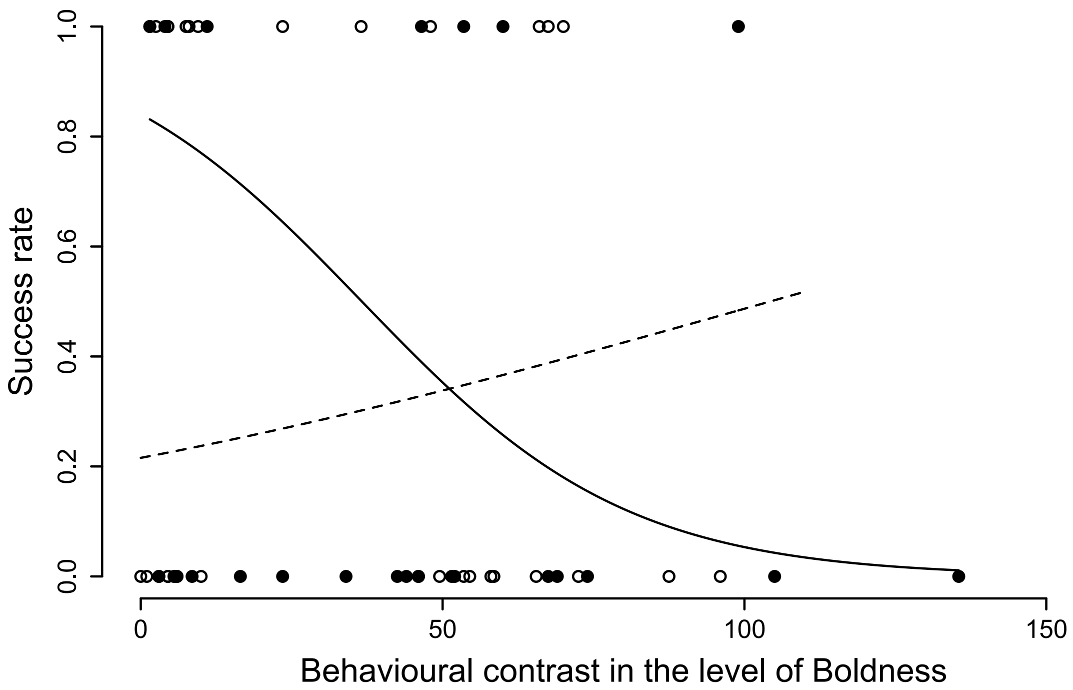
Supplemental Material 2. Graphical illustrations for the results of (G)LMMs testing for directional effects of (a) male and (b) female behaviour, and (c) male-female behavioural contrasts on reproductive success. Effects shown for the level (solid lines) and inconsistency (dashed lines) (in the contrast) of the behaviour. Insignificant effects indicated by grey lines. For significant effects (significance level of 0.05) and trends (black lines) the direction of effect is indicated by "+" (positive) or "-" (negative), brackets indicate the effect tended to be significant. Cycles indicate an interactive effect and (a-b)"*" indicates an interaction between male and female behaviour.

CHAPTER 6 - SUPPLEMENTAL MATERIAL 3



Supplemental Material 3. Male (continuous line, black data points) and female inconsistency (dashed line, data points with white filling) in the time spent near the intruder had an interdependent effect on the number of offspring produced; significance level = 0.05.

CHAPTER 6 - SUPPLEMENTAL MATERIAL 4



Supplemental Material 4. The success rate (likelihood to produce fry) was affected by a breeding pair's contrast in the level of pre-determined boldness in interaction with their contrast in inconsistency; significance level = 0.05. Statistical analysis was performed with continuous variables but for this graphical illustration we divided the contrast in inconsistency into similar (continuous line, black data points) and dissimilar (dashed line, data points with white filling) using a 50% cut-off.

CHAPTER 7

GENERAL DISCUSSION

In this thesis, I aimed to investigate three questions concerning the effect of sexual selection on the evolution of personality variation in the rainbow krib. These were: (I) is male personality sexually selected via female mate choice? (II) Is female personality sexually selected via male mate choice (for boldness only)? And (III) what are the reproductive consequences being associated with the preference pattern (behavioural vs. genetic benefits)? See below for a trait-specific (boldness and aggression) discussion of my results and general conclusions.

Sexual selection and boldness

In my correlative female mate choice study, I found females to prefer males of a dis-similar level and a similar consistency of boldness (Chapter 3). Female preference for dis-assortment in the level of boldness did translate into reproductive success. That is, breeding pairs that were formed dis-assortatively with respect to their level of pre-determined boldness had more offspring compared to assortatively formed breeding pairs (Chapter 6). I proposed, this preference pattern may result in behavioural benefits during offspring care with dis-assortment in the behavioural level facilitating parental role division and assortment in consistency easing the negotiation over fixed vs. flexible roles (discussed in more detail in Chapter 3). But this does not seem to be the case in the rainbow krib as pre-determined boldness did not relate to parental care behaviours (Chapter 6). Instead, dis-assortment in the level of boldness may have genetic advantages, such as increased offspring viability (Charlesworth and Charlesworth 1987; Dingemanse et al. 2004).

Conversely to the above reproductive advantage of dis-assortment in the level of boldness, and conflicting with female preference for dis-assortment in the level of boldness, I found positive assortment in the level of boldness-like parental care behaviour during the breeding (activity in the presence of an animated intruder) to be associated with increased reproductive success, i.e. larger offspring (Chapter 6). I term this parental behaviour 'boldness-like' because the assessment method slightly differed from the original 'boldness' measure regarding the social context (alone vs. family) and regarding the location (test tank vs. current home tank). Despite these differences in the assessment method, I consider both 'boldness' and 'boldness-like' measurements to be biologically meaningful measures that reflect an individual's willingness to engage into risky behaviour (which is the definition of boldness; Wilson et al. 1994). As discussed in **Chapter 6**, these two fitness consequences (i.e. more offspring resulting from dis-assortment and larger offspring resulting from positive assortment) may pose an important reproductive trade-off, which may promote behavioural convergence between the two members of a breeding pair. Importantly, behavioural converge could also explain why pre-determined boldness did not predict boldness-like parental care behaviour. The hypothesis of behavioural (or emotional) convergence is not new in psychological research on humans (e.g. Anderson et al. 2003; Gonzaga et al. 2007; Neumann and Strack 2000) but has received very little attention in non-human animals (but see Laubu et al. 2016). The few empirical work that is available on the topic of behavioural convergence in breeding pairs as well as the potentially important role it may play during the breeding pose the necessity for further empirical work.

Also, my results may imply that there are different investment strategies: assortative mating for few but large offspring vs. dis-assortative mating for many small offspring (**Chapter 6**). That is, larger offspring are generally assumed to have higher survival rates (Rollinson and Hutchings 2013). But producing many, small offspring may also be advantageous if, for example, predation risk is high (Schrader and Travis 2012) or food is very abundant (Winemiller and Rose 1993). Choosing between these investment strategies may allow individuals to adapt to varying environmental conditions (Schrader and Travis 2012).

Female preference for similar consistency of boldness did not translate into reproductive success (**Chapter 6**). Considering that I did find effects of behavioural consistency on reproductive success without the conservative significance level adjustment that I applied due to multiple testings, this could likely be caused by the relatively low number of successfully reproducing breeding pairs. The few studies that considered a potential effect of behavioural consistency on reproductive success found reproductive success to increase with (assortment in) consistency (Botero et al. 2009; Byers 2006; Schuett et al. 2011).

I did not find evidence of male mate choice for female boldness (**Chapter 5**). This is surprising given that dis-assortment in the level of boldness was linked to reproductive success (**Chapter 6**). Thus, female behaviour (in combination with male behaviour) did affect reproductive success. An explanation for the lack of male choice could be that the selective pressure on male choice is lower (compared to female choice) because the costs of reproduction are lower for males. That is, I expected males to be choosy because they invest into costly parental care (discussed in **Chapter 1** and **5**). Yet, reproductive costs might be higher for females due to costly egg production and spawning (Balshine-Earn 1995; Blumer 1986; Trivers 1972). For example, Balshine-Earn (1995) showed that parental care in the Galilee St Peter's fish, *Sarotherodon galilaeus*, was energetically costly for both sexes but females suffered from additional reproductive costs in terms of subsequently decreased fecundity.

Notably, I could not confirm the female preference pattern I found in the correlational experiment (Chapter 2) when performing a non-correlational female mate choice for boldness experiment (Chapter 4). However, female preference for the apparent bold male increased with increasing behavioural contrast in the apparent level of boldness between the two stimulus males that were presented to a female (Chapter 4). This result suggests a causal link between female choice and male boldness but the behavioural contrast between stimulus males may have been not large enough to provoke a response (discussed in Chapter 4). Therefore, it seems worthwhile to conduct a follow-up experiment using a refined methodology.

Sexual selection and aggression

I found female rainbow kribs to prefer consistent high-aggression males (Chapter 2). Further, I found a sex difference in the consistency of aggression with only males showing stable personality variation in aggressiveness but not females (Chapter 2). The sex difference in consistency of aggression is confirmed by another data set that was obtained from the same population (B. Very and W. Schuett, unpublished data). Both the sex difference in consistency and female preference for consistent high-aggression males may be explained by a stronger conservation of male aggressiveness (level and consistency) due to its relevance during parental care (Royle et al. 2010; Schuett et al. 2010) (i.e. territory defence behaviour). In cichlids, breeding pairs have often been described to show a sex-specific role division with males performing more defence behaviours and females providing more direct offspring care (e.g. Itzkowitz 1984; Lavery and Reebs 1994; McKaye and Murry 2008; Richter et al. 2010). Thus, sexual selection may favour the evolution of consistent high-aggression males in the rainbow krib due to behavioural benefits in terms of higher parental quality (i.e. high and constant offspring protection via territory defence), which in turn increases offspring survival. Unfortunately, I could not test this hypothesis because male and female aggressive behaviour did not show stable personality variation when setting up the breeding experiment (Chapter 6). However, the aggression-like parental care behaviour that I obtained during the breeding (time spent near an animated intruder) was repeatable. Similar to the above 'boldness-like' parental care behaviour, I use the suffix '-like' as to indicate that the assessment method slightly differed from the original aggression assessment with respect to context and location (see above). Unlike indicated by the female preference pattern, I found positive assortment in aggression-like parental care behaviour to be associated with increased reproductive success. Notably, I did not test whether pre-determined aggression predicts aggression-like parental care behaviour (as aggression was not repeatable) and can therefore not conclusively say whether and how these two measures are linked. Boldness did not predict boldness-like parental care behaviour indicating that possibly also aggression and aggression-like parental care behaviour may not be correlated.

Only shortly mentioned in **Chapter 2**, I found empirical support for the hypothesis that male consistency of aggression is favoured by female choice but I have to be cautious with respect to the interpretation regarding the level of male aggression. That is because also low aggression males received above average preference scores if they were additionally consistent in their expression of aggression (**Chapter 2**) - seemingly conflicting the above selection trajectory. In my female mate choice experiment, females could always choose between two males. The two males that were presented to a female were classified into consistent vs. inconsistent and low vs. highly aggressive relative to each other. That is, consistent low-aggression males were always paired with inconsistent high-aggression males (vice versa consistent high-aggression males were always paired with inconsistent low-aggression males). Thus, there was no treatment where females could choose between low- and high-aggression males while controlling for consistency (i.e. no fully crossed design). The results provided do therefore not allow me to conclusively interpret female choice for the level of male aggression.

However, I conducted a follow-up experiment where I did test female mating preference for the level of male aggression while controlling for differences in consistency (**Chapter 4**). Conversely, I also set up a treatment where I tested female preference for the consistency of male aggression while controlling for the level of behaviour. Furthermore, I manipulated the level and consistency of male behaviour in this mate choice experiment allowing me to test for causality. Unlike expected, I did not detect female preference for the level or consistency of male aggression. But, I emphasis to cautiously interpret these results as the methodology I was applying here (manipulation of aggression via definition of the distance towards an animated opponent using small plastic cylinders) represents pioneering-work that needs further development (discussed in **Chapter 4**). Alternatively, these results may imply that there is no causal link between female preference and male aggression indicating that aggressiveness of male rainbow kribs is not sexually selected.

Notably, I was working with two different measures for aggressiveness throughout my experimental work: in the beginning, I used the number of aggressive behaviours performed towards an actual opponent (**Chapter 2**, **Appendix 2**) and later, I switched to the time spent in immediate proximity to an animated opponent as an indirect assessment method (**Chapter 5**, **Chapter 6**, **Appendix 3**). For the latter, I found either high - (**Chapter 5**) or no repeatability (**Chapter 6**) for both sexes. These two results contrast each other as well as the results obtained from the first measure of aggressiveness (sex difference in the repeatability of the number of aggressive behaviours, see above). This seems surprising given that holding conditions remained constant throughout and that the results of the two assessment approaches highly correlate with each other (**Appendix 3**). For my experiments, I used a series of three generations of stock fish. Additionally, I had to buy fish (mainly females because of a highly skewed sex ratio in our stock) from several external suppliers. Possibly, differences in the stability of the social environment or in the genetic background (discussed in **Chapter 1**) may have caused the discrepancy in the repeatability of aggressiveness. However, my measure of boldness was repeatable throughout (**Chapter 3-6**).

Conclusions

Central to my studies was (a) to identify the direction of selection acting on male and female personality differences (directional mate choice for compatibility vs. (dis-) assortative mate choice for compatibility) and (b) to identify the reproductive benefit(s) being associated with parental personality differences (behavioural vs. genetic benefits). Direction of selection and associated reproductive benefits differed between pre-determined personality and personality measures obtained during the breeding.

During the breeding, I found positive assortment in aggression- and boldness-like parental behaviours (time spent near an animated intruder and activity in the presence of the intruder) to be associated with increased reproductive success (offspring size) (**Chapter 6**). This finding is in line with other studies that found an assortative mating preference for behavioural traits (Kralj-Fišer et al. 2013; Montiglio et al. 2016) or increased reproductive success of assortative breeding (Ariyomo and Watt 2013; Both et al. 2005; Laubu et al. 2016; Schuett et al. 2011). Behavioural benefits arising from assortment in parental care are likely (Schuett et al. 2010), yet, also genetic benefits are possible as breeding pairs raised their genetic offspring and repeatable parental care behaviour has a genetic component (Bendesky et al. 2017; Freeman-Gallant and Rothstein 1999; Maccoll and Hatchwell 2003). Further experimental work using a cross-fostering approach may provide valuable insights in resolving genetic from behavioural benefits.

For the level of boldness determined before the breeding, I found dis-assortment to be advantageous, i.e. females showed a dis-assortative mating preference for the level of male boldness (**Chapter 3**), which translated into increased reproductive success in terms of a higher number of offspring (**Chapter 6**). In a meta-analysis on genetic and phenotypic traits in varies taxa, Jiang et al. (2013) found dis-assortment to be very rare; and their simulations suggested these might be false positives. Yet, I found dis-assortment for the level of boldness to be beneficial in two separate experiments (**Chapter 3** and **6**) indicating the effect is more than a false positive. I did not find behavioural benefits for dis-assortment. But dis-assortment may have genetic benefits, e.g. it may lead to heterozygote offspring that is more viable (Schuett et al. 2010). However, I cannot conclusively say what the mechanism is that links reproductive success and pre-determined boldness with each other; calling for further investigation.

I found the direction of female choice to differ between the two personality traits of interest (directional for aggression and (dis-) assortment for boldness). This is especially interesting since aggression and boldness are often reported to be correlated in an aggression-boldness syndrome (Koolhaas et al. 1999; Norton and Bally-Cuif 2012; but see Moss et al. 2015). I aimed to test for such an aggression-boldness syndrome in my breeding experiment (**Chapter 6**) but had to omit this test because aggression was not repeatable in that experiment (see above). Repeatability of behaviour is a precondition for behavioural traits to form a syndrome (Dingemanse et al. 2010; Sih et al. 2004a; Sih et al. 2004b). However, I did find boldness-like and aggression-like parental care measures to be correlated with each other (**Chapter 6**, Supplemental Material 1) indicating that also pre-determined boldness and - aggression might be correlated. Future work on personality differences in the rainbow krib may investigate the repeatability of aggression in more detail and may test for a potential aggression-boldness syndrome, including the implications resulting from it.

Taken together, my results provide evidence that sexual selection affects personality differences in the rainbow krib. However, the overall direction of selection is not that clear. I found positive assortment in the level of aggression- and boldness-like parental care behaviour to be associated with larger offspring (possibly due to behavioural benefits). But dis-assortment in pre-determined boldness was associated with a higher number of offspring (possibly du to genetic benefits). On the long run, positive assortment could have a stabilising effect on personality variation while dis-assortment may erode variation (given there are no other selective pressures; Schuett et al. 2010). Importantly, offspring size and – number were negatively correlated with each other indicating a reproductive trade-off. Dis-assortment for many but small offspring and positive assortment for few but large offspring may represent different investment strategies. However, the effects of behavioural assortment vs. dis-assortment relate to different contexts (parental care vs. pre-determined behaviour) and boldness shown in these two contexts was not correlated, i.e. breeding pairs may mediate between the reproductive trade-off via behavioural convergence. Cleary, further investigations are needed to elucidate the possible reproductive strategies and their implications. Further, my female mate choice experiments indicate that not only the level but also the consistency of both personality traits of interest (aggression and boldness) is under sexual selection though the reproductive consequences of consistency remain to be shown. Similarly, it remains to be tested whether and how female preference for the level of male aggressiveness translates into reproductive success. The strength of selection acting on male and female behaviour may differ due to differences in choosiness between the sexes. The results presented here are a first step in understanding how sexual selection shapes personality variation in the rainbow krib; but further work is needed for a better understanding of the complex relationship between personality differences and sexual selection in this species.

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APPENDIX 1

Artificial Visible Implant Elastomer (VIE) tags of different colour and symmetry do not influence mate choice in a cichlid

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Abstract

Experimental individuals are frequently marked with coloured tags for individual identification. Except for birds, the consequences of such artificial tagging on mate choice have been rarely investigated even though individuals often prefer naturally brightly coloured or symmetrically ornamented mates. We tested whether differently coloured Visible Implant Elastomer (VIE) tags influence female mate choice in rainbow kribs, *Pelvicachromis pulcher*. Females were allowed to simultaneously choose between a control and a VIE-marked male. The VIE-marked male carried two tags of the same colour (red, blue, green or white) set symmetrically or asymmetrically. Females did not show a preference for or avoidance of males carrying any of the colours compared to control males, no matter if the tags had been set symmetrically or asymmetrically. Although we found no discrimination for or against colour-tags, we highlight the importance of considering potential influences of colour-marks on mate choice in behavioural and evolutionary studies.

1. INTRODUCTION

Females often choose their mate on the basis of secondary sexual traits, such as size or conspicuous, extravagant ornamentation (Andersson 1994). For example, female sticklebacks, *Gasterosteus aculeatus* (Milinski and Bakker 1990), and female Trinidadian guppies, *Poecilia reticulata* (Godin and Dugatkin 1996), favour brightly coloured over dull males, and pied flycatchers, *Ficedula hypoleuca*, prefer males with large ornaments (Sirkiä and Laaksonen 2009). In addition, females generally show a preference for bilateral symmetry in those secondary sexual traits that tend to be naturally symmetric (Little et al. 2008; Moller and Thornhill 1998; Simmons et al. 2004; Swaddle and Cuthill 1994a). Bilateral symmetry is thought to be a quality indicator (Brookes and Pomiankowski 1994; Little et al. 2008), as it likely shows the ability to resist stress during environment (e.g. Parsons 1990; Parsons 1992). Similarly, elaborate, extravagant ornaments are assumed to be a reliable quality indicator, since they are linked to condition (Milinski and Bakker 1990) and can be costly to produce and maintain (Godin and McDonough 2003; Zuk and Kolluru 1998).

Research in behavioural or evolutionary ecology often requires visual identification of study animals. Frequently, individuals are marked with differently coloured marks or tags, for instance differently coloured leg bands, ear-clips or subcutaneously injected marks. Previous studies on zebra finches, *Taeniopygia guttata* (reviewed in Schuett and Dall 2010; but see Seguin and Forstmeier 2012), and other bird species (Goforth and Baskett 1965; Hagan and Reed 1988) have demonstrated that this can be problematic, since artificial colour marks can act in the same way as natural ornaments and can consequently affect mate choice, other social interactions and life-history traits. Zebra finches in the wild (Burley 1988) and in the laboratory (Burley et al. 1982; but see Jennions 1998), for example, preferred red-ringed over unringed males but avoided males carrying light-blue or light-green rings. Red-cockaded woodpeckers, *Picoides borealis*, with red rings had lower reproductive success than males without red rings (Hagan and Reed 1988). More generally, colours, which occur naturally in the species' ornament might be beneficial, those that are atypical might be detrimental during mate choice (Burley 1986). Studies have also shown that females prefer males that carry symmetric leg bands over males with asymmetric coloured leg bands in zebra finches (Swaddle and Cuthill 1994b; but see Jennions 1998) and bluethroats, *Luscinia s. svecica*, (Fiske and Amundsen 1997). Similarly, male bluethroats prefer females with symmetric coloured leg bands over females with asymmetric ones (Hansen et al. 1999).

Visible Implant Elastomers (VIE; VIE-Northwest Marine Technology, Shaw Island, WA, USA) are frequently used to mark individuals for visual identification. This marking method has been applied in a wide range of vertebrate and invertebrate taxa, for instance fish (Bailey et al. 1998; Bruyndoncx et al. 2002; Woods and Martin-Smith 2004), cephalopods (Brewer and Norcross 2012), decapods (Godin et al. 1996), arachnids (Chapin 2011; Melo et al. 2013), echinoderms (Martinez et al. 2013), amphibians (Bailey 2004; Sapsford et al. 2015), reptiles (Penney et al. 2001), insects (Moffatt 2013) and earthworms (Butt and Lowe 2007). Previous studies have assessed the influence of VIE marking on growth (Olsen and Vøllestad 2001; Simon and Dörner 2011; Soula et al. 2012), survival

(Olsen and Vøllestad 2001; Roberts and Angermeier 2004; Simon and Dörner 2011), predation (Bouska and Paukert 2010; Reeves and Buckmeier 2009) and stress response (Fürtbauer et al. 2015). Yet, to our best of knowledge, no study has ever tested the potential influence of VIEs on mate choice. This is surprising, given the above mentioned influences of other colour marking methods on mate choice in birds, the general role of colouration and symmetry of coloured secondary sexual traits in mate choice as well as the frequent use of VIEs in behavioural studies (Bertucci et al. 2010; Casalini et al. 2010; Colléter and Brown 2011; King et al. 2014; Zhou and Fuller 2016). Only two studies to date have assessed the influence of VIEs on social behaviour in a non-mating context: shoal-choice. While Croft et al. (2004) showed no influence of VIE tags on shoaling in guppies, *P. reticulata*, Frommen et al. (2015) showed that zebrafish, *Danio rerio*, spent more time with a shoal consisting of VIE-tagged individuals than with a shoal consisting of untagged individuals. The latter finding again highlights the importance of assessing the influence of VIE colour tags on mate choice.

Here, we tested the influence of differently coloured VIEs as well as asymmetrical versus symmetrical application of VIEs on female mate choice in a bi-parental and territorial West-African cichlid, the rainbow krib, *Pelvicachromis pulcher*. In eight different treatment groups females could choose between control (unmarked) and marked (with one of four colours) males in binary choice tests; the tags of the marked males were set either symmetrically or asymmetrically. Rainbow kribs are highly suitable for this study since they are very colourful and sexually dimorphic in colour patterns. Such characteristics often suggest mate choice based on colouration (e.g. Andersson 1994). Furthermore, male rainbow kribs show a colour polymorphism that is linked to different male reproductive tactics (Martin and Taborsky 1997), suggesting that female choice on male colouration may directly affect female reproductive success and should therefore be important for females.

2. MATERIAL & METHODS

2.1. Study animals and holding conditions

Experimental fish were obtained from a captive breeding stock at the University of Hamburg. Due to a very strong male-biased sex ratio in our captive population, about 50% of the focal females were obtained from a commercial supplier. Fish were maintained in groups of siblings (individuals from breeding stock) or groups of origin (individuals from supplier), respectively. All fish were kept under standardised holding conditions (100 x 50 x 25 cm or 50 x 50 x 25 cm tanks, 26 ± 1°C water temperature, aerated and filtered water, weekly water changes, 12:12 h light/dark cycle with full spectrum fluorescent light) and were fed 5 days a week with *Artemia* ssp. During experiments, females were kept in single-sex groups (50 x 50 x 25 cm tanks); males were maintained individually (50 x 25 x 25 cm tanks). Sexes were distinguished based on their dimorphism in colouration, size (Martin and Taborsky 1997) and body shape. Colouration of a typical *P. pulcher* male in our population is shown in Figure 1a. The mean ± SE total length of experimental males was 50.89 ± 0.68 mm and of experimental females 44.70 ± 1.13 mm.

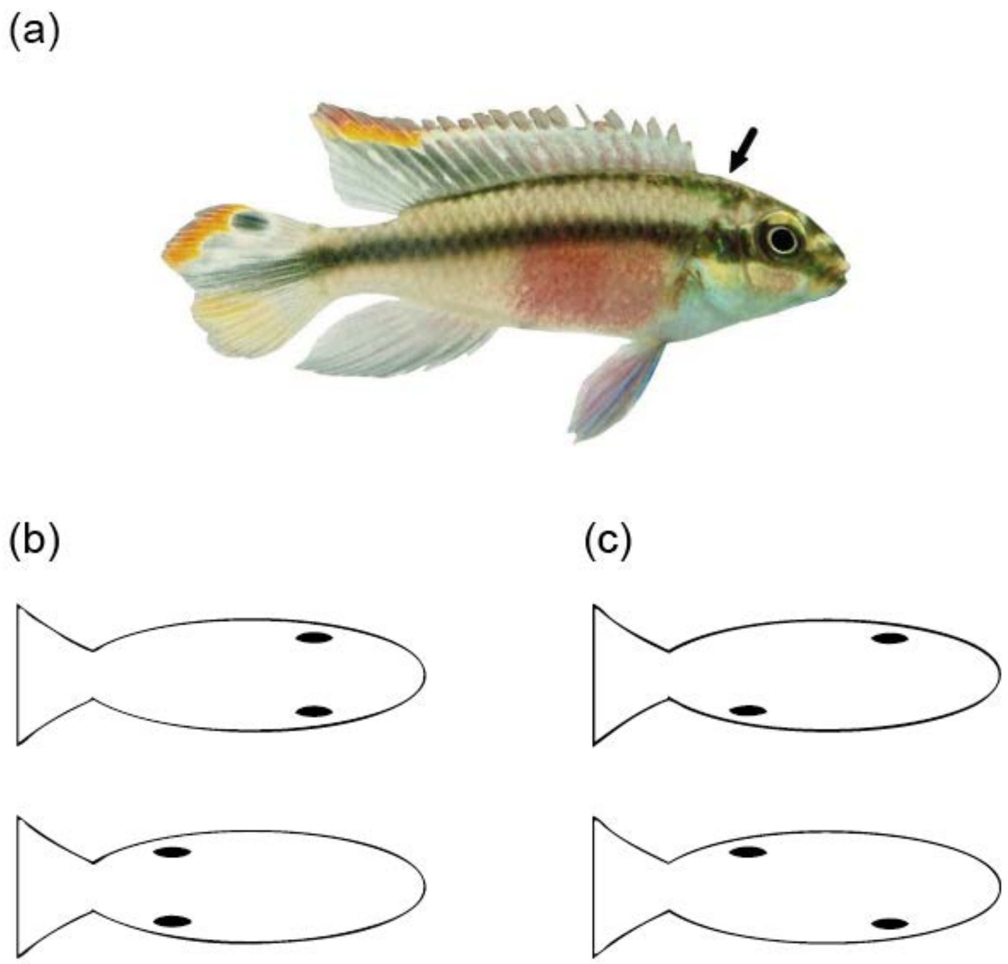


Figure 1: (a) A typical male from our captive *Pelvicachromis pulcher* population, carrying a green VIE-tag (indicated by arrow). Positions of VIE-tags (black dots) in (b) symmetrical and (c) asymmetrical treatments. A fish received two tags of the same colour (red, blue, green or white) at either of the two shown combinations of positions of the symmetrical and asymmetrical treatment, respectively. (b), (c) Schematic view of fish from above.

2.2. Experimental procedure

Females (N = 22) were allowed to simultaneously choose between two males, one VIE-marked male and one unmarked male. Each marked male (N = 64) carried two VIE tags of the same colour: red (N = 16), blue (N = 16), green (N = 16) or white (N = 16). The VIE tags were arranged either symmetrically (N = 8 males per colour; Figure 1b) or asymmetrically (N = 8 males per colour; Figure 1c), leading to eight different treatment groups. VIEs had been set subcutaneously (length approx. 2–3 mm; see Figure 1a) at least 24 h before mate choice trials. Unmarked males (N = 64) had been handled in the same way as marked males: a needle was briefly inserted under their skin to ensure any treatment effects were not caused by different handling. Males within a test pair were matched for family and size, with a size difference of <4.5% in total length (mean ± SE difference: 1.1 ± 0.001%), but were otherwise randomly chosen. We conducted 16 trials per treatment: each male pair (N = 64 pairs) was used in two trials, each with a different, unrelated female, resulting in a total number of 128 mate choice tests. The two females that saw the same male pair were not related to each other (i.e., no siblings). Within a treatment, a different female was used for each trial. Among treatments, females were tested multiple times (range 2–8 times), since we did not have enough females to use different sets of females. We tested female mate preferences via binary choice test (standard procedure for testing mate preferences; Schlupp et al. 1994; Schlüter et al. 1998). The experimental tank (100 x 35 x 30 cm, water level: 10 cm) was partitioned into three compartments: a female compartment (60 x 35 cm) in the middle and two male compartments on the outer sides (20 x 35 cm). Before a trial, the compartments were separated using clear dividers (permanent) and opaque dividers (removable). The female compartment was divided into three zones, with a neutral zone (40 x 35 cm) in the middle and two preference zones at the adjacent sides (width of preference zones corresponded to two fish lengths, 10 x 35 cm), next to the male compartments.

To begin a trial, the focal female was introduced into the middle of the neutral zone using a clear Plexiglas cylinder (diameter 10 cm) and the males of a randomly chosen pair were placed into a male compartment each. After an acclimatisation period of 10 min, the opaque dividers were removed and the female was allowed to watch the males for 1 min. Then, the cylinder was removed and the first trial part of 17 min began. After this first part of the trial, positions of the males were switched to test for any side-bias by the female. After switching the positions of the males, all three fish were allowed to acclimate again for 10 min (following 1 min in the cylinder with visual contact) before the second part of the trial began, lasting again 17 min. In order to avoid disturbances, the experimental tank was surrounded with black plastic foil (Schlüter et al. 1998) and no observer was present during trials. Instead, all trials were video-recorded from above.

We determined the association time with each male, i.e., the time a female spent in the preference zone of a male, from videos using the software EthoVision XT 11 (Noldus, Wageningen, The Netherlands). The association time is a decent predictor for mating preference in cichlid fishes (Dechaume-Moncharmont et al. 2011; Thünken et al. 2007). Tracking always started 2 min after the start of a trial part for two reasons: first, removal of the cylinder could disturb the experimental individuals briefly; second, the removal of the cylinder sometimes caused small undulations of the water, interfering with the tracking of the software. Therefore, we tracked focal females for 15 min, leading to a total of 30 min analysed video data per trial.

2.3. Data analysis

We assessed whether VIE marking influenced mate choice using two linear mixed effects models (LMMs) with the total association time with each male as response variable. We checked that model assumptions were not violated using diagnostic plots. In both models we included the male ID nested within male test pair and nested within male family as well as female ID nested within female family as random terms. In the first analysis we assessed whether there was a general preference for or avoidance of colour-marked males, regardless of the treatment. The type of male (marked versus control male) was added as fixed term. Even though we had matched males for size, we added male total length as covariate to account for any potential effect of even small size differences between males on mate choice. In the second analysis, we assessed whether the treatments differently influenced mate

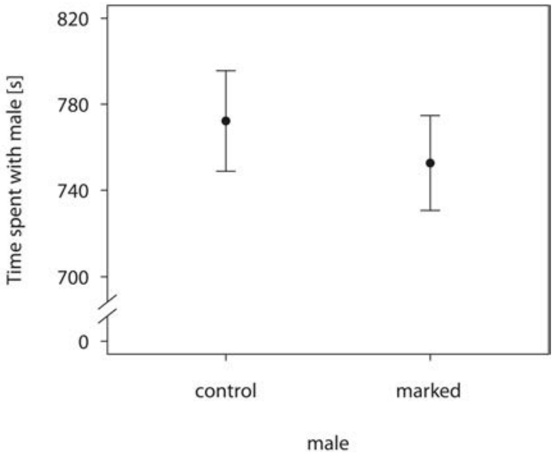


Figure 2: The mean (± SE) time females spent with a control and VIE-marked male during mate choice trials. Mean (± SE) based on raw data. N = 121 trials.

choice. This was important since some colours could positively, some negatively influence mate choice, resulting in no overall preference for or avoidance of colouration. In the second analysis we added the three-way interaction between the type of male, the colour of VIE tags of the marked male (blue, green, red, white) and the arrangement of the VIE tags of the marked male (symmetric versus asymmetric) as well as their two-way interactions and their main effects as fixed terms. Note that in this analysis only results of interactions including the type of male are meaningful, since colour and symmetry treatment by themselves are only characteristics of the trial and not necessarily of the individual (which is characterised via 'type of male'). Male size was not included as covariate in this analysis since there was no effect of size in the first analysis (see '*Results*').

Maximal models were step-wise simplified using likelihood ratio tests, starting with highest level interactions (Crawley 2007). All analyses were conducted in R (R Core Team 2016). Seven mate choice trials were excluded from analyses because of female side-bias, lost VIE tag or wrong sexing of an individual, leaving 121 mate choice trials for analyses. A female was defined as being side-biased within a trial when she spent more than 80% of the trial in just one preference zone, regardless of which male was placed there (Schlupp and Ryan 1997; Schlüter et al. 1998).

3. RESULTS

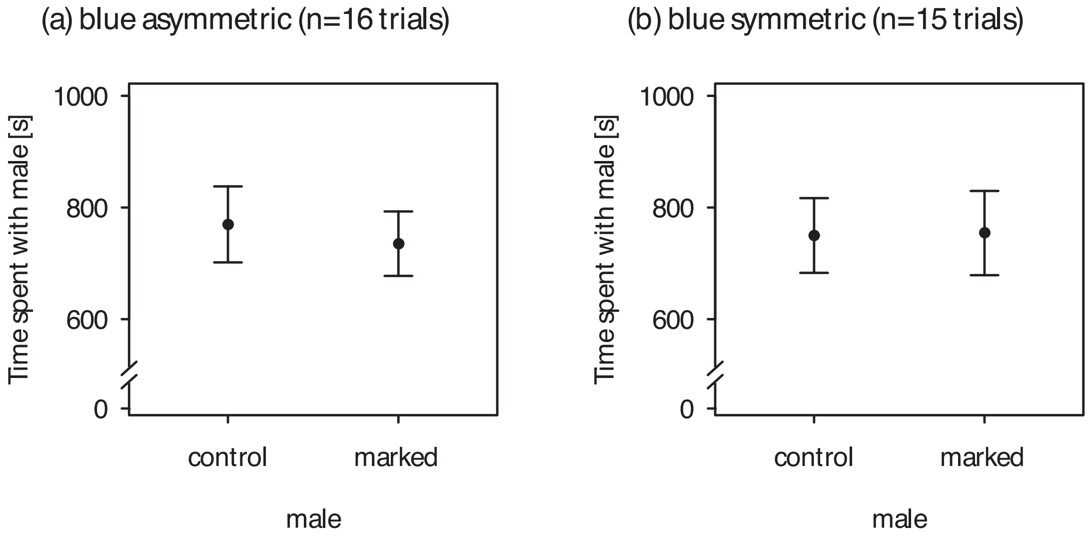
Females spent on average 84.9 ± 0.01% (mean ± SE) of the time with either male. Overall, females did not show a preference for marked or unmarked males (Figure 2; Table 1, model 1). Male size did not affect female choice (Table 1, model 1). Also, female mate choice was independent of the combination of colouration and arrangement, the specific VIE colouration and whether VIEs were arranged symmetrically or asymmetrically (male type ± colouration x arrangement; male type x arrangement; male type x colouration; Figure 3; Table 1, model 2).

4. DISCUSSION

We found no evidence that female *P. pulcher* consider artificial VIE colour marks during mate choice: neither the colouration nor the arrangement of marks (asymmetric or symmetric) influenced female mate choice. One interpretation of these results could be that colouration is not important for mate choice in *P. pulcher* (at least those colours we used for marking). This explanation, however, seems very unlikely because there is strong sexual dimorphism in colouration between the sexes in *P. pulcher*, and different male colour morphs show different mating tactics (Martin and Taborsky 1997), suggesting that female rainbow kribs should consider male colouration during mate choice. Colouration seems generally important for mate choice in cichlid species (Martin 2013; Salzburger et

Table 1. Summary of test statistics from LMMs with the time a female spent with each male (control versus VIE-marked male) as response.

Model	Fixed effect	Factor level	Coefficient	SE	χ^2	df	*p*
1	(Mean)	[Male type = control]	741.47	124.13			
	Male type		-19.54	33.50	0.35	1	0.56
	Male total length		0.29	2.34	<0.01	1	0.98
2	(Mean)	[Male type = control; colour = blue; arrangement: asymmetric]	751.22	69.26			
	Male type		-34.56	92.23	0.35	1	0.56
	Colouration				(1.32)	(3)	(0.72)
		Green	-96.58	93.65			
		Red	60.22	92.27			
		White	-8.47	92.27			
	Arrangement				(<0.01)	(1)	(0.98)
		Symmetric	-19.41	93.60			
	Male type: colouration				5.07	3	0.17
		Marked male: green	191.49	132.36			
		Marked male: red	-62.69	130.43			
		Marked male: white	68.88	130.43			
	Male type: arrangement				1.00	1	0.32
		Marked male: symmetric	39.53	132.36			
	Colouration: arrangement				(0.18)	(3)	(0.98)
		Green: symmetric	60.63	135.88			
		Red: symmetric	-41.11	133.71			
		White: symmetric	177.04	131.40			
	Male type: colouration: arrangement				6.27	3	0.10
		Marked male: green: symmetric	-166.71	192.16			
		Marked male: red: symmetric	83.86	189.06			
		Marked male: white: symmetric	-324.53	185.82			



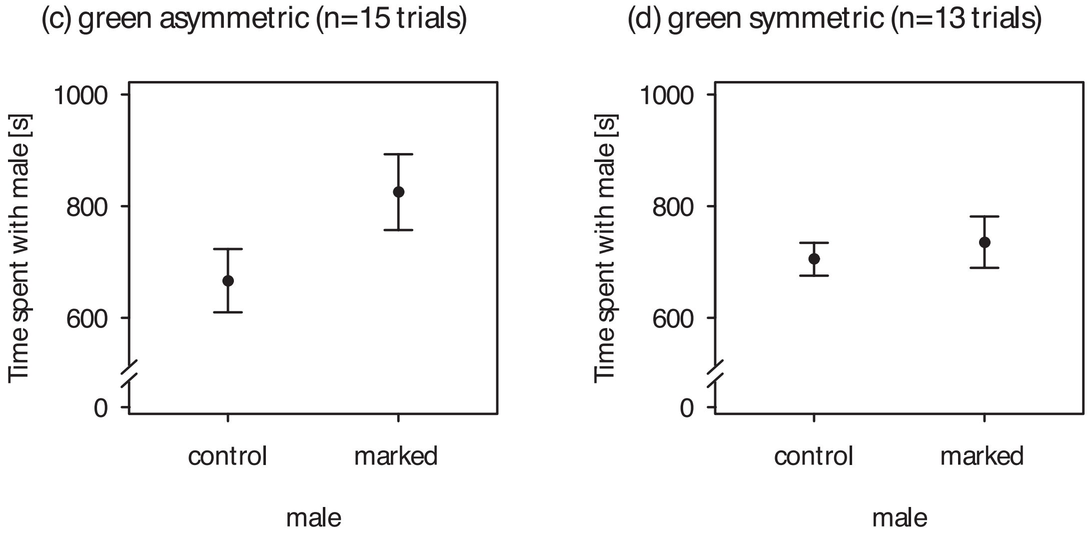
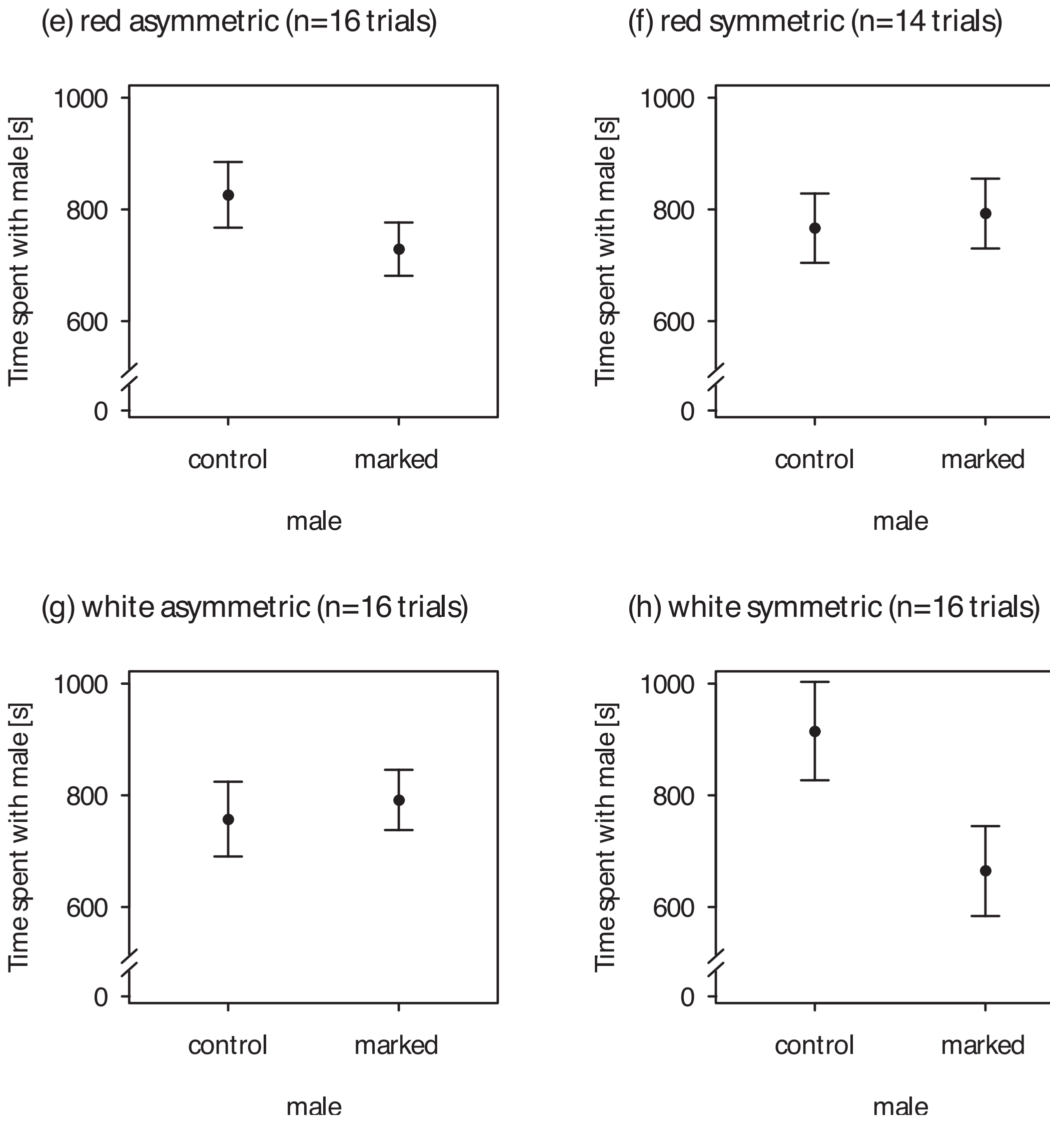


Figure 3. The mean (± SE) time a female spent with a control and VIE-marked male during mate choice trials. Marked males carried two blue (a, b), green (c, d), red (e, f) or white (g, h) VIE marks in an asymmetrical (a, c, e, g) or symmetrical (b, d, f, h) manner. Mean (± SE) based on raw data.

al. 2006; Seehausen and van Alphen 1998; Selz et al. 2014), including the closely related, similarly ornamented sister species *P. taeniatus* (Baldauf et al. 2009). Furthermore, the colours we used (blue, green, red and white) are to some extent (red and white more, blue and green less) included in the natural ornamentation of *P. pulcher* (at least to the human eye). An absence of mate choice based on artificial colour marks could also arise if females are not motivated to choose a mate. Yet, females with strong side-bias were excluded from analysis and females spent about 85% of time close to the males. While a high association time with males could also indicate a mere social preference, association times near males have been shown to predict female preferences for spawning partners in cichlids (e.g. Dechaume-Moncharmont et al. 2011).

In contrast to our study on fish, some studies on birds found strong effects of colour marks on mate choice (e.g. Burley et al. 1982; Fiske and Amundsen 1997; reviewed in Schuett and Dall 2010). These studies used other marking methods, mainly leg bands. This could indicate that different


(e) red asymmetric (n=16 trials)

(f) red symmetric (n=14 trials)

(g) white asymmetric (n=16 trials)

(h) white symmetric (n=16 trials)

Figure 3. Continued.

colour marking methods vary in their impact on mate choice. Nevertheless, effects of coloured leg bands on mate choice in birds are also ambiguous: while some studies found effects, others did not (reviewed in Schuett and Dall 2010). Consequently, the marking method alone cannot explain different results. Factors, such as the location or the size of the marking might also play an important role. For instance, the size of the marking relative to the body or ornament size of the study species might be crucial. Furthermore, different species vary in their visual system and colour vision, including the colour spectrum that can be detected (Endler 1990). Therefore, the same colour tags likely influence mate choice of different taxa differently. Effects likely also vary with the environmental conditions, such as conspicuousness against the background, light regimes or transmission properties of the habitat (e.g., aquatic versus terrestrial, Endler 1990). This makes it difficult to generalise the effect of artificial colour marking on mate choice across species, for instance, from terrestrial to aquatic species. Thus, when effects of colour marks are unknown under test conditions and in the study species, potential effects of the marks on behaviour should be ideally tested prior to the study. If this is not possible, we recommend at least using colours that are not part of the natural ornamentation, equally distributing the colours across treatments and statistically controlling for potential effects in analyses. If there is large between-individual variation in ornamentation, natural colour patterns might be used for individual identification rather than artificial colour marking. Yet, unless individual differences in ornamentation are really distinct, using natural colour patterns to distinguish among individuals might be unreliable and prone to error. *P. pulcher* individuals, for instance, can quickly change the intensity of their colouration, when competing for a resource (W. Schuett and U. Scherer, personal observations), making visual identification based on natural colour patterns very difficult in this species.

Here, we tested for the first time whether VIE colour marks affect mate choice. Our results suggest that artificial VIE colour marks do not influence mate choice of *P. pulcher*. These results are quite promising for future mate choice and behavioural studies, which often require individual identification. Nevertheless, more studies are now needed to shore up our findings and to identify species as well as environmental conditions in which VIE colour tags do or do not influence behaviour. In general, neutrality of VIE colour tags or any other marking method should be validated for each species prior to behavioural studies, especially under those circumstances in which colouration is generally known to impact behaviour, such as during mate choice.

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APPENDIX 2

Lateralisation in agonistic encounters: Do mirror tests reflect aggressive behaviour? A study on a West African cichlid

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ABSTRACT

In this study, population level lateralisation and the suitability of mirror tests as a test of natural aggressive behaviour in male rainbow kribs *Pelvicachromis pulcher* was investigated. Aggressive behaviour in live agonistic trials correlated positively with behaviours towards a mirror image and no visual lateralisation was detected.

BRIEF COMMUNICATION

Mirror tests are widely used to assess agonistic behaviour in a broad range of taxa, including amphibians (Furtado and Nomura 2014), mammals (DeCaluwe et al. 2013), reptiles (Farrell et al. 2002) and birds (Schuett et al. 2011). Especially in behavioural research on fishes, the method is frequently used to determine aggressive behaviour towards conspecifics (Archard and Braithwaite 2011; Vøllestad and Quinn 2003). There is no evidence for an ability of self-recognition in a mirror in fishes, making mirror tests potentially suitable for assessing aggressive behaviour (Desjardins and Fernald 2010). Indeed, replacing a real opponent with a mirror image does have some striking advantages. The (apparent) opponent is completely matched in size, behavioural variance of the focal fish that is caused by the opponent's behaviour is reduced, less study animals are needed and stressful side effects (*e.g.* marking and injury) are avoided (Balzarini et al. 2014; Johnsson et al. 2003). The ubiquitous suitability of mirror tests, however, has recently been questioned (Arnott et al. 2011; Balzarini et al. 2014; Earley et al. 2000; Elwood et al. 2014). Behavioural responses towards a mirror image and towards an actual opponent correlated in some species (Ariyomo and Watt 2013; Balzarini et al. 2014) but not in others (Balzarini et al. 2014). Also, responses towards a mirror image and an actual opponent were associated with different hormonal responses (Oliveira et al. 2005) and different brain activities (Desjardins and Fernald 2010). A potential reason for discrepancies in results among studies and species might be differences in lateralisation of aggressive behaviours (Ariyomo and Watt 2013; Arnott et al. 2011).

Cerebral lateralisation has been detected in different species (Rogers 2002). That is, the left and right hemisphere of the brain differ in their cognitive functions, leading to a cerebral asymmetry, which affects the processing of visual information (since the sensory input coming from the left and right eye are processed in different, contralateral hemispheres). As a consequence, visual lateralisation can cause a preferential use of either the left or right eye for different tasks in a variety of vertebrates (de Santi et al. 2001; Rogers et al. 2004).

During agonistic encounters of fishes, a population-level right eye dominance has often been reported (Arnott et al. 2011; but for the left eye dominance see Sovrano 1999). This visual lateralisation is thought to facilitate the commonly observed head to tail alignment between two fish in an agonistic encounter (Arnott et al. 2011). The advantage of such a formation is information gathering about size and fighting ability of the opponent (Arnott and Elwood 2009), information important for assessing the chance of success in a potential fight and avoidance of unnecessary escalation (Arnott et al. 2011). Such ritualised lateralisation, however, cannot be performed with a mirror image (Arnott et al. 2011).

Taken together, mirror tests might reflect aggressive behaviour only in those species with no lateralisation during agonistic displays (Ariyomo and Watt 2013; Arnott et al. 2011). Yet, there are only few studies analysing the interplay between the level of lateralisation and the suitability of mirror tests (Ariyomo and Watt 2013). In this study, population-level lateralisation in aggression (i.e. differential use of each body side during agonistic encounters) was investigated in males of a territorial and bi-parental West African cichlid, the rainbow krib *Pelvicachromis pulcher* (Boulenger 1901). Furthermore, behavioural responses towards a mirror image and towards an opponent were compared. In a study on 16 species, originating from 13 families, Bisazza et al. (2000) found lateralisation to be present in all gregarious species, but only in 40% of the non-gregarious species. Further research on cichlids has supported the results of Bisazza et al. (2000) by detecting no population-level lateralisation in less social cichlids (Reddon and Balshine 2010), including the species of interest, *P. pulcher* (A. R. Reddon and P. L. Hurd, unpublished data). Therefore, lateralisation was predicted to be absent or weak in *P. pulcher*. Consequently, a positive correlation between the response in the mirror test and towards a live same-sex conspecific was predicted given the above reasoning.

Pelvicachromis pulcher males ($n = 36$) were obtained from a captive breeding stock at the University of Hamburg. Study individuals were maintained in family groups under standardised holding conditions (50 cm x 50 cm x 25 cm tanks, 26° C, range ± 1° C water temperature, aerated and filtered water, weekly water changes, 12L:12D) and were fed once a day, 5 days a week with *Artemia* spp. On experimentation days, individuals were fed after the observations.

Each male was measured once for its behaviour towards a mirror and on the subsequent day for its behaviour towards an actual opponent. As discussed in Bell (2012), randomised and fixed testing orders both have advantages and disadvantages, and which testing order to choose depends on the objective and the experimental power. In this study, individuals were tested in a fixed order because the experimental design did not provide sufficient power to statistically correct for carry-over effects. A fixed design here provided the advantage of lowering the influence of carry-over effects on correlations by causing the same experiential level for all individuals. Carry-over effects were expected to be small and to have more or less the same effect on all individuals. Prior to mirror tests, males were measured for their standard length (L_S, range: 38.6–69.4 mm) and transferred into individual observation tanks (50 cm x 25 cm x 25 cm). Randomly chosen, size-matched pairs ($n = 18$) with a maximum difference of 10% in L_S were placed into adjacent tanks, visually separated by opaque dividers. Each tank was provided with 1 cm of sand and half a clay pot as shelter (8 cm x 8 cm x 4 cm) with the opening towards the mirror and opponent's side. Study individuals were allowed to acclimate overnight and were tested against the mirror the next day. A mirror (50 cm x 25 cm) was introduced to the long side of the tank, covering the whole length of the tank. After an acclimation of 2 min, the focal male was video-recorded for 10 min (from the short side of the tank). The next day, aggressive behaviour towards a real opponent was tested in the same manner but instead of introducing a mirror, the visual partition between the two adjacent tanks of size-matched pairs was removed. The behaviour of both males was video-recorded for 10 min (after an acclimation of 2 min). During trials, no human was in the experimental room to avoid disturbances. Pairs of test individuals originated from different holding tanks and from different families to avoid possible effects of familiarity (Frostman and Sherman 2004).

The number of all agonistic behaviours was determined from the videos following the ethogram Riebli et al. (2011). Accordingly, two categories of aggressive behaviour were classified: restrained aggression (including: fast approaching the

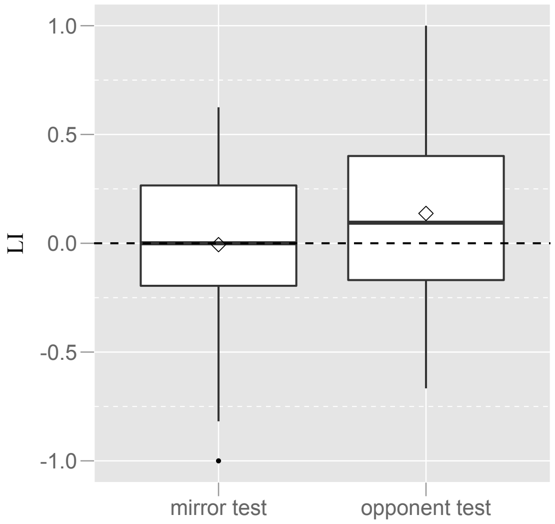

Figure 1. Medians, means (◊), quantiles and ranges for lateralisation index (LI) in the mirror and opponent test. Positive values indicate a right eye preference, negative values indicate a left eye preference and zero means left and right lateral displays were used equally often.

mirror or opponent, frontal display, left lateral display, right lateral display and s-shaped bending) and overt aggression (bites, *i.e.* mouth contact with the mirror or glass plate).

To assess lateralisation in the mirror and opponent test, the lateralisation index (L_l) was calculated and analysed according to (Arnott et al. 2011). The L_l is a relative index determining the proportional use of left and right lateral displays [calculation: (right – left) divided by (right + left)]. L_l values were normally distributed (Kolmogorov–Smirnov tests with Lilliefors correction). Thus, one-sample *t*-tests against zero were performed to test for a preference in the usage of one particular body side in both the mirror and opponent test. Eight individuals in the mirror test and six individuals in the opponent test were excluded from this analysis because they did not show any lateral displays ($n = 28$ and $n = 30$). Also, a difference in the L_l between mirror and opponent test was investigated using a paired *t*-test ($n = 27$). Power analyses for *t*-tests were computed using the R package *pwr* (Champely 2015).

Aggressive behaviours in the mirror and opponent test were compared following Balzarini et al. (2014) and Elwood et al. (2014). Due to deviations from a normal distribution (Kolmogorov–Smirnov tests with Lilliefors correction), Spearman rank correlation tests were used to assess whether the number of aggressive behaviours (restrained and overt aggression, respectively) correlated between the live agonistic trial and the mirror test. Wilcoxon matched-pairs signed-rank tests were used to test whether the level of aggression (number of restrained and overt aggressions, respectively) towards the mirror and against the opponent differed. To test for a potential effect of the opponent's behaviour on the subject's behaviour, Spearman rank correlations between the behaviour of the two individuals were used. All statistical analyses were performed using R version 3.2. (R Core Team 2015).

Regardless of the testing method, no difference in the proportional use of left and right lateral displays could be detected (one-sample *t*-test on the L_l; mirror test: $t_{27} = 1.4659$, $P > 0.05$, power = 0.2934; opponent test: $t_{29} = -0.0876$, $P > 0.05$, power = 0.0508; Figure 1). Also, the L_l did not differ between mirror and opponent test (paired *t*-test; $t_{26} = -1.3528$, $P > 0.05$, power = 0.3071; Figure 1). A difference in L_l between methods could be masked, however, by contra-direct

Restrained and overt aggression shown towards the mirror and against the opponent positively correlated (Spearman rank correlation; $n = 36$; restrained aggression: $r_s = 0.4331$, $P < 0.01$; overt aggression: $r_s = 0.4838$, $P < 0.01$; Figure 2) and responsiveness did not differ between methods (Wilcoxon matched-pairs signed-

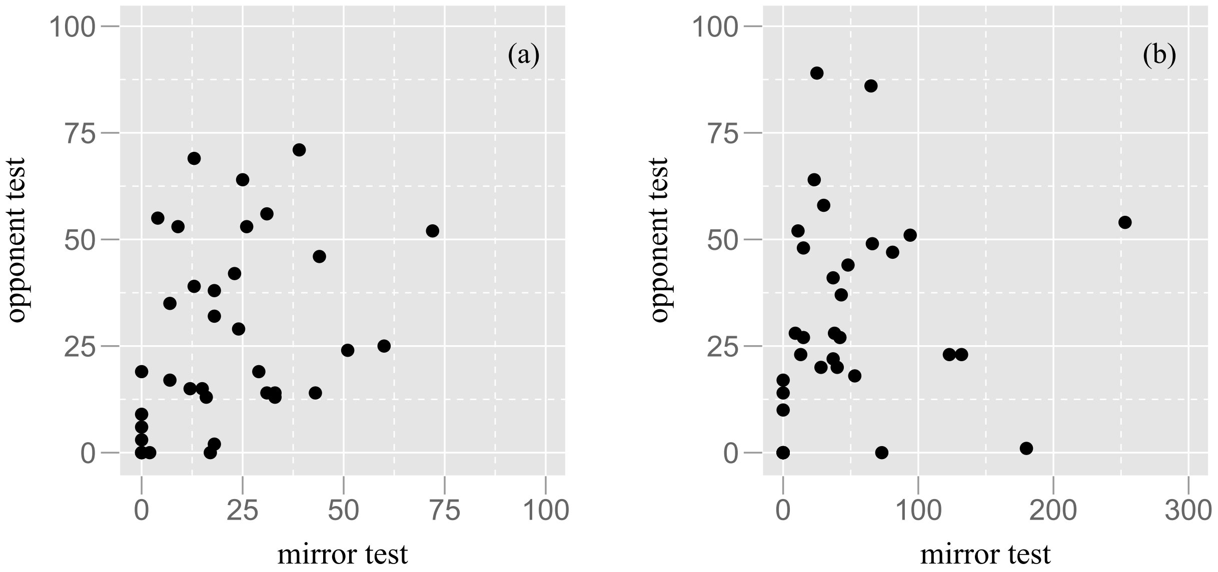

Figure 2. Number of aggressive behaviours shown towards the mirror image and towards a real opponent: (a) Restrained aggression and (b) overt aggression. Please note, scales are different.

rank test; $n = 36$; restrained aggression: $V = 185.5$, $P > 0.05$; overt aggression: $V = 260.0$, $P > 0.05$). In the opponent test, the behaviour of both males correlated positively (Spearman rank correlation; $n = 18$; restrained aggression: $r_s = 0.5039$, $P < 0.05$; overt aggression: $r_s = 0.8075$, $P < 0.0001$; Figure 3).

In agreement with the prediction, a population-level lateralisation could not be detected in *P. pulcher*. Therefore, this study supports the results of (Bisazza et al. 2000); lateralisation may have evolved to better co-ordinate behaviour in a social context, *e.g.* shoaling behaviour (Rogers 1989). Thus, laterality might potentially be absent in the aggressive, territorial *P. Pulcher* due to a lack of a complex social community structure.

Aggressive behaviours of *P. Pulcher* correlated positively between mirror and opponent tests. Also, there was no difference in the responsiveness between methods, indicating that *P. Pulcher* males do not differentiate between a mirror image and real intruder. Therefore, mirror tests represent a suitable method to test aggressive behaviour in *P. pulcher*. In the opponent test, the focal male's behaviour was strongly influenced by the opponent's behaviour (in agreement with previous findings in other species; Balzarini et al. 2014; Earley et al. 2000). Such effects make it difficult to standardise aggression tests using a live stimulus and to ensure repeatability of test results.

There are exceptions and limitations, however, for the general validity of the prediction that less social species are suitable for mirror tests because they are not lateralised. Balzarini et al. (2014) investigated the method in three species of African cichlids, differing in their social complexity. Aggressive behaviours during mirror and opponent tests only correlated in the most social species, the daffodil cichlid *Neolamprologus pulcher* (Trewavas & Poll 1952). Although *N. Pulcher* shows a population-level lateralisation towards social stimuli (Reddon and Balshine 2010), the species does not show lateralisation in aggression (neither parallel swimming nor lateral displays during agonistic encounters; Balzarini et al. 2014). While these results

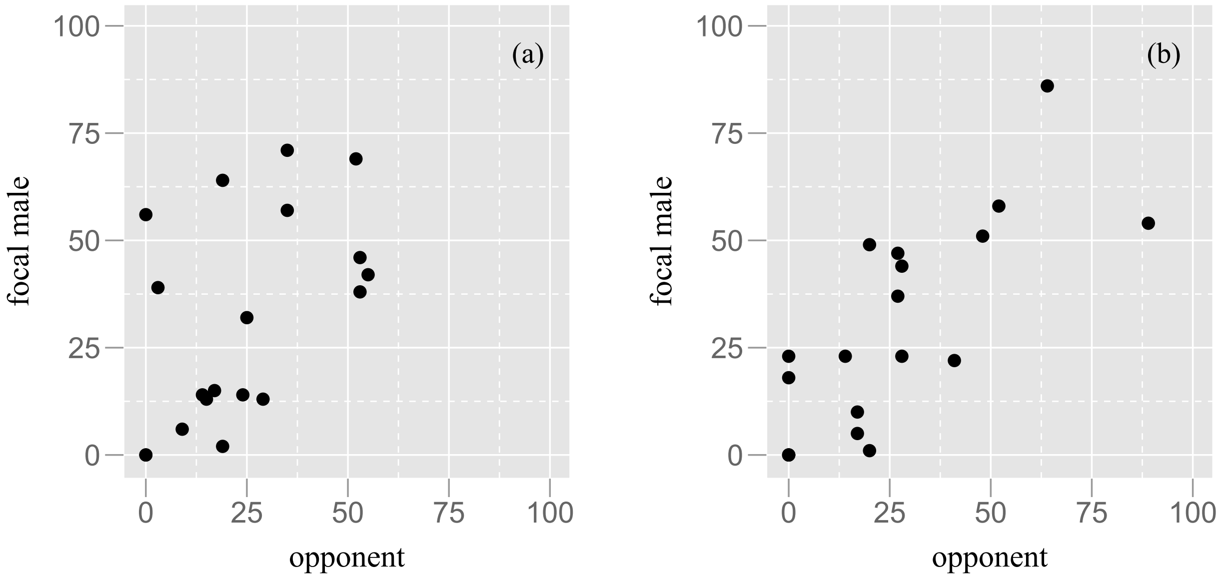

Figure 3. Number of aggressive behaviours shown by the opponents in a live agonistic trial: (a) restrained aggression and (b) overt aggression.

support a link between sociality and lateralisation, they clearly indicate that a species is not lateralised *per se*. Behaviours in social interactions might be lateralised independently of each other, *i.e.* a species that shows laterality during shoaling activities does not necessarily have to be lateralised during agonistic encounters.

Finally, sociality may influence the suitability of mirror tests for other reasons than lateralisation. Individuals of highly social species are possibly more sensitive in the recognition of behavioural abnormalities caused by unusual reactions of the mirror image. Further investigations are needed to ascertain how sociality and lateralisation associate and how they impact the suitability of mirror tests.

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APPENDIX 3

Validation of 2D-animated pictures as an investigative tool in the behavioural sciences: A case study with a West African cichlid fish, *Pelvicachromis pulcher*

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ABSTRACT

Virtual stimuli represent an increasingly popular tool in the study of animal behaviour. Modern techniques have the potential to simplify and improve traditional experiments using live stimuli. However, the increasing availability of diverse techniques is associated with problems and limitations. Although many new methods have been developed, their validation remains largely untested. In the present study, we therefore performed two experiments to test whether 2-D animations of predators and conspecifics elicit biologically appropriate behavioural responses in male rainbow kribs, *Pelvicachromis pulcher*. Individual responses towards a sympatric natural fish predator, *Parachanna obscura*, were tested using live predators and still colour photographs, animated using PowerPoint©. Compared to control trials (empty aquarium and white computer screen, respectively), individuals decreased their activity in response to both live and animated predators. We found no difference in activity between live and animation trials. Further, we tested individual aggression (frequency of aggressive behaviours) exhibited towards live and animated conspecifics. Individual aggressive behaviours shown towards live and animated conspecifics were positively correlated. Moreover, an individual's mean distance towards the opponent was a suitable proxy for individual aggression permitting the facilitation and standardisation of an individual's aggression through the use of a tracking software compared with the more laborious, traditional manual assessment. Our results show that simple, inexpensive animation techniques have the potential to provide an easy-to-apply and useful technological advance in animal behaviour research.

1 INTRODUCTION

Video and animation techniques represent an increasingly popular and frequently used tool to investigate principles of sexual selection (Mehlis et al. 2008), aggression (McGinley and Taylor 2016), visual communication (e.g. species and kin recognition: Macedonia et al. 2015; Van Dyk and Evans 2007), anti-predator responses and boldness (Fischer et al. 2014), social perception (Watanabe and Troje 2006) and shoaling preferences (Nakayasu and Watanabe 2014). Such techniques include the use of photographs, videos, 2-D animations, 3-D animations and 3-D models (reviewed in Baldauf et al. 2008; Woo and Rieucau 2011) and have been used to investigate intra- and interspecific behavioural responses in taxonomically-diverse taxa, including birds (Moravec et al. 2010), mammals (Campbell et al. 2009), reptiles (Macedonia et al. 2015), arachnids (Uetz et al. 2011) and fishes (Mazzi et al. 2003).

Compared with traditional behavioural experiments using live stimuli, the use of videos and animations has some striking advantages. In line with the ‘3R framework’ (replacement, reduction, refinement) of research animal use proposed by Russell and Burch (1959), virtual stimuli can contribute to animal welfare and have the potential to enhance experimental manipulations. For example, replacing live stimuli with virtual analogues reduces the number of animals needed for experiments and technological progress makes invasive techniques like the surgical manipulation of morphological traits redundant (Gierszewski et al. 2016; Woo and Rieucau 2011). Modern methods enable selective manipulations of single traits in morphology, colouration or behaviour (reviewed in Rosenthal 1999; Rosenthal 2000; Stevens et al. 2007) and therefore allow for a decoupled testing of correlated traits, thereby facilitating the testing of causality (Veen et al. 2013). Furthermore, live stimuli vary naturally in their behaviour (e.g. activity and position within and between trials; Fischer et al. 2014), making it difficult to ensure equal conditions for all test individuals; in contrast, virtual stimuli can be standardised.

However, there are several problems and limitations associated with the use of videos or animations in animal behaviour research (reviewed in Chounard-Thuly et al. 2016). One of the major concerns regards fundamental differences in the visual systems of humans and other animals. Species vary in the number and type of photoreceptors, leading to differences in their perception of brightness, contrast and colour (reviewed in D'Eath 1998; Fleishman and Endler 2000; Fleishman et al. 1998; Oliveira et al. 2000). However, electronic devices (e.g. cameras, processing programs, computer screens) used for the production and presentation of virtual stimuli are tuned to the human eye, clearly causing spectral restrictions for non-human species (e.g. for UV-sensitive species). Species also differ in their perception of motion (D'Eath 1998; Künzler and Bakker 2001). Here, the critical flicker-fusion frequency (CFF) and transition time between video frames are important parameters. CFFs are species-specific and represent the crucial frame rate needed for a species to perceive a flickering object as continuous (Künzler and Bakker 2001; Oliveira et al. 2000). In fishes, for example, the CFF is assumed to be less than 30 frames per second (Fleishman and Endler 2000; Oliveira et al. 2000). The transition time refers to a restriction of video monitors - it is the time a LCD/TFT, LED or plasma display needs to switch from 10% to 90% grey-to-grey on a scale of 0% = black and 100% = white (Baldauf et al. 2008). Accordingly, a low transition time blurs moving objects. Also, the viewing angle of a screen has to be taken into consideration when selecting a monitor (Baldauf et al. 2008). It should come as close to 180° as possible. Furthermore, videos and animations are often limited to presenting solely visual cues, even though olfactory, chemical or acoustic cues are important in animal behaviour and individual recognition (Baldauf et al. 2008; Balshine-Earn and Lotem 1998; Hesse et al. 2015). Another limitation of the use virtual stimuli is that they usually do not allow for interactions with test individuals (Fischer et al. 2014; but see Butkowski et al. 2011 for an interactive approach).

Despite their limitations, virtual stimuli have been proven to be a valuable instrument in the behavioural sciences and have been successfully applied in numerous studies (Clark and Stephenson 1999; Gierszewski et al. 2018; Qin et al. 2014). Yet, there are studies in which test subjects did not respond to video stimuli (Gonçalves et al. 2000; Patterson-Kane et al. 1997). Other studies could elucidate differential responses to natural vs. virtual stimuli (Balshine-Earn and Lotem 1998; Robinson-Wolrath 2006; Trainor and Basolo 2000). Taken together, behavioural responses to virtual stimuli are likely species-specific and depend on the quality and suitability of the technical equipment. Often, the production of a virtual stimulus is not trivial and requires either specific expertise and/or is costly (Veen et al. 2013), although there are also simple and easy-to-apply techniques (Fischer et al. 2014). But do those simple techniques keep what they promise? In the present study, we tested the suitability of PowerPoint© as a simple animation technique facilitating standardisation in behavioural studies. In two separate and complementary experiments, we compared behavioural responses of males of a territorial and aggressive West African cichlid, *Pelvicachromis pulcher*, towards live and virtual stimuli.

In a first experiment, we tested the suitability of PowerPoint© animations of 2-D fish images as method to assess the boldness level (i.e. propensity to engage in risky behaviour; Wilson et al. 1994) of individual fish. We exposed *P. Pulcher* males to both a live and a virtual predator and compared their behavioural responses (activity, avoidance, inspection behaviour) towards these predator stimuli to their behaviour in predator-free control trials. As a predator, we used the African obscure snakehead, *Parachanna obscura*, a carnivorous fish predator that occurs sympatrically with natural populations of *P. pulcher*. Fishes commonly respond to predators with anti-predator behaviours such as reduced activity (McLean and Godin 1989; O'Connor et al. 2015), increased distance to the predator (Broom and Ruxton 2005; Cooper and Martín 2016) and escape behaviour (Lima and Dill 1990). They may also cautiously approach or 'inspect' a potential predator at a distance (Dugatkin et al. 2005; Godin and Dugatkin 1996; Pitcher et al. 1986), which is a risky behaviour (Dugatkin and Godin 1992), so as to gather information on and assess the actual threat (Hesse et al. 2015; Magurran and Higham 1988) and even to deter attack in some cases (Godin and Davis 1995). Anti-predator behaviour has not been described for *P. Pulcher* yet. Nonetheless, we expected individuals to show typical anti-predator behaviour (reduced activity and predator avoidance) towards both a live and animated predator. In addition, predator inspection behaviour could be expected as it has been described for a sister species, *Pelvicachromis taeniatus* (Hesse et al. 2015).

However, individuals could potentially perceive live and animated predators rather as a novel (and potentially risky) object than identifying them as predators. Therefore, fear or even curiosity arousing from the presentation of our stimuli could also represent a novel-object response. While we are here testing the suitability of predator animations to measure general boldness, we cannot differentiate between anti-predator vs. novel-object response. Validation of the use of PowerPoint© animations to quantify individual boldness (i) would be revealed by an (expected) positive correlation between behavioural responses towards the live and the virtual predator. Validation of the use of the behaviour of interest being a suitable measure of boldness (ii) would be revealed by a significant difference in the magnitude of behavioural response between predator (live and animation) trials and respective control trials.

In a second experiment, we tested the suitability of PowerPoint©-animated photographs of male conspecifics to incite aggressive behaviour in male *P. pulcher*. Further, we tested for a correlation between individual aggression (frequency of aggressive behaviours) and other behavioural measures (activity, time spent in close proximity to the opponent) obtained using an animal tracking software. Such behavioural correlations would allow us to automate and standardise the assessment of individual aggression. Visual observing and manually counting the number of aggressive behavioural acts is time consuming and subject to observer bias, whereas the assessment of aggression via tracking software would be more rapid, objective and independent of observer-specific effects. Validation of the suitability of video-animated opponents to measure aggression (iii) would be revealed by an (expected) significant positive correlation between the aggression level exhibited by individual focal fish towards a live conspecific and their aggression level shown towards a video-animated conspecific. Further, validation of the use of automation for the assessment of individual aggression (iv) by using a focal individual's activity level or time spent in close proximity to an opponent (either virtual or live) would be indicated by significant positive correlations between the latter behaviours and the frequency of the focal individual's actual agonistic displays exhibited towards the opponent.

2 METHODS

2.1 Test individuals and holding conditions

We tested male *P. pulcher* originating from a captive breeding stock at the University of Hamburg (ancestor individuals obtained from local suppliers). Holding conditions were standardised for all individuals. We maintained individuals in sibling groups in separate holding aquaria (100 x 50 x 25 cm) filled with aerated and filtered water (26 ± 1°C) and exposed to overhead full spectrum fluorescent lighting (Osram L 30W 11–860 Lumilux daylight) on a 12 hr L:12 hr D illumination cycle. Aquaria water was changed weekly. Fish were fed once daily for 5 days per week with live brine shrimp *Artemia* spp. One week prior to experimentation, we measured all fish for their total length using *ImageJ* (Schneider et al. 2012) and transferred all individuals to smaller individual home aquaria (50 x 25 x 25 cm). These aquaria were endowed with half a clay pot (8 x 8 x 4 cm) as shelter and an internal water filter. Water conditions were similar to those described above. Jolles, Taylor and Manica (2016) demonstrated that the social environment affects repeatability of boldness, with socially-housed fish showing reduced behavioural stability. Therefore, all individuals remained in individual aquaria for the duration of the experiments (described below). During the experimentation period, fish were fed once daily for 7 days a week. On experimentation days, individuals were fed after being tested for their behaviour.

2.2 Production of animations

We produced PowerPoint© exemplar animations of predator (Experiment 1) and conspecific male specimens Experiment 2) following the protocol of Fischer et al. (2014). *Parachanna obscura* (N = 4 exemplars, $\bar{x}$ ± SE total length = 19.1 ± 0.3 cm) and *P. pulcher* individuals (N = 6, $\bar{x}$ ± SE total length = 5.8 ± 0.0 cm) were photographed under standardised light conditions using a Nikon D80 digital camera with a Nikon AF-S DX Nikkor objective. Each individual was placed in a small glass container (22 x 18 x 25 cm) and a photograph of either the left or the right side of the fish was taken. Stimulus *P. pulcher* individuals were additionally exposed to a mirror simulating a social stimulus that elicits aggressive displays, and a photograph of the lateral side of the stimulus fish was taken when he was in a lateral display posture. Lateral displays are a common aggressive behaviour in cichlid fishes (Riebli et al. 2011). Using photos of displaying conspecifics for animated virtual stimuli provides a more naturalistic context because this mimics the behaviour of an actual opponent, which is likely to elicit a behavioural response in the focal fish. Fish images were cut out of digital photos using Preview for MacOS version 8.0 (Apple Inc., Cupertino, CA, USA) and transferred to a Power Point© presentation (Power Point© for Mac version 14.5.9; Microsoft Corporation, Redmond, WA, USA). Power Point© presentations were created with a white background. Predator (Supplemental Material 1 Figure (a) for an example) and conspecific (Supplemental Material 1 Figure (b) for an example) photos were animated to move across the entire width of a computer screen (see below) at a constant speed (1 cm/s) over 43 s in an alternating sequence (from the left to right and then back from the right to the left, and so on). The size of the animated photo was scaled to the original size of the live fish that was photographed.

2.3 Experiment 1

We repeatedly tested individual *P. pulcher* males (*N* = 36, $\bar{x}$ ± *SE* total length = 5.7 ± 0.1 cm) for their boldness level in each of four stimulus treatments: a live predator (*P. obscura* specimen presented in a separate predator aquarium), a live control (only empty predator aquarium), an animation of the predator (computer screen showing the predator animation), and an animation control (only the white computer screen) were presented. During paired live and animation trials for any given focal fish, the same (but randomly chosen) predator specimen was presented to the focal male. Treatment order was randomised, with 4 days elapsed between successive individual trials.

To begin a trial, we introduced two focal individuals into the experimental aquarium (Figure 1a), each into one of two test compartments. Test compartments were physically separated using a partition of white Plexiglas, allowing the testing of two focal fish simultaneously. Water exchange between test compartments was only possible through approximately 1 mm fissures (we used a Plexiglas guide rail to maintain the Plexiglas plate in its position). Individual focal fish were initially constrained in a clear Plexiglas cylinder for 10 min. Following this acclimation period, we allowed focal fish full view of either the live predator, the predator animation, the empty predator aquarium or the white computer screen by removing an opaque screen located between the experimental aquarium and either a computer monitor (Dell, UltraSharp U2412M, 1,920 x 1,200 pixel, 61-cm screen, USA, Round Rock) or the predator aquarium that were set up on one of the short sides of the experimental aquarium. The monitor and predator aquarium covered the entire width of the experimental aquarium's side allowing both focal fish in the two test compartments to view the predator (Figure 1a). After 45 s (corresponding to the duration of one iteration of the animation on the computer monitor), the cylinders were gently raised allowing the focal fish to swim freely in their respective test compartment, and a test period of 11 min started. Before the onset of live predator tests, the live fish predator was placed in a clear Plexiglas cylinder in the centre of its aquarium and was allowed to acclimatise for 10 min. The predator was kept in this cylinder, ensuring that he would be in full view of both focal test fish throughout the behavioural trial. Predators were able to turn around in their cylinder and did not behaviour. For each treatment, individual fish were tested at the same time of day (± 30 min) to control for any potential effects of hunger level and time of day on

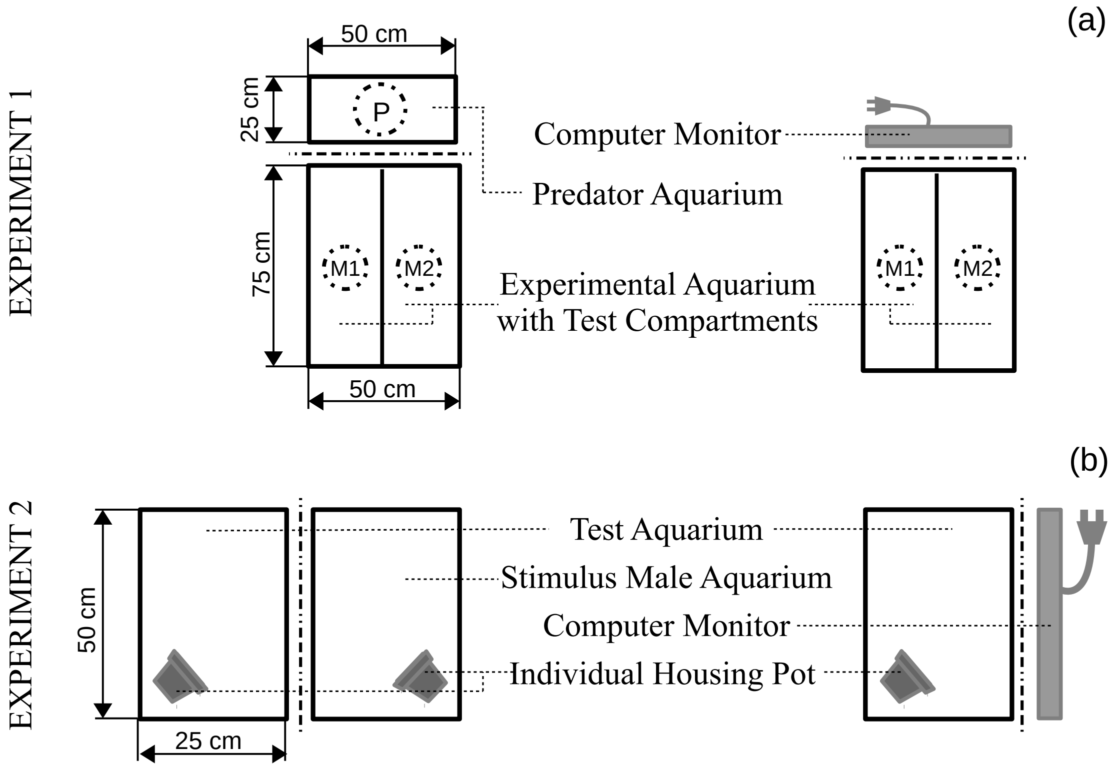

Figure 1. Experimental set-up for testing (a) individual boldness and (b) aggressiveness towards live (left) and animated (right) predators (Experiment 1) and conspecific opponents (Experiment 2). Test aquaria were visually separated from stimuli (computer monitor, predator and conspecific aquaria) using removable, white opaque screens (dashed line). Experiment 1: focal males (M) were kept in clear Plexiglas cylinders (diameter = 11 cm) during the acclimation period, predators (P) were kept in clear Plexiglas cylinders (diameter = 20 cm) during the entire trial.

individual behaviour (Ariyomo and Watt 2015; MacPhail et al. 2009). A complete water change of experimental aquaria was performed after every three trials. In all experimental aquaria, water temperature (26 ± 1°C) and water level (10 cm) maintained constant. We video-recorded all trials from above using a digital video camera (Sony HD-CX405), with no person present in the experimental room during behavioural trials. The experimental room was equipped with overhead full spectrum fluorescent lighting (Osram L 30W 11–860 Lumilux daylight). All fish were predator naive before the experiment.

For each individual focal fish, we later tracked its activity (total distance [cm] moved), avoidance behaviour (mean distance [cm] to the predator) and predator inspection behaviour (amount of time [s] spent in a 25-cm wide 'inspection zone' nearest the monitor or predator aquarium, following Hesse et al. (2015) from videos using the software Ethovision XT 11 (Noldus, Wageningen, The Netherlands). Videos were analysed for 10 min, starting 1 min after the start of the video. Owing to a very high correlation observed between time spent in the inspection zone and the mean distance of the focal fish to the predator for each stimulus treatment (Spearman rank correlations: r_s [live] = -.858, r_s [control live] = -.841, r_s [animation] = -.932, r_s [control animation] = -.882; all $p <$ 0.0001, each N = 36 males), we present here only the results for mean distance to the predator as a measure of spatial use to avoid redundancy.

2.4 Experiment 2

Pelvicachromis pulcher males (*N* = 34, $\bar{x}$ ± *SE* total length = 5.7 ± 0.1 cm), different from the ones used in Experiment 1, were tested for their aggressiveness towards a live and an animated male conspecific in randomised order. During both live conspecific and animation trials, the same but randomly chosen stimulus male (*N* = 6 exemplars, $\bar{x}$ ± *SE* total length = 5.7 ± 0.0 cm) was presented to a focal male. In total, each stimulus male was used five or six times and not more than once per day.

To begin a live conspecific trial, we placed a focal male in a test aquarium and the opponent in an adjacent, identical aquarium. These two aquaria were facing each other along their longer axis and were visually separated using a white opaque screen (Figure 1b). Individuals were transferred to these test aquaria with the clay pot of their respective individual home aquarium. We expected that transferring fish within their clay pot would encourage the expression of territorial, aggressive behaviour in the test aquaria. Focal and stimulus fish were allowed to acclimatise for 20 min. Following this period, the opaque screen was removed, allowing both focal and stimulus fish to view each other, and a test trial of 12 min was started. The procedure for the animation trial was similar: a test individual and its clay pot were transferred to a test aquarium (Figure 1b). On its long side, this aquarium faced a computer monitor (Dell, UltraSharp U2412M, 1,920 x 1,200 pixel, 61-cm screen, Round Rock, TX, USA). Initially, a white opaque screen was placed between the monitor and the test aquarium. After a 20-min acclimation period, the screen was removed and a test trial of 12 min was started. During this period, the monitor showed the animation of a displaying virtual conspecific opponent.

All experimental aquaria used for live conspecific and animation trials were surrounded with white Plexiglas plates, except for one short side of each aquarium, which was left open for filming using a digital video camera. A second video camera was placed above each test aquarium. During both live conspecific and animation trials, all fish (focal and stimulus males) were concurrently filmed from above and the side. No observer was present in the room during trials. In both trials, focal males were tested at the same time of day ± 30 min. Water temperature in the test aquaria was maintained at 26 ± 1°C. We changed the water in the test aquarium after each trial. Also, we added some water of an individual's (focal and stimulus males) home aquarium to the respective test aquarium (10% of the water volume) before each trial to facilitate territorial aggression. Experimental aquaria were illuminated from above (full spectrum fluorescent lighting; Osram L 30W 11–860 Lumilux daylight).

Aggressive behaviour was assessed manually for all focal and stimulus fish from the videos taken from the side. Following Riebli et al. (2011) and Scherer et al. (2016), we recorded the number of all restrained (fast approach towards the opponent, frontal display, left lateral display, right lateral display, s-shaped body bending) and overt (bites, i.e. mouth contact with aquarium glass wall in front of opponent) aggressive behaviours for 10 min, starting 2 min after the beginning of the video playback. The aggressiveness of each focal and stimulus fish was calculated as the sum of all restrained and overt aggressive behaviours exhibited over 10 min. Using Ethovision XT 11, we recorded the mean distance to the opponent (monitor or live conspecific aquarium), amount of time spent within one fish body length (6 cm) of the opponent (hereafter, time in aggression zone) and activity (total distance moved, cm) for all focal and stimulus individuals from the videos taken above the aquaria. Based on the behaviour shown in their home aquarium, we had selected stimulus males that were aggressive so as to ensure that focal males would assess the stimulus male presented as a potential territorial intruder and respond to it aggressively. During the experimental trials, the live stimulus males were active and aggressive as expected ($\bar{x}$ ± *SE*, total aggression = 489 ± 27 behavioural acts, activity = 2,450.1 ± 146.4 cm, mean distance to focal male = 3.8 ± 0.3 cm). Similarly to Experiment 1, we found a very strong correlation between the mean distance of the focal male to the stimulus male and the amount of time he spent in the aggression zone (Spearman rank correlations: r_s [live] = −.980, r_s[animation] = −.968; both $p < .0001$, $N = 34$ males). We therefore present results only for the mean distance to the stimulus as a measure of spatial use to avoid redundancy.

2.5 Data analyses

2.5.1 Experiment 1

All data analyses were performed using R 3.2.3 (R Core Team 2015). To test our first objective (validation of 2-D animations to assess individual boldness), we ran two linear mixed effects models (LMMs), one for each of the behaviours recorded during the live stimulus trials (i.e. activity, and mean distance to the predator; for each model N = 36 focal males) as the response variable. We included the respective behaviour recorded during the animation trial (activity or mean distance to the predator, respectively) as fixed effect and predator ID and treatment order as random effects. To test our second objective (validation for our measure of boldness), we performed two additional LMMs. Each model contained one of the behaviours recorded (either activity or mean distance to the predator; for each model N = 144 [36 males, each in 4 treatments]) as the response variable. Both models included the treatment (animation, animation control, live predator, live predator control) as fixed effect, and predator ID, treatment order, and focal ID as random effects. Adjusted *p*-values for treatment groups were calculated via single-step multiple post hoc comparisons with Tukey contrasts using the *multcomp*-package (Hothorn et al. 2008).

2.5.2 Experiment 2

To test our third objective (validation of 2-D animations to assess individual aggressiveness), we fitted a LMM for each of the behaviours recorded separately (i.e. total aggression, activity, and mean distance to the stimulus; for each model N = 34 focal males). Each model contained the focal male's behaviour recorded during the live stimulus trial as the response variable and the respective focal male's behaviour recorded during the animation trial as fixed effect. Further, in each model, we included the stimulus male's behaviour recorded during the live trial as fixed effect, and stimulus male ID and treatment order (live trial first vs. animation trial first) as random effects. We also tested for a difference in the magnitude of focal fish aggressiveness between the live and animation treatment by fitting a LMM with individual total aggression as the response variable. The LMM included the treatment (live vs. animation) as fixed effect and focal male ID, stimulus male ID and treatment order as random effects (N = 68 live and animation trials of 34 focal males). To test our fourth objective (suitability of tracking software variables as proxy measures of individual aggression), we fitted a LMM on each of the behavioural variables recorded with the tracking software (i.e. activity, and mean distance to the stimulus; N = 68 live and animation trials of 34 focal males). As fixed effects, we included in each model individual total aggression and the treatment (live vs. animation) and the interaction between total aggression and treatment, because correlations between total aggression and behaviours from the tracking software variables might differ between treatments. As random effects, we included the focal male ID, the stimulus male ID and treatment order.

We applied LMMs on our behavioural data using the *lme4*-package (Bates et al. 2015). Minimal adequate models were identified via stepwise backward model selection. Partial R^2 with CL (confidence level) for significant predictors were calculated using the *r2glmm*-package (Jaeger 2016). Model assumptions were visually verified using residual and q-q plots. Whenever necessary to meet model assumptions, we power-transformed our response variable (Experiment 1: mean distance to the stimulus, lambda = 0.37; Experiment 2: activity, lambda = −0.96) using the *car*-package (Fox and Weisberg 2011).

3 RESULTS

3.1 Validation of PowerPoint© animations to quantify individual boldness

The activity of focal males exhibited towards a stimulus predator in the live and animation trials were positively correlated (LMM; χ^2_1 = 7.841, p = .005, coefficient = 0.415; partial R^2 = .202; CL = [0.028, 0.447]; N = 36; Figure 2). However, the mean distance of focal males to the stimulus predator was not correlated between live and animation trials (LMM; χ^2_1 = .002, p = .969; N = 36).

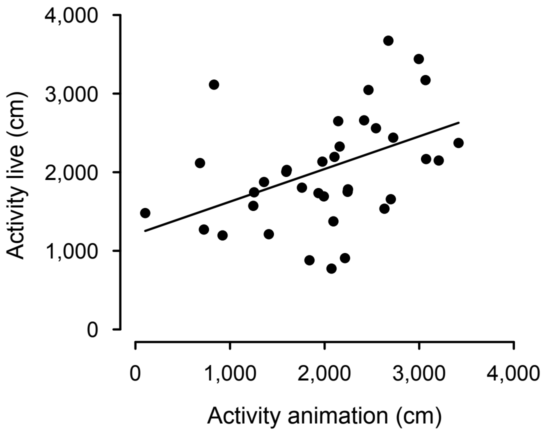

Figure 2. Relationship between the activity levels of individual fish expressed during live and computer-animated predator trials (Experiment 1).

3.2 Validation for our measure of boldness

The activity of individual focal males differed significantly between treatments (LMM; $\chi^2_1 = 37.728$, $p < .0001$; partial $R^2 = .204$; CL = [0.111, 0.330]; $N = 144$ trials); in the presence of either an animated or live predator, activity was lower compared to respective control trials, while no difference could be detected between control treatments (animation control, live control) and stimulus treatments (animation, live), respectively (Table 1; Figure 3a). The mean distance of focal males to the stimulus predator also varied significantly between treatments (LMM; $\chi^2_1 = 26.805$, $p < .0001$; partial $R^2 = .126$; CL = [0.053, 0.247]; $N = 144$ trials). For animation trials, we found no difference in the mean distance of focal males to the predator stimulus between the animation and animation control trials; in contrast, focal males approached a live predator significantly more closely compared to an empty predator aquarium (live control), which resulted in a significant difference in the focal males' distance response towards live vs. animated predators (Table 1; Figure 3b).

3.3 Validation of PowerPoint© animations to quantify individual aggression

The total aggression of focal males directed towards a live conspecific stimulus male positively correlated with their total aggression directed towards an animated stimulus male (LMM; $\chi^2_1 = 5.996$, $p = .014$, coefficient = 0.487; partial $R^2 = .171$; CL = [0.013, 0.424]; $N = 34$; Figure 4a) and did not depend on the total aggression of the stimulus male in the live trials (LMM; $\chi^2_1 = 0.832$, $p = .362$; $N = 34$). The activity of focal males during the live trials positively correlated with their activity during the animation trials (LMM; $\chi^2_1 = 10.397$, $p = .001$, coefficient on transformed scale = -1.115×10^{-7}; partial $R^2 = .293$; CL = [0.081, 0.537]; $N = 34$; Figure 4b) and did not depend on activity level of the stimulus male in the live trial (LMM; $\chi^2_1 = 0.623$, $p = .431$; $N = 34$). Further, the mean distance of focal males to the stimulus male positively correlated between live and animation trials (LMM; $\chi^2_1 = 10.512$, $p = .001$, coefficient = 0.445; partial $R^2 = .267$; CL = [0.063, 0.515]; $N = 34$; Figure 4c) and did not depend on the stimulus male's mean distance to the focal male in the live trials (LMM; $\chi^2_1 = 0.021$, $p = .886$; $N = 34$). Lastly, the total aggression of focal males during live trials was higher than during animation trials (LMM; $\chi^2_1 = 43.393$, $p < .0001$, coefficient = 150.740; partial $R^2 = .418$; CL = [0.258, 0.573]; $N = 68$; Figure 5a).

Table 1. Summary of post hoc analyses using linear mixed effect models on the behavioural responses of focal test males to a stimulus predator in each treatment of Experiment 1.

Response variable	Group comparison	Estimate	Std. error	*z*-value	*P*-value
Activity	cA - A	841.640	154.600	5.444	<0.001
	cL - L	-581.500	154.600	-3.761	<0.001
	A - L	1.910	154.600	0.012	1.0000
	cA - cL	-258.230	154.600	-1.670	0.3390
Mean distance to predator	cA - A	-0.045	0.038	-1.199	0.6274
	cL - L	-0.148	0.038	-3.916	<0.001
	A - L	-0.200	0.038	-5.303	<0.001
	cA - cL	-0.007	0.038	-0.187	0.9977

3.4 Suitability of tracking software variables as proxy measures of aggression

The activity of focal males, as quantified using Ethovision, was not correlated with their total aggression (LMM; $\chi^2_1 = 1.030$, $p = .310$; $N = 68$) and was higher in the live treatment compared to the animation treatment (LMM; $\chi^2_1 = 26.747$, $p < .0001$, coefficient = 3.661; partial $R^2 = .228$; CL = [0.403, 0.082]; $N = 68$; Figure 5b). However, the mean distance of focal males to the stimulus male negatively correlated with their total aggression (LMM; $\chi^2_1 = 31.101$, $p < .0001$, coefficient = −0.016; partial $R^2 = .325$; CL = [0.167, 0.494]; $N = 68$; Figure 6) and did not differ between the treatments (LMM, $\chi^2_1 = 0.038$, $p = .845$; $N = 68$). The interaction between total aggression and treatment neither influenced the activity level (LMM: $\chi^2_1 = 0.009$, $p = .923$; $N = 68$) nor the mean distance of focal males to the stimulus male (LMM: $\chi^2_1 = 0.374$, $p = .790$; $N = 68$).

4 DISCUSSION

Using a cichlid fish species (*P. pulcher*) as a model study system, we demonstrated here that PowerPoint© animation of still digital photographs of stimulus fish is a suitable method to reliably assess boldness measured as activity under simulated predation risk, but not boldness measured as inspection/avoidance behaviour. Further, we could incite territorial aggression of focal males towards animated virtual conspecific male intruders. Also, we identified suitable (distance to an opponent) and unsuitable (activity) proxy measures for aggressiveness. We observed high concordance in measures of boldness and aggressiveness when directed by focal fish towards live and animated (virtual) predators and conspecific intruders, respectively.

More specifically, in Experiment 1, individual focal males similarly decreased their activity in the presence of both live and animated predators, and there was no difference in their activity level when exposed to live vs. animated predators, suggesting that the predator animation effectively induced timid behaviour in male *P. Pulcher* to a similar degree as a live fish predator did. A reduction in activity under a perceived increase in predation risk is a common anti-predator behaviour, which decreases an individual's risk of mortality to predation, in animals in general (Godin 1997; Lima and Dill 1990) and in cichlids in particular (O'Connor et al. 2015). Along a continuum, low and high activity levels are common in shy (timid) and bold animals, respectively (Réale et al. 2007; Sih and Bell 2008). It should nevertheless be noted that our experimental design does not allow us to conclusively differentiate between anti-predator behaviour and general timidity-boldness (e.g. towards a novel object). Thus, it is possible that our predator animation was not perceived as a predator but just as a potentially risky novel object, eliciting a similar behavioural response.

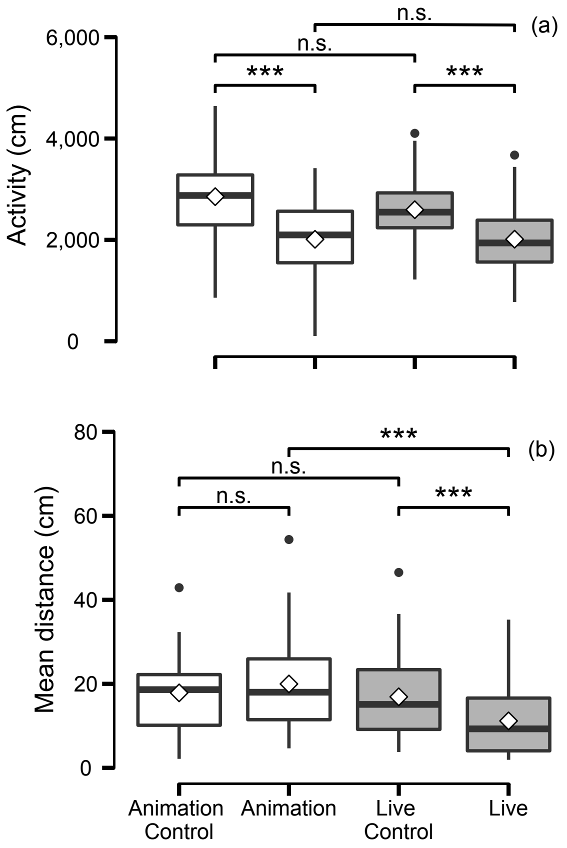

Figure 3. Variation among the experimental stimulus treatments (Experiment 1) in the (a) activity and (b) mean distance of individual focal males to a predator stimulus and control stimulus. Box plots show medians, means (◊), quantiles, 1.5 interquartile ranges and the significance level p < .0001 (***). The figure depicted is based on original data, whereas the statistical analysis was carried out on transformed data.

The mean distance of focal males to the stimulus predator, taken as a measure of predator inspection, did not correlate between live and animated predator trials. Focal males responded differently to live and animated predators, in that they reduced their mean distance to the predator when a live predator was present but not in the presence of an animated stimulus predator. This result suggests that male *P. Pulcher* exhibit inspection behaviour, a behaviour that has also been described for the sister species *P. taeniatus* (Hesse et al. 2015), but only towards a live fish predator and not an animated one. In approaching a potential predatory threat in this manner, an individual prey might gather information about the nature and level of potential predatory threat (Dugatkin and Godin 1992), such as the attack motivation of the predator, and behave accordingly and (or) deter predator attack (Godin and Davis 1995). An accurate assessment of predation risk is advantageous because anti-predator behaviour is costly in terms of energy expenditure and lost opportunities (Houston et al. 1993; Lima and Dill 1990; Skelly 1992). Predatory threats therefore can negatively affect reproduction (Lima and Dill 1990), habitat use (Oliveira et al. 2016) and feeding behaviour in prey animals (Dixon and Baker 1988; Hughes and Ward 1993; Pérez-Tris et al. 2004). The observed difference in the mean distance of focal males to a live fish predator and an animated fish predator could potentially be an artefact of our experimental set-up. During experimental trials, the live fish predator was constrained

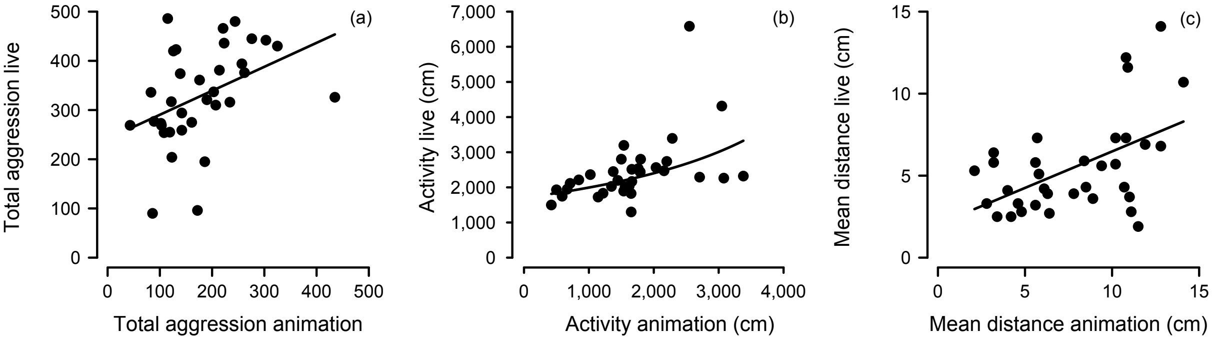

Figure 4. Relationships between the behaviours of individual focal fish directed towards an animated and a live conspecific opponent (Experiment 2). Behaviours depicted are (a) total aggression (total number of aggressive behavioural acts), (b) activity (predicted line back-transformed from model output), and (c) mean distance to the opponent.

in a clear cylinder and thus fixed in a particular position to ensure its visibility to the two focal fish being simultaneously tested. The activity of the predator inside the cylinder was consequently limited. In comparison, the animated virtual predator moved continuously back and forth, at a constant speed, across the width of the computer monitor. This difference in the movement (e.g. general activity level, swimming speed/pattern) of live and animated stimulus predators represents a behavioural mismatch, which might have led to a different perception of the actual threat by the focal fish and consequently to the observed difference in their predator inspection behaviour between the live and animal stimulus predator trials.

In Experiment 2, we found that PowerPoint© animations of still photographs of male conspecifics incited aggressive behaviour in male *P. pulcher*. All of the behavioural variables we recorded (total aggression, activity, mean distance towards the opponent) correlated positively between live and animated stimulus fish trials. The mean distance of focal males to the stimulus fish did not differ between live and animated stimulus male trials. Notwithstanding their significant concordance as noted above, total aggression and individual activity levels in focal males were higher when they were exposed to a live opponent compared to an animated (virtual) opponent. Furthermore, because the mean distance of focal males to the stimulus male opponent negatively correlated with their total aggression directed at the stimulus male, average distance to a perceived territorial intruder, as measured by our tracking software, can be used a reliable proxy measure of male aggressiveness, at least in *P. pulcher*. In contrast, the activity level of individual focal males, as quantified using the tracking software, did not correlate with their aggressiveness towards an intruder stimulus male and is therefore not a reliable proxy measure of aggression.

The observed difference in total aggression directed by focal males towards live vs. animated opponents could be due to differences in the duration of aggressive behavioural bouts. During video analyses, we noticed differences in the lateral displays of focal males between the live and animation trials. When exposed to animated opponents, individual focal males tended to perform prolonged lateral displays of up to approximately 1–2 min per bout, but much shorter displays towards live opponents. Such an apparent difference in the duration of aggressive displays might have led to more frequent, but shorter, display bouts during live stimulus male trials compared to fewer, but longer, display bouts during animated stimulus male trials, raising the possibility that focal males may not have differed significantly in their overall aggressive effort directed towards a perceived intruder male in the live vs. animation trials. Prolonged display durations in the animation trials may have been incited through the constant (persistent) displaying behaviour of the animated (virtual) male opponent. Scherer et al. (2016) have shown that the aggressive behaviour of *P. pulcher* males covaries positively with an opponent's aggressiveness. Similarly, the difference in activity between live and animation trials could be caused by differences in the activity of live vs. animated opponents. Although animated opponents were continuously moving at 1 cm/s (resulting in a total monitor distance travelled of 600 cm during a trial), live opponents exhibited a much higher level of (more varied) activity in the test aquarium.

Interestingly, we did not detect a general effect of the opponent's behaviour on the focal fish's behaviour, which is contrary to our previous finding with this same model species of fish (Scherer et al. 2016). Aggressive behaviour of live stimulus males was relatively similar (i.e. high) in the current study; thus, it may not have been sufficiently diverse to engender differences in the focal males' aggressive responses.

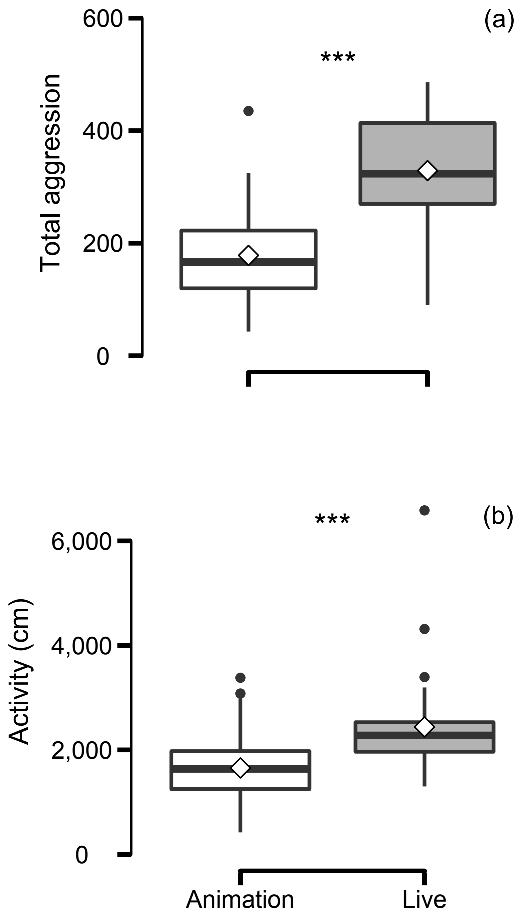

Figure 5. Differences in the levels of (a) total aggression and (b) activity of individual focal males in response to live and animated opponents (Experiment 2). The figure depicted is based on original data, whereas activity was box-cox transformed for statistical analysis. Box plots show medians (-), means (◊), quantiles, 1.5 interquartile ranges, and the significance level p < .0001 (***).

5 CONCLUSIONS

In summary, using PowerPoint© to animate still digital photographs of stimulus animals to present in a standardised manner to test subjects is an easy-to-apply and inexpensive method that can deliver biologically appropriate results. We found PowerPoint© animations of a natural fish predator and conspecific male intruder to be a suitable tool to reliably assess shyness–boldness (as measured by activity under simulated predation risk) and territorial aggressiveness, respectively, in individual males of a cichlid species, *P. pulcher*. We further showed that the use of a video tracking software allowed for a rapid and standardised assessment of the aggressiveness of individual subjects by

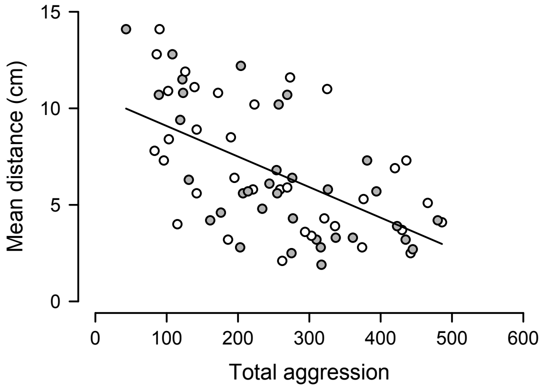

Figure 6. Relationship between total aggression (total number of aggressive behavioural acts) and mean distance to the stimulus of individual focal males during animation (open circles) and live (filled circles) stimulus trials (Experiment 2).

automatically calculating the mean distance of a subject to an opponent as a reliable proxy measure of the actual frequency of aggressive behavioural acts exhibited by a subject towards a perceived territorial intruder. However, while computer animations were suitable to assess some behaviours (activity, aggression), we found other behaviours to differ between live and animated stimuli boldness Experiment 1, individuals only decreased their average distance to a live predator but not to an animated one. In the aggression Experiment 2, the aggressiveness level of subjects was correlated between live and animated opponent trials, but aggressiveness was lower when subjects were exposed to an animated opponent compared to a live one. As discussed above, such differences might be accounted for by, e.g. differences in the behaviour of live and animated stimuli (e.g. differences in activity). Our results here show that the use of simple 2-D animated virtual stimuli can be a reliable and dependable alternative to the conventional use of live animals in experimental studies of animal behaviour and offers a number of advantages (e.g. stimulus standardisation, specific manipulations, reduction in use of live experimental animals, time and monetary savings), but requires careful validation before implementation.

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APPENDIX 3 – SUPPLEMENTAL MATERIAL 1

Supplemental Material 1. Photograph of a typical (a) predator - and (b) conspecific specimen used for the production of animations.

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❤

DECLARATIONS

Certificate of originality

I hereby declare that the contents of this thesis entitled 'Does sexual selection favour consistent behavioural differences in bi-parental cichlids?' are a record of my own original work, except where other contributors are named. The contributions of others are listed in the below 'Contribution statement'.

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst, und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Mein Anteil bei Konzeption, Durchführung und Berichtsabfassung sowie die Beiträge Anderer sind im 'Contribution statement' im Einzelnen dargelegt.

Datum, Ort Ulrike Scherer

Ethical approval

All applicable guidelines for the care and use of study animals were followed. All procedures performed in the studies were in accordance with the ethical standards of the German 'Behörde für Gesundheit und Verbraucherschutz Hamburg' (permission number 52/16).

Contribution statement

CHAPTER 1 | General introduction

I, Ulrike Scherer, wrote the general introduction. Wiebke Schuett made improving comments.

CHAPTER 2 | Predictability is attractive: Female preference for behaviourally consistent males but no preference for the level of male aggression in a bi-parental cichlid

Ulrike Scherer, Mira Kuhnhardt, and Wiebke Schuett

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The experiment was designed and planned by me, Mira Kuhnhardt, and Wiebke Schuett. I and Mira Kuhnhardt executed the experiment. I analysed the data and wrote the first version of the manuscript. Wiebke Schuett made edits.

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CHAPTER 3 | Different or alike? Female rainbow kribs choose males of similar consistency and dissimilar level of boldness

Ulrike Scherer, Mira Kuhnhardt, and Wiebke Schuett
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CHAPTER 4 | Do female rainbow kribs choose males on the basis of their apparent aggression and boldness? A non-correlational mate choice study

Ulrike Scherer, Jean-Guy J. Godin, and Wiebke Schuett
(*Submitted manuscript*)

The two experiments presented were designed and planned by me, Jean-Guy J. Godin, and Wiebke Schuett. I executed the experiments, I analysed the data, and I wrote the first version of the manuscript. Wiebke Schuett and J Jean-Guy J. Godin edited the manuscript.

CHAPTER 5 | No male mate choice for female boldness in a bi-parental West African cichlid, the rainbow krib (*Pelvicachromis pulcher*)

Ulrike Scherer and Wiebke Schuett
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CHAPTER 6 | Personality differences, parental care behaviour and reproductive success in the bi-parental rainbow krib (*Pelvicachromis pulcher*)

Ulrike Scherer and Wiebke Schuett

The experiment was designed and planned by me and Wiebke Schuett. I executed the experiment, I analysed the data, and I wrote the first version of the manuscript. Wiebke Schuett edited the manuscript.

CHAPTER 7 | General Discussion

I wrote the general discussion. Wiebke Schuett made improving comments.

APPENDIX 1 | Artificial Visible Implant Elastomer (VIE) tags of different colour and symmetry do not influence mate choice in a cichlid

Wiebke Schuett, Teresa F. Nava, Neele Rahmlow, and Ulrike Scherer
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The experiment was designed and planned by Wiebke Schuett and me. Teresa F. Nava and Neele Rahmlow executed experimental trials. I made all VIE tags. Wiebke Schuett analysed the data and wrote the introduction, result and discussion section of the manuscript. I wrote the methods section of the manuscript. All authors edited the manuscript.

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APPENDIX 2 | Lateralisation in agonistic encounters: Do mirror tests reflect aggressive behaviour? A study on a West African cichlid

Ulrike Scherer, Melanie Buck, and Wiebke Schuett
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The experiment was designed and planned by Melanie Buck and Wiebke Schuett. Melanie Buck executed the experiment. I analysed the data and wrote the first version of the manuscript. Wiebke Schuett edited the manuscript.

APPENDIX 3 | Validation of 2D-animated pictures as an investigative tool in the sciences – A case study with a West African cichlid fish, *Pelvicachromis pulcher*

Ulrike Scherer, Jean-Guy J. Godin, and Wiebke Schuett
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The two experiments presented were designed and planned by me, Jean-Guy J. Godin, and Wiebke Schuett. I executed the experiments, I analysed the data, and I wrote the first version of the manuscript. Wiebke Schuett and J Jean-Guy J. Godin edited the manuscript.

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